Asthma, atopy, and lung function in young adults after hospitalization for bronchiolitis in infancy

Karen Galta Sørensen

Thesis for the degree of Philosophiae Doctor (PhD)
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1. PREFACE

1.1 Scientific environment and funding

This thesis is based on data from the LAMA-study (Lung function, AsthMa, and Atopy in young adults after hospitalization for bronchiolitis in infancy) carried out at Stavanger University Hospital and Haukeland University Hospital during 2015-2020. The project originated from the Pediatric Research Group at Stavanger University Hospital led by Professor Knut Øymar, and was also included in the Research Group for Pediatric follow-up studies at the Department of Clinical Science, University of Bergen, currently led by Professor Camilla Tøndel.

I signed a PhD-contract with the University of Bergen, Faculty of Medicine in 2017, and received a grant from the Western Norway Regional Health authority in 2019 (F-12502). Stavanger University Hospital, The Kloster Foundation, The Norwegian Allergology and Immunopathology Association, and The Norwegian Asthma and Allergy Association all contributed financially to the conduction of the clinical examinations.

Main supervisor has been Ingvild Bruun Mikalsen, Associate Professor at the University of Bergen. Co-supervisors have been Knut Øymar and Thomas Halvorsen, both Professors at the University of Bergen.

1.2 Acknowledgements

First of all, I am very grateful to all children, young adults, and parents who have taken part in this study. Without their participation and patience, this study would not have been possible.

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Our former and current director Henning Garsjø and Oddny Hovtun Bjorland at the Division of Obstetrics Gynecology and Pediatrics, head of the Department of Pediatric and Adolescent Medicine Berit Helgeland Kyllevik, and the director at the Department of Research at Stavanger University Hospital Svein Skeie deserve acknowledgements for their support and assistance in facilitating the research at the Pediatric department. I am thankful to the Leading Clinician at the Pediatric ward, Ann Marit Gilje, for her flexibility and encouragement for my research, and for making me feel appreciated and desired back in clinical work.

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the world of research, for taking far more than his share of “home-with-a-sick-child-days”, and for giving me the opportunity to work at all hours if needed. Last but not least, I want to thank our wonderful children Filip (8), Aksel (7), and Pia (4) for all the hugs, laughter and fun, and for constantly reminding me of what is most important in life.

Stavanger, April 2023
Karen Galta Sørensen

1.3 Sammendrag

Bakgrunn:

Bronkiolitt er en viral nedre luftveisinfeksjon som ofte rammer spedbarn. Respiratorisk syncytialt virus er det vanligste viruset ved bronkiolitt, etterfulgt av rhinovirus. Bronkiolitt er assosiert med økt risiko for astma og nedsatt lungefunksjon i barndomsårene, mens sammenhengen med atopi er mer uklar. Vi har mindre kunnskap om langtidsutfall i ung voksen alder etter bronkiolitt, men det kan se ut til at den økte risikoen for luftveissykdom vedvarer. Årsaksmekanismene bak dette, inkludert betydningen av eosinofil inflammasjon, vekst og fettmetabolisme, er imidlertid ikke tilstrekkelig kartlagt. Studier tyder på at ulike virus har ulik betydning.

Formål:

Vårt hovedmål var å bidra til ny kunnskap om langtids-konsekvenser etter bronkiolitt. Vi ville studere sammenhengen mellom bronkiolitt i spedbarnsalder og astma, atopi og lungefunksjon i ung voksen alder, og hvordan disse sammenhengene påvirkes av kjønn og viral etiologi. Videre ville vi studere utviklingen av lungefunksjon over tid, og ulike mekanismer som kan påvirke langtids-konsekvensene av bronkiolitt inkludert eosinofil inflammasjon, vekst og fettmetabolisme.

Metoder:

Norsk oppfølgings-studie ved 17-20 års alder som inkluderte 225 deltakere innlagt på sykehus for bronkiolitt i spedbarnsalderen i 1996–2001, hvor av 60 deltakere hadde resultater fra lungefunksjonsmåling fra en oppfølging ved 11 år. I tillegg inkluderte studien to ulike kontrollgrupper; den første inkluderte 40 deltakere som også hadde deltatt i 11-års oppfølgingen, mens den andre inkluderte 167 matchede deltakere rekruttert til oppfølgingen ved 17-20 år. Oppfølgingen inkluderte spørreskjema for astma, og undersøkelser av lungefunksjon, atopi, kroppsmasseindeks og markører for fettmetabolisme.

Resultater:

Unge voksne med tidligere bronkiolitt hadde høyere forekomst av astma, men ikke atopi, og et mer obstruktivt lungefunksjons-mønster enn kontrolldeltakerne. Banene for utvikling av lungefunksjon fra 11 til 18 år lå lavere, men parallelt med kontrollgruppen. Antall eosinofile blodceller målt under bronkiolitt var negativt assosiert med lungefunksjon i ung voksen alder, men ikke med atopi eller astma. Vekst og fettmetabolisme så ikke ut til å forklare den økte sykeligheten etter bronkiolitt.

Konklusjon:

Unge voksne med tidligere bronkiolitt hadde økt forekomst av astma og redusert lungefunksjon. Det er behov for videre forskning for å forstå mekanismene som ligger bak disse sammenhengene.

Konsekvenser:

Økt kunnskap om langtidskonsekvenser etter bronkiolitt kan danne grunnlag for å utvikle mer persontilpassede retningslinjer for oppfølging. Dette kan gi mulighet til å igangsette forebyggende tiltak for å modifisere utviklingen mot senere luftveissykdom.

1.4 Summary of thesis

Background:

Bronchiolitis is a viral lower respiratory tract infection commonly seen in infants. Respiratory syncytial virus is the most commonly detected virus, followed by rhinovirus. Hospitalization for bronchiolitis is associated with increased risk of asthma and impaired lung function during childhood, whereas the association with atopy is less consistent. Long-term outcomes beyond childhood are less described, but studies indicate persistence of respiratory morbidity until young adulthood. The mechanisms behind these associations are poorly understood, including the role of eosinophil inflammation, growth, and fat metabolism. Studies suggest that the virus involved might play a role.

Aim:

Our main aim was to provide new knowledge on long-term outcomes after bronchiolitis. Specifically, we aimed to study associations between bronchiolitis in infancy and subsequent asthma, atopy, and lung function in young adult age, particularly addressing potential modifying factors including sex and viral etiology. Further, we aimed to study trajectories of lung function after bronchiolitis, and potential underlying mechanisms of the long-term outcomes including eosinophil inflammation, growth, and fat metabolism.

Methods:

This Norwegian cohort study enrolled 225 young adults (age 17-20 years) hospitalized for bronchiolitis in infancy during 1996–2001, of whom 60 subjects had acceptable results for spirometry from participation in a follow-up at 11 years of age. Additionally, there were two different control groups; the first included 40 subjects who had participated also in the 11-year follow-up, while the second included 167 matched control subjects recruited particularly for this follow-up at young adult age. The evaluation at 17-20 years included questionnaires for asthma, and examinations of lung function, atopy, body mass index, and markers of fat metabolism.

Results:

Young adults hospitalized for bronchiolitis had higher prevalence of asthma, but not atopy, and a more obstructive lung function pattern compared to control subjects. Their lung function trajectories from 11 to 18 years were lower, but parallel to those of the control group. The level of eosinophils during bronchiolitis was negatively associated with lung function, but not atopy or asthma. Growth and fat metabolism did not seem to explain the increased respiratory morbidity after bronchiolitis.

Conclusion:

Young adults hospitalized for bronchiolitis in their first year of life had higher occurrence of asthma and impaired lung function. Further research is needed to understand the mechanisms behind these associations.

Consequences:

Better knowledge of the long-term outcomes after bronchiolitis may form a basis for developing more personalized guidelines for follow-up, which in turn may provide an opportunity to initiate preventive measures to modify the development towards disabling respiratory morbidity in older age.

1.5 List of papers

1. Sørensen KG, Øymar K, Dalen I, Halvorsen T, Mikalsen IB.
   Asthma, atopy and lung function in young adults after hospitalization for bronchiolitis in infancy: impact of virus and sex.
   BMJ Open Respir Res. 2022 Jan;9(1):e001095. doi: 10.1136/bmjresp-2021-001095.

2. Sørensen KG, Øymar K, Dalen I, Halvorsen T, Mikalsen IB.
   Lung function and bronchial hyper-reactivity from 11 to 18 years in children with bronchiolitis in infancy.
   Pediatr Allergy Immunol. 2020 Jan;31(1):57-65. doi: 10.1111/pai.13137.
   Erratum in: Pediatr Allergy Immunol. 2022 Dec;33(12): PMID: 31595542.

3. Sørensen KG, Øymar K, Dalen I, Halvorsen T, Mikalsen IB.
   Blood eosinophils during bronchiolitis: Associations with atopy, asthma and lung function in young adults.
   Acta Paediatr. 2023 Apr;112(4):820-829. doi: 10.1111/apa.16666.

4. Sørensen KG, Øymar K, Jonsson G, Dalen I, Halvorsen T, Mikalsen IB.
   Are BMI and adipokines associated with asthma, atopy and lung function in young adults previously hospitalized for bronchiolitis?
   Respir Med. 2023 Apr;209:107149. doi: 10.1016/j.rmed.2023.107149.

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1.6 Abbreviations

α	Significance level
β	Regression coefficient
API	Asthma Predictive Index
BHR	Bronchial hyperresponsiveness
BMI	Body mass index
CDHR3	Cadherin-related family member 3
CI	Confidence interval
COPD	Chronic obstructive pulmonary disease
COPSAC	Copenhagen Prospective Studies on Asthma in Childhood
DRS	Dose response slope
EISL	International Study of Wheezing in Infants
FEF_{25-75}	Forced expiratory flow between 25-75% of the forced vital capacity
FeNO	Fractional exhaled nitric oxide
FEV_1	Forced expiratory volume in first second
FVC	Forced vital capacity
GAN	Global Asthma Network
GLI	Global Lung Initiative
ICD	International Classification of Diseases
ICS	Inhaled corticosteroid
Ig	Immunoglobulin
IFN	Interferon
IL	Interleukin
LRTI	Lower respiratory tract infection
ISAAC	International Study of Asthma and Allergy in Childhood
NICE	National Institute for Health and Care Excellence (United Kingdom)
OR	Odds ratio
PCR	Polymerase chain reaction
RRR	Relative risk ratio
RSV	Respiratory syncytial virus
RV	Rhinovirus
SD	Standard deviation
SIGN	Scottish Intercollegiate Guidelines Network
SPT	Skin prick test
Th	T-helper cell
UK	United Kingdom
USA	United States of America

2. INTRODUCTION

Acute bronchiolitis, a viral infection of the lower respiratory tract, is one of the most substantial health burdens for infants worldwide [1 2]. In developed countries, this is the most common cause for hospitalization during the first year of life [3]. In South Rogaland, Norway, 3.4% of all infants less than one year of age were hospitalized for bronchiolitis during 2008 to 2012 [4]. Hence, a substantial group of young adults will bear this in their medical history. How does this affect them? Which subsequent consequences can young adults face after this transient event early in life, and why? These questions have been the starting point for my research, which aims to increase the knowledge of long-term outcomes after bronchiolitis.

In the pages that follow, I will elucidate viral bronchiolitis more thoroughly before describing different outcomes that may be affected in young adult age. Further, I will present an overview of what is known about long-term consequences of bronchiolitis, and finally provide a background for the possible underlying mechanisms for these consequences, emphasizing those assessed in my research.

Since the beginning of this project, guidelines have been updated and new studies on the long-term consequences after bronchiolitis have been published. The section describing methods for clinical examinations is based on literature and guidelines available before our study was commenced in 2015. This also applies to the introduction for literature that is directly related to our objectives. More recent studies will in this case be addressed in the discussion section when interpreting our results. A structured search for literature on long-term outcomes after bronchiolitis was performed at 13th of December 2022 in Medline, EMBASE, and Cochrane Library.

2.1 Viral bronchiolitis

2.1.1 Definition and clinical characteristics

There is no uniform definition of bronchiolitis worldwide, and comparison between studies is challenging particularly due to differences in age limits and symptomatology. The upper age limit for inclusion in different post-bronchiolitis

studies varies from 12 months [5-8] to 24 months [9-11] or even 36 months [12]. Also, definitions from different European countries tend to focus more on crackles being an important part of the clinical picture, whereas the guidelines from the United States of America (USA) focus on wheezing, and probably includes clinical pictures that in Europe rather would be referred to as virus-induced wheeze or early onset asthma [13]. The American Academy of Pediatrics' guideline from 2014 defined bronchiolitis as a viral upper respiratory tract prodrome followed by increased respiratory effort and wheezing in children from 1 month through 23 months of age [13 14]. In Europe, The Scottish Intercollegiate Guidelines Network (SIGN) published a guideline for bronchiolitis in 2006 [15]. This guideline was withdrawn in 2016, but was still in use when our study was planned, and emphasized that bronchiolitis was a seasonal clinical diagnosis caused by a viral infection and characterized by fever, nasal discharge and wheezy cough with fine inspiratory crackles and/or expiratory wheeze on examination [15]. The National Institute for Health and Care Excellence (NICE) in the United Kingdom (UK) published a guideline for bronchiolitis including a definition similar to that of SIGN shortly before the SIGN guideline was withdrawn [13]. The NICE guideline was first published in 2015, last updated in 2021, and defines bronchiolitis as a coryzal prodrome followed by cough and tachypnea±chest recessions, and wheeze±crackles on chest auscultation [13 16]. Both the SIGN and NICE guidelines state that bronchiolitis affects children under 2 years of age, but most commonly infants in their first year of life, with the incidence peaking between 3 and 6 months of age [15 16].

Even though there is no uniform definition, most clinicians share a common perception of bronchiolitis as a virus induced inflammation of the bronchioles in young children leading to symptoms from the lower respiratory tract after a prodromal phase of upper respiratory tract symptoms with rhinorrhea with or without fever [1]. Symptoms may include cough, increased work of breathing, apnea, rales, wheeze, and impaired feeding [15 17]. On clinical examination, crackles on auscultation, expiratory wheeze, increased respiratory rate, prolonged expiration, recessions, use of accessory muscles, cyanosis, and decreased general condition may be present [15 17]. The median duration of symptoms from the onset of acute bronchiolitis is around two weeks, with 10–20% of infants still having symptoms at 3 weeks after onset [1].

2.1.2 Epidemiology

Bronchiolitis is common, and associated with high morbidity, but low mortality in high-income countries. A systematic analysis of the global burden of acute lower respiratory tract infections (LRTI) due to respiratory syncytial virus (RSV) estimated that in children aged 0-12 months in 2019, there were 12.9 million new episodes worldwide, of which 2.2 million were severe episodes requiring hospitalization, and 66 300 deaths were attributable to RSV [2]. In high-income countries, the corresponding estimates were 515 000 new episodes, 294 000 hospitalizations, 400 in-hospital deaths, and 900 RSV-attributable overall deaths of which 200 were in the first four weeks of life [2]. In high-income countries, the in-hospital case fatality ratio is 0.1% [2]. Hence, mortality occurs predominantly in the developing world, and with the highest rates in the youngest.

Bronchiolitis is a seasonal infection which in the tempered northern hemisphere tends to peak during the winter months [1 15 17]. This period corresponds to the occurrence of RSV infection which peaks during epidemics from October until May in the temperate northern hemisphere [17]. Possible explanations for this seasonal variation are climate-depending behavior with indoor crowding during the cooler months facilitating viral transmission, and increased exposure to air pollutant such as ozone and traffic pollutants [1]. Pollutants together with weather-related factors such as inhalation of cold and dry air, might affect the airways in ways that make them more vulnerable and susceptible for viral infections.

2.1.3 Risk factors for severe bronchiolitis

Severe bronchiolitis is in the literature often defined as cases in which hospitalization are needed. Infants < 3 months of age, and children with congenital heart disease, neuromuscular impairment, or chronic lung disease including ex-prematures and a variety of “syndroms” (e.g. trisomy 21) have increased risk of a severe course of bronchiolitis [17-19]. Even though children with pre-existing medical conditions are over-represented, bronchiolitis is generally a frequently occurring

disease, and hence the majority of patients will still be born at full term and otherwise healthy [18 20]. Studies on the impact of different social factors on the risk of being hospitalized for bronchiolitis have shown breastfeeding to be protective, and parental smoking and having siblings at home or attending nursery or day-care attendance to be risk factors [15]. In addition, boys have a higher risk of bronchiolitis than girls [21 22].

2.1.4 Viral etiology

Bronchiolitis is an infection in which the viral etiology is highly dependent on age, with RSV dominating in infants and rhinovirus (RV) in particular being more frequent with increasing age [23 24] (Figure 1). RSV is detected in 60-80% of cases in patients below 12 months of age [17]. RV is the second most common virus (14-30%), thereafter bocavirus (14-15%), metapneumovirus (3-12%), entero-, adeno-, corona, and influenza viruses (1-8%) [17]. Co-infections are common, and previous studies have found up to 30% of children with bronchiolitis to be infected with more than one virus [18 23 25]. The Norwegian Bronchiolitis ALL-study on virus type and genomic load during acute bronchiolitis found viral co-infection to be even more common, with two or more viruses (maximum 7) detected in 61% of infants [26].

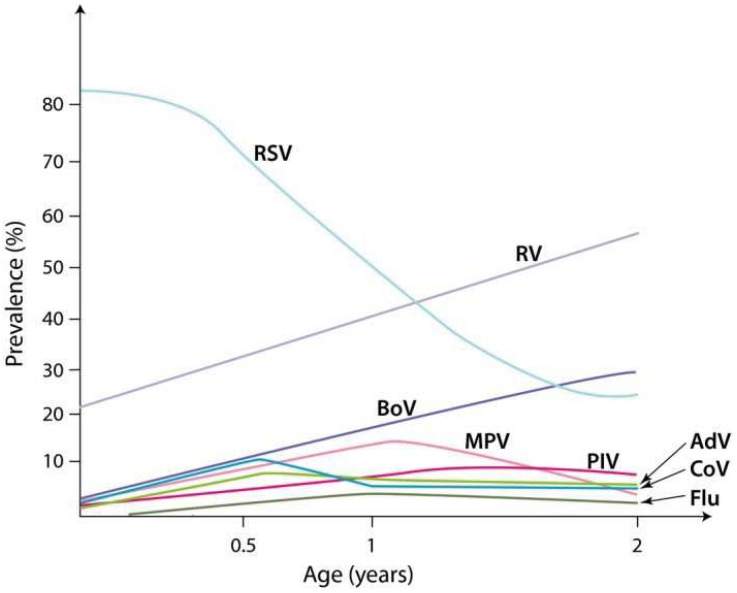

Figure 1. The frequency of viral etiologic agents according to the age of the hospitalized patients with the first episode of bronchiolitis or wheezing. Viral diagnostics were based on PCR (including rhinovirus C species) except for human bocavirus which was based on serology.
Abbreviations: RSV, respiratory syncytial virus; RV, rhinovirus; BoV, human bocavirus 1; MPV, metapneumovirus; PIV, parainfluenzavirus; AdV, adenovirus; CoV, coronavirus; Flu, influenza.
Reprinted from Jartti et al. [24]. © 2018 The Authors. Allergy. Published by John Wiley & Sons Ltd. (Open access, CC BY-NC 4.0).

2.1.5 Pathophysiology

Bronchiolitis is an acute inflammation of the bronchioles characterized by extensive inflammation, edema, increased mucus production, and necrosis of epithelial cells lining small airways leading to cellular debris [14 17 18]. This leads to airflow obstruction, distal air trapping, and potentially lobar collapse or atelectasis [17 18]. Smooth-muscle constriction seems to play a minor role [27].

Damage to the airways during bronchiolitis may occur either directly by injuries inflicted by the viral infection itself, or indirectly by activation of the immune system. The type of immune response of acute bronchiolitis is dependent on viral etiology, with a T-helper (Th) 1-response in the majority of RSV-infected infants, and a high Th2/Th1-ratio with higher levels of Th2 type interleukins in the majority of RV-infected infants [28 29]. In the following, the immune response is described for RSV and RV separately with a graphical overview in Figure 2.

RSV bronchiolitis

In addition to a Th1-type response, the initial immune response of RSV is an innate interferon (IFN) response with induction of type I and III IFNs and further induction of various immune cells, including neutrophils (Figure 2A). A neutrophilic inflammation dominated in a study of bronchoalveolar lavage cellularity in infants with severe RSV-bronchiolitis [30]. Consistent with this, a genetic study in children with RSV reported upregulation of genes involved in neutrophil recruitment and activation, and downregulation of genes involved in T-cell activation and differentiation [31]. Low IFN-responses have been found to be associated with increased severity in infants hospitalized for RSV-bronchiolitis, corroborating this as an important response [32].

RSV-infection is common in the first months of life when the immune system is still immature and dependent on the innate immune system in response to a toll-like-receptor ligation and maternal-derived antibodies [24] (Figure 2A). In the youngest infants, the Th1 cell response seems to develop rather slowly, but an increased IL-17-production is more prominent and leads to increased mucus production [24].

RV bronchiolitis

In healthy children, RV also targets the airway epithelial cells and can induce a Th1-type response and a Type I and III IFN-response with further induction of various immune cells (Figure 2B). On the other hand, children at risk of severe RV infection, particularly children with atopic predisposition, may have low interferon responses [33]. Deficient interferon responses to RV infection have been found both in atopic asthma, non-atopic asthma, and in atopic subjects without asthma, suggesting this response to be present in Th2-oriented conditions in general [34]. The immune response in these children tends to be dominated by induction of type 2 innate cytokines, such as IL-25 and IL-33, which subsequently boost a Th2-response and eosinophil inflammation via IL-5 and IL-13 among others [24]. Consistent with this, an observational study assessing cytokine responses reported higher concentrations of both IL-5 and IL-13 in children with early wheezing due to RV compared to RSV wheezing [28].

Other viruses

The pathophysiology of bronchiolitis caused by other viruses such as bocavirus and metapneumovirus are less studied, but they have been suggested to constitute a third cluster within bronchiolitis (in addition to RSV-bronchiolitis and RV-bronchiolitis) both clinically and pathophysiologically [24]. This type of bronchiolitis is probably less severe, and entails a lower risk of long-term sequelae [24].

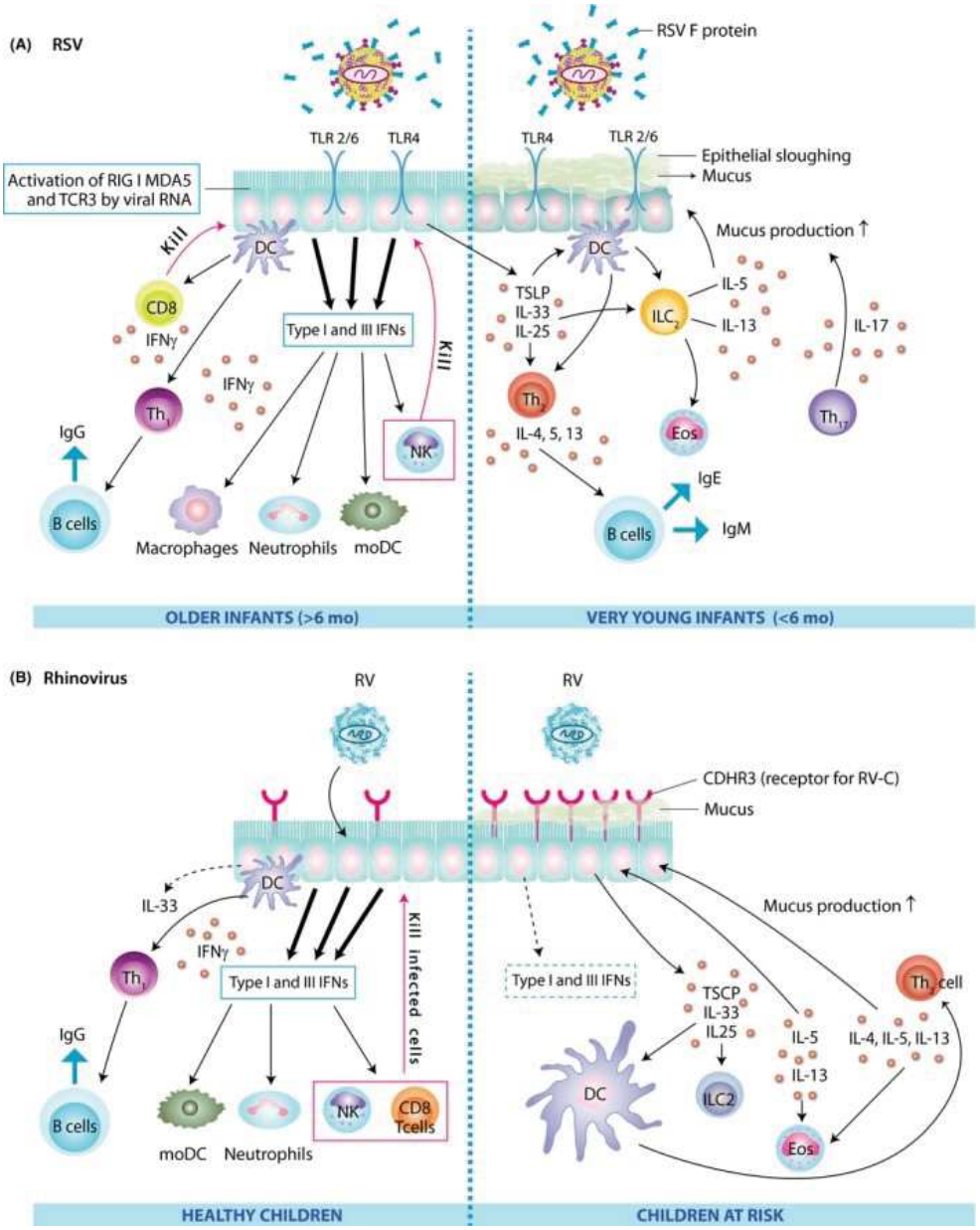

Figure 2. Pathogenesis of respiratory syncytial virus **(A)** and rhinovirus infection **(B)** in the airway epithelial cells of healthy children and those at risk.
Abbreviations: CDHR3, cadherin-related family member 3; DC, dendritic cell; Eos, eosinophil; IFN, interferon; Ig, immunoglobulin; IL, interleukin; ILC, innate lymphoid cell; MDA, melanoma differentiation-associated protein; moDC, monocyte-derived dendritic cells; NK, natural killer cell; RIG, retinoic acid-inducible gene; RSV, respiratory syncytial virus; RV, rhinovirus; TCE3, third T-cell receptor; Th, T helper cell; TLR, toll-like receptor; TSLP, thymic stromal lymphopoietin.

2.2 Asthma

2.2.1 Definition

The term asthma is now considered an umbrella diagnosis for several diseases with distinct mechanistic pathways (endotypes) and variable clinical presentations (phenotypes) [35]. The Global Initiative for Asthma (GINA) guideline from 2022 defines asthma as follows: *"Asthma is a heterogeneous disease, usually characterized by chronic airway inflammation. It is defined by the history of respiratory symptoms, such as wheeze, shortness of breath, chest tightness and cough, that vary over time and in intensity, together with variable expiratory airflow limitation."* [36] There is no single diagnostic test for asthma. The diagnosis is based on clinical assessment supported by objective tests that seek to demonstrate variable airflow obstruction or the presence of airway inflammation [37].

2.2.2 Epidemiology

Asthma is a common chronic condition that causes substantial morbidity globally [38], but there is a considerable heterogeneity in the definitions used in epidemiological studies contributing to highly variable estimates of asthma prevalence [39]. Studies often use information from questionnaires with self-reporting of asthma, with a distinction between "asthma ever" (lifetime prevalence) and "current asthma" (point prevalence). A study on different asthma definitions in children found 122 papers to yield 60 different definitions, with prevalence estimates varying between 15.1% and 51.1% depending on the asthma definition used [40]. In adults, lifetime asthma, diagnosed asthma, and current asthma were in 117 papers defined in 8, 12, and 29 different ways, respectively [39]. Hence, comparisons between studies are challenging.

The European Community Respiratory Health Survey, the International Study of Wheezing in Infants (EISL), the International Study of Asthma and Allergies in Childhood (ISAAC), and the World Health Survey have all collected data on asthma prevalence [41]. However, except for EISL which has reported data from 2012, none of these report data recorded after 2003 [41]. The Global Asthma Network (GAN), a multi-

country cross-sectional population-based study using the same core methodology as the ISAAC, has recently reported an overall global prevalence in adults for current wheeze of 6.6% and asthma ever of 4.4%, with the highest prevalence in high-income countries with a prevalence for current wheeze of 10.6% and asthma ever 10.2% [38]. "Current wheeze" was in the GAN-study defined as a positive answer to the question "Have you had wheezing or whistling in the chest in the past 12 months?" whereas "Asthma ever" was defined as a positive answer to the question "Have you ever had asthma?" [38]. A GAN-study in children found a world total prevalence for adolescents aged 13-14 years of 10.4% for current wheeze and 11% for asthma ever with a stable prevalence of current wheeze over 10 years in high-income countries [42].

In Norway, there are no recent nationally representative surveys on the prevalence of asthma. According to the Public Health Report, figures from the Norwegian Prescription Database show that the proportion of the population aged 5-44 who have collected at least one asthma medication within one year indicates that the prevalence of asthma has remained stable at around 5-6% in the period 2010-2020 [43].

In general, the risk of asthma is related to sex, and the distribution between sexes varies with age. During childhood, the prevalence of asthma is higher in males, but after a switch during puberty, females have a higher prevalence in adulthood [44-46]. Also the GAN-study reported higher prevalence of asthma in female than male adults [38]. Sex hormones, social and environmental factors, and genetic and epigenetic variations are important factors in the sex differences observed in asthma [46]. While atopic predisposition is a risk factor for asthma in both sexes during childhood, the influence of atopic predisposition on the development of asthma seems more pronounced in boys than in girls [47].

2.2.3 Pathophysiology

Asthma is usually associated with airway inflammation and bronchial hyperresponsiveness (BHR) [36].

Airway inflammation

Based on the mechanisms behind the heterogeneity of airway inflammation, asthma can be divided into two major endotypes: Th2-high (eosinophilic) and Th2-low (non-eosinophilic) asthma [35]. Whereas up to 25% of adults with asthma have neutrophilic inflammation, this is rarely described in children [48].

Th-2-high

Eosinophils are the cardinal cell type associated with Th-2-high asthma, and also central effectors of allergic inflammation [23]. A Th2-dominated eosinophilic inflammation is important in the pathophysiology of eosinophilic asthma, which may or may not be related to atopy [49]. Diagnosis of an eosinophilic phenotype may be based on deep lung tissue exploration (bronchoalveolar lavage or biopsy), induced sputum, blood assessment, or eosinophil cell-derived markers such as fractional exhaled nitrogen oxide (FeNO) [48]. Peripheral blood eosinophils may be closely correlated to eosinophil inflammation in deep lung tissue [48], but this finding is not consistent [35]. However, peripheral blood eosinophils have recently been highlighted as a biomarker useful to evaluate response to inhaled corticosteroids (ICS) in the treatment of asthma [50-52].

Th-2-low

Th2-low asthma is linked to activation of Th1 and/or Th17 cells, and is generally characterized by neutrophilic or paucigranulocytic (i.e., normal sputum levels of both eosinophils and neutrophils) inflammation [35]. The mechanisms underlying recruitment and maintenance of neutrophilic airway inflammation are yet unknown, but severe neutrophilic asthma has been associated with chronic infection with atypical bacteria, obesity, smoking, and poorly understood underlying smooth muscle abnormalities [35]. A Th2-low asthma is associated with resistance to treatment with ICS [35 48].

Bronchial hyperresponsiveness

In the ERS technical standard on bronchial challenge testing, airway hyperresponsiveness is defined as: "*an increased sensitivity and exaggerated response to non-allergenic stimuli that cause airway narrowing"* [53]. While most commonly associated with asthma, different degrees of BHR are also seen in other diseases associated with airway inflammation or obstruction, and in healthy subjects [53]. The degree of BHR may increase during exacerbations and decrease during treatment with anti-inflammatory medications such as ICS, and may be absent during asymptomatic periods [53]. BHR is assessed by bronchial challenge tests or broncho-provocation tests where subjects are exposed to increasing doses of a bronco-constrictor paralleled by repeated measurements of lung function. These tests are categorized as direct or indirect by the way that airway smooth muscles are stimulated to cause bronchoconstriction [53]. Whereas methacholine and histamine act directly on the airway smooth muscle, exercise, cold air, mannitol eucapnic hyperventilation, and hypertonic saline act indirectly to cause airway narrowing [53]. Studies have found that BHR can be detected before asthma symptoms are reported, with BHR during childhood acting as a predictor of subsequent asthma in adolescence or adulthood [54-56].

2.3 Atopy

According to a report of the Nomenclature Review Committee of the World Allergy Organization which describes recommended nomenclature for allergy for global use, atopy is defined as follows: *"Atopy is a personal and/or familial tendency to become sensitized and produce immunoglobulin (Ig) E antibodies in response to ordinary exposures to allergens, usually proteins"* [57]. This report further elaborates that the term atopy should be used to describe the genetic predisposition to become IgE-sensitized to otherwise harmless allergens commonly occurring in the environment, and states that this term can only be used if an IgE sensitization has been documented. This may be accomplished *in vivo* by measurement of IgE antibodies in serum from peripheral blood samples, or *in vitro* by a positive skin prick test (SPT) in which a fine needle is passed through a drop of allergen extract before the size of a reactive skin wheal is recorded after a standardized time span [58].

Atopy, or allergic sensitization, is common. A study of an unselected population in Belgium demonstrated a 40.3% prevalence of a positive SPT to one or more common aeroallergens [59], and data from The National Health and Nutrition Examination Survey in the United States 2005-2006 found that 44.6% of the population aged 6 years and older had positive specific IgE for at least one allergen [60]. In the Environment and Childhood Asthma study in Oslo based on a Norwegian population-based birth cohort, 49% had allergic sensitization at a 16-year follow-up [54].

2.4 Lung function

The main function of the lungs is the process of gas exchange by breathing. Figure 3 gives an overview of the various lung volumes that can be measured by plethysmography. Total lung capacity consists of the vital capacity which is the maximal volume of air possible to exhale after maximal inhalation, and a residual volume that remains in the lungs even after a maximal exhalation. The vital capacity is further divided into the tidal volume, which is the volume that is inhaled and exhaled at rest, the inspiratory reserve capacity which can be recruited by maximal inhalation, and the expiratory reserve volume which can be recruited during maximal exhalation.

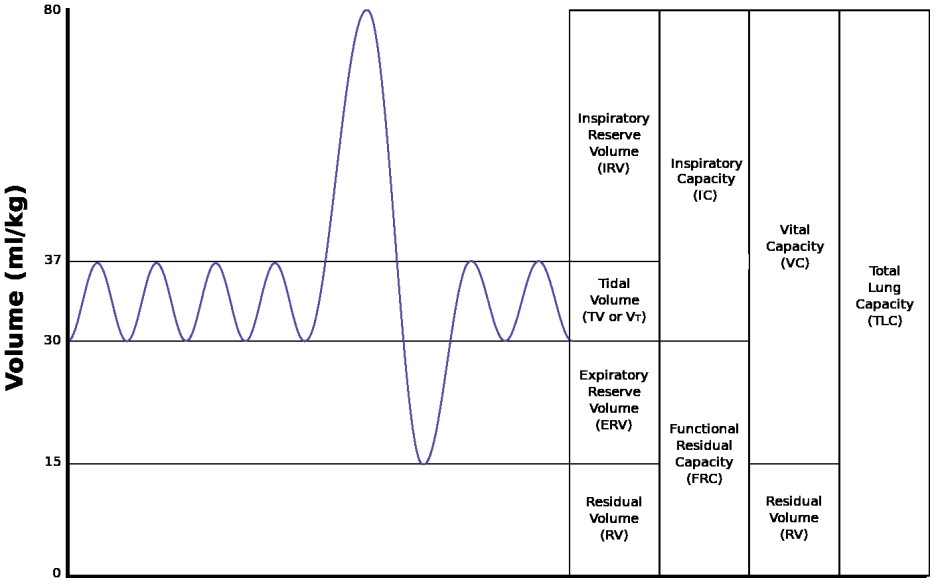

Figure 3. Lung volumes.
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Lung function is most commonly measured by spirometry which is a physiological test that measures the maximal volume of air that an individual can inspire and expire with maximal effort [61] . The primary signal measured in spirometry is either volume or flow as a function of time [61]. From this, a flow-volume loop can be constructed, as illustrated in Figure 4, in which the curve below the x-axis represents the inspiration and the curve above the x-axis represents the expiration. In cases of respiratory morbidity in the lower airways, such as in asthma, it will be airflow in the expiratory phase that is most affected. A description of the most commonly used lung function variables derived from spirometry is given in Table 1.

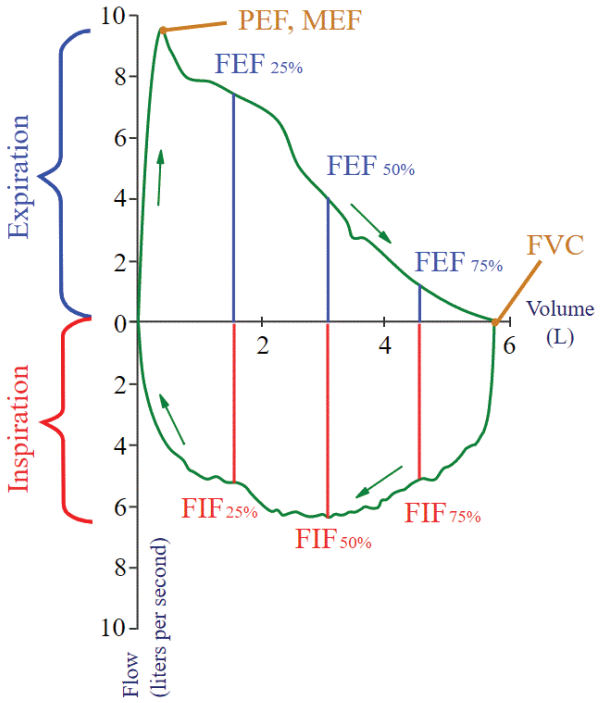

Figure 4. Flow-volume loop as measured by spirometry. Abbreviations explained in Table 1.
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Table 1. Lung function variables.

Abbreviation	Description	Explanation
PEF	Peak expiratory flow	The maximal flow (volume/time) achieved during a maximal forced expiration.
FVC	Forced vital capacity	The volume of air that can be exhaled after a maximal inspiration.
FEV_1	Forced vital capacity in first second	The volume of air that is exhaled during the first second of a forced expiration.
FEV_1/FVC	Forced vital capacity in first second/Forced vital capacity	The volume of air that is exhaled during the first second of a forced expiration as a proportion of the total exhaled volume. Important measurement for obstruction.
$FEF_{25\%}$ $FEF_{50\%}$ $FEF_{75\%}$ $FIF_{25\%, 50\%, 75\%}$	Forced expiratory flow Forced inspiratory flow	The forced expiratory flow (volume/time) when 25%, 50%, or 75% respectively, of the volume (FVC) has been exhaled. Forced inspiratory flows ($FIF_{25\%, 50\%, 75\%}$) are the corresponding variables for inspiration.
FEF_{25-75}	Forced expiratory flow between 25-75% of the forced vital capacity	The average flow (volume/time) from 25% to 75% of the volume (FVC) has been exhaled.

Lung function varies with age, height, sex, and ethnicity, and thus the test results need to be compared to values predicted from data obtained in presumably healthy and otherwise comparable populations. The Global Lung Function Initiative (GLI) has collected respiratory function outcomes from around the world, and produced reference equations for this purpose [62].

2.4.1 Trajectories of lung function and chronic obstructive lung disease

The lung function changes during a life course in three main phases. First is a growth phase with gradually increasing lung function as the lungs mature and grow. The most excessive lung growth occurs during puberty in close relation to the pubertal height growth [63]. After this period of accelerated growth, the lung function reaches a peak at age 20-25 years. The second phase is a plateau-phase lasting for a few years, and lastly, the third phase is characterized by a decline in lung function due to physiological ageing [64]. All of these phases may be altered by different genetic or environmental factors giving rise to different lung function trajectories for the individual throughout a life course (Figure 5). Genetic predispositions, maternal tobacco smoking or undernutrition, intrauterine growth restriction, preterm birth, bronchopulmonary dysplasia, air pollution exposures, and respiratory tract infections

such as bronchiolitis are all factors with a potential to alter the lung function in utero or early in life [64 65]. This might result in a lung function below normal, which may or may not show catch-up towards a normal lung function trajectory later in life (Figure 5).

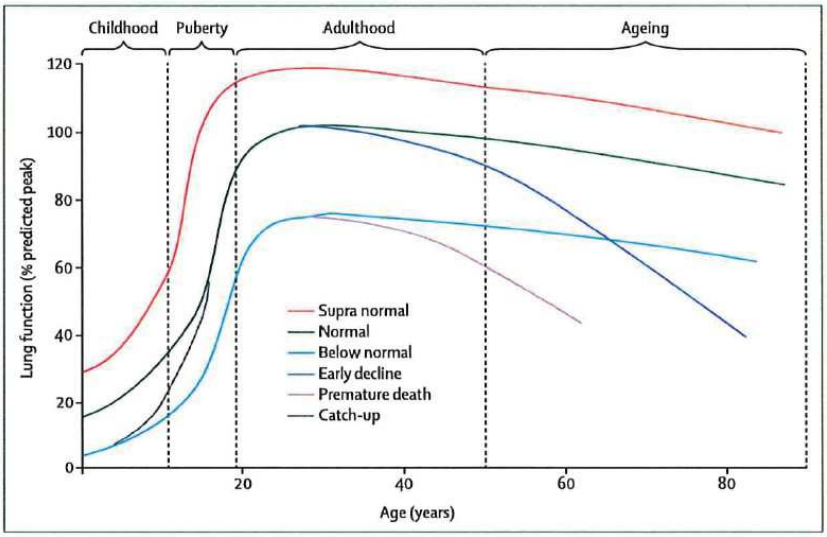

Figure 5. Potential lung function trajectories throughout the life course.
Reprinted from Agusti et al. [64] with permission from the publisher. © 2019 Elsevier Ltd.

Population-based birth cohort studies have confirmed that abnormal lung function trajectories characterized by repeated measurements of FEV_1 below normal in adulthood, may in fact originate in early life [66 67], and hence it has been hypothesized that chronic obstructive pulmonary disease (COPD) may begin in childhood [65]. Failure to attain maximal lung function at its plateau may lead to COPD later in life, even when the physiological rate of decline of lung function is maintained. COPD is a heterogeneous lung condition characterized by chronic respiratory symptoms due to abnormalities of the airways that cause persistent, and often progressive, airflow obstruction [68]. COPD is a major public health problem with extensive healthcare and economic costs [69]. Knowledge of factors that influence disease development is therefore valuable, and previous research suggest respiratory tract infections in early life to be significant risk factors for COPD [65 70 71].

2.5 Long-term outcomes after bronchiolitis

As this section is directly related to our objectives, only literature available before our study was commenced in 2015 is included.

There is a growing body of literature recognizing that children hospitalized for bronchiolitis have an increased risk of respiratory morbidity persisting towards young adulthood [72-74], but only four post-bronchiolitis follow-up-studies have continued from inclusion in early childhood until adulthood (Table 2). In addition, some birth cohort studies have followed their participants until adulthood, and assessed the associations between wheeze or LRTI in young age and subsequent respiratory morbidity. In the UK, 100 infants of atopic parents were recruited to a birth cohort during 1976-77, and 63 subjects were followed until 22 years of age [75 76]. The Tucson Children's Respiratory Study in the USA enrolled healthy infants at birth in a longitudinal non-selected birth cohort study of the early origins of respiratory disease during 1980-84. Physician-confirmed (but not necessarily hospitalized) LTRIs with RSV < 3 years of age were recorded, and airway function and prevalence of asthma were measured at 22 years of age [55 67].

Table 2. Overview of cohort studies of children hospitalized for bronchiolitis followed until adult age. This table displays studies that include subjects aged 17 years and older. Follow-up studies in which all subjects are 16 years or younger are left out. Figures in superscript in the column for number of subjects at follow-up represent the references of the associated published papers.
Abbreviations: RSV, respiratory syncytial virus; RV, rhinovirus.

Project initiator	City, Country, Year	Original number of subjects	Age at follow-up	Number of subjects at follow-up	Control group	Age	Virus
Matti Korrpi	Kuopio, Finland, 1981/82	83	18-21 26-29 28-31	54[77 78]/52[79] 59[80] 48[81 82]	1. Longitudinal age-matched control group from non-atopic families. 2. Population-based control group matched on sex and birth month.	< 24 months	RSV, non-RSV
Göran Wennergren	Göteborg, Sweden, 1984/85	101	17-21 25-28	89[83]/55[10] 82[84]	Population-based age-matched control group.	< 24 months	RSV, non-RSV
Nele Sigurs	Borås, Sweden, 1989/90	47	18	46[5]	Longitudinal control group matched on age and sex.	< 12 months	RSV only
Tiina M. Reijonen	Kuopio, Finland, 1992/93	100	15-18	67[11 85]	Population-based control group matched on age and sex.	< 24 months	RSV, RV, others

2.5.1 Asthma

All of the post-bronchiolitis cohort studies described in Table 2 report an increased prevalence of asthma in adult age of 20-64% depending on different definitions of asthma [5 11 77 80 81 83 84]. Persistent wheezing in early life (i.e. children who had wheeze during LRTI before age 3 and were still wheezing at age 6) was independently associated with asthma at 22 years of age in the Tucson birth cohort [55], whereas wheeze before the second birthday was not a risk factor for asthma at 22 years of age in the birth cohort from the UK [75]. The risk of asthma after bronchiolitis seems to be related to the virus involved, with the highest risk being observed after bronchiolitis caused by other viruses than RSV, particularly by RV [8 11 86].

2.5.2 Atopy

In most post-bronchiolitis cohort studies, the prevalence of atopy in subjects hospitalized for bronchiolitis do not differ from that of subjects in the respective control group [77 81 83], with one exception. Sigurs et al. found both allergic rhinoconjunctivitis and allergic sensitization towards airborne allergens to be more common in the post-bronchiolitis group compared to the control group [5]. The post-bronchiolitis group followed by Sigurs consists of RSV positive cases only. Others find allergy or atopy to be most prevalent after non-RSV-bronchiolitis, particularly RV-bronchiolitis [28].

2.5.3 Lung function and bronchial hyperresponsiveness

Impaired lung function and an obstructive lung function pattern have been reported up till young adult age after bronchiolitis [5 10 77]. In the 28-31-year follow-up of the Finnish cohort from 1981/82, Backman et al. found irreversible airway obstruction in 21% of the post-bronchiolitis group [82]. Irreversible obstruction was in this study defined as a post-bronchodilation FEV_1/FVC-ratio below 88% of the predicted value. Some studies have examined BHR in adult age after bronchiolitis by

either direct bronchial challenge tests by methacholine provocation tests [77], or indirect tests by isocapnic dry air hyperventilation challenges [10], but none of these reported significantly increased BHR compared to the control group. However, in our follow-up at 11 years of age, children in the post-bronchiolitis group had higher BHR than control subjects [8].

2.6 Mechanisms of respiratory morbidity after bronchiolitis

Taken together, the studies described above support the notion that children hospitalized for bronchiolitis have an increased risk of asthma and impaired lung function persisting into young adulthood. However, the underlying mechanisms of these associations are complex and not yet fully understood, and there is heterogeneity within the "bronchiolitis" diagnosis to take into consideration. Viral etiology seems to play an important role with different pathophysiological mechanisms associated with different viruses as described in section 2.1.4. Sex, atopic predisposition, exposure to smoking, and other premorbid and life-style factors may also be involved to varying degrees [21 22 24]. Lately, genetics and microbiome have both been subjects of increasing interest as described in more details in the following. And finally, as both bronchiolitis and asthma are heterogeneous conditions that show diversity linked to different types of inflammation, it is interesting to study possible interaction effects between these variables. The background for both eosinophil inflammation and inflammation linked to fat metabolism will round off this section.

2.6.1 Genetics

Both RSV, RV, and the other viruses that cause bronchiolitis are common environmental exposures, and in relation to the substantial number of children that are exposed to these viruses, only a small proportion require hospitalization for severe bronchiolitis. This suggests individual host factors such as genetics to be important, but the current knowledge of how genetic risk factors are linked to bronchiolitis is limited. Genome-wide-association studies have not found any genome-wide polymorphisms associated with bronchiolitis [87], but in infants with bronchiolitis, toll-

like receptor gene polymorphisms were found to be associated with subsequent asthma development [88]. Different candidate gene studies have also been conducted, often focusing on genes or polymorphisms that might be common for both bronchiolitis and asthma. Genetic variants at the 17q21 locus are linked to asthma in general, and have also been found to be associated with asthma in children after RV wheezing [89]. Variants of the cadherin-related family member 3 (CDHR3)-gene have also been associated with asthma [90]. CDHR3 is suggested to function as a receptor for RV subtype C, and has been shown to be associated with RV-infections, but not with RSV bronchiolitis [91 92]. A recent study from Norway analyzed different type 2 cytokine genes after viral bronchiolitis in early childhood, and found IL-33 to be positively associated with allergic asthma [93]. IL-33 is a cytokine that triggers expression of a number of Th2 cytokines and thereby increases eosinophilic inflammation [93].

2.6.2 Microbiome

The complex sum of the microbes and their activities inhabiting all parts of the human body is called the microbiome. The microbial colonization of mucosal tissues early in life plays an important role in the development of the immune system, and early life events may have long-term consequences [94]. Infections, use of antibiotics, and environmental factors including delivery mode, nutrition/breastmilk, having siblings, or attending daycare centers may all affect the composition of the microbiome early in life [95].

Airway and gut microbiome are related to the pathogenesis of respiratory tract infections, seem to play an immunomodulatory role during and after bronchiolitis [95], and are associated with childhood asthma [96-98]. However, the role of the microbiome in the association between bronchiolitis and subsequent respiratory morbidity is not fully understood. A recent study with data from a multicenter cohort of infants with bronchiolitis demonstrated complex interplays between the genetic host response, microbial composition and function, and their contribution to disease severity [99]. RSV and RV infection showed different microbiome composition and function, and also

different genetic host response profiles [100]. This further corroborates that the pathophysiology differs between RSV and RV infection.

2.6.3 Eosinophil inflammation

Eosinophil blood cells are central effectors of allergic inflammation, linked to atopy, and play a major role in the pathophysiology of Th2-high asthma as described in section 2.2.3. High levels of eosinophils in early life are considered to be a predictive marker for subsequent asthma. In the Asthma Predictive Index (API), which is based on data from the Tucson Children's Respiratory Study, eosinophilia ≥ 4% of the total white blood cells is included as one of the criteria [101]. This clinical index is based on eosinophils measured at follow-ups in healthy state approximately at the age of 11 months. Bronchiolitis, on the other hand, is an acute viral infection in which the expected response is a suppression of blood eosinophils [102]. However, a subset of infants with bronchiolitis have normal or even elevated levels [103]. Based on this, it is interesting to explore whether eosinophil blood cell counts at the time of acute bronchiolitis are associated with subsequent atopy and respiratory morbidity. This is often the only available result in everyday clinical life at the paediatric ward, and knowledge of how and if eosinophils during bronchiolitis may predict the future respiratory health of the child would be valuable.

Follow-up-studies during childhood have reported that the level of eosinophils during bronchiolitis was associated with subsequent asthma[104 105] and impaired lung function [105]. There is little published data on associations between the level of eosinophils during bronchiolitis and respiratory outcomes in young adult age, and hence it is not fully known if this association persists. Studies from one of the Finnish post-bronchiolitis cohorts found that the level of eosinophils during bronchiolitis predicted subsequent asthma at 6–7 years [106], but not at 11–13 years of age [107]. The other Finnish cohort initiated by Matti Korppi (Table 2) measured blood eosinophils both during the acute admission for bronchiolitis and during convalescence 4-6 weeks later, and found that elevated blood eosinophils during convalescence predicted increased asthma risk at 2-3 years, 3-4 years, and 8.5-10 years, but not at 13-16 years

or 18-20 years [108]. Eosinophil blood cell count during the hospitalization for bronchiolitis did in this study not predict asthma at any age.

Studies have shown that subjects infected by RSV have lower levels of blood eosinophils during the hospitalization for bronchiolitis compared to those testing negative for RSV [108 109].

2.6.4 Growth and fat metabolism

Body mass index (BMI)

Obesity is an increasing problem worldwide [110 111]. In 2015, a total of 107.7 million children and 603.7 million adults were obese, and elevated BMI accounted for 4 million deaths [110]. Elevated BMI and obesity have been found to be major risk factors for asthma, but also underweight populations have increased risk of asthma, giving the asthma–BMI curve a J-shape [112]. Asthma associated with obesity has previously been described as a predominantly non-atopic non-eosinophilic asthma with neutrophil airway inflammation [113]. The most consistently reported effect of obesity on lung function in adults is a decrease in the functional residual capacity and expiratory reserve volume due to increased intra-abdominal pressure on the diaphragm and fat mass on the chest wall [112]. Other lung function variables may also be affected, resulting in both restrictive and obstructive lung function patterns, but this has been less consistent in the literature [112 113]. A study estimating the causal effect of BMI on different outcomes using Mendelian randomization, found BMI to be related to a higher prevalence of asthma and decreased FVC and FEV_1, but not to atopy [114]. Consistent with this, also a study in young adults based on a Brazilian birth cohort found adiposity to be associated with lower lung function [115].

Few studies have focused attention on growth in adult post-bronchiolitis populations, but the two Finnish cohorts have explored the association between overweight and asthma. None of them found a significant association between overweight or obesity and asthma in young adult age [80 85]. However, as the authors point out, the number of cases with asthma is limited in both studies, and the results must therefore be interpreted with caution due to risk of false negative results.

The pathogenesis of respiratory morbidity related to elevated BMI is complex and not fully understood, but seems to include both mechanical factors caused by the excess weight and altered inflammation [112].

Adipokines

Adipose tissue is immunologically active and produces a large number of different proteins called adipokines. Among these, adiponectin, leptin, and resistin have been suggested to play a role in lung function impairment and inflammatory airway conditions such as asthma [116 117]. Ghrelin is not mainly produced in adipocytes, and is therefore strictly speaking not an adipokine. However, as Ghrelin is also an appetite-modulating hormone which has been suggested to be an inflammatory marker of asthma, it has been included among the regulatory peptides alongside with the other adipokines described [118-120]. An overview of the different adipokines' functional roles, synthesis, and levels in obesity is given in Table 3. Associations between each adipokine and asthma, atopy, and lung function found in general populations are described in the following paragraphs. No studies have assessed these associations in a post-bronchiolitis population.

Table 3. Overview of the different adipokines' location of synthesis, level in obesity, and main functions.

	Adiponectin	Leptin	Resistin	Ghrelin
Location of main synthesis	Adipose tissue	Adipose tissue	Adipose tissue	Stomach
Level in obesity	↓	↑	↑	↓
Main functions	Decreases insulin resistance and blood glucose levels.	Suppresses appetite and affects energy expenditure.	Actions in humans are not fully understood, but Resistin increases insulin resistance in rodents.	Stimulates appetite, enhances use of carbohydrates, and reduces fat utilization.

Adiponectin

Adiponectin is a predominantly anti-inflammatory adipokine [116]. Some studies find adiponectin to be negatively associated with asthma, but this is not consistent [116 121-123]. A study in children found lower levels of adiponectin in subjects with allergic rhinitis compared to healthy control subjects [124], but another study reported no correlation between adiponectin and markers of atopy [125]. Serum adiponectin was in a

large follow-up study positively associated with lung function in young adults, independent of obesity [126]. However, previous research on the association between adiponectin and lung function is inconsistent [127].

Leptin

Leptin acts pro-inflammatory, and has previously been reported to be positively associated with asthma in certain populations, such as prepubertal boys, peripubertal or postpubertal girls, and premenopausal women [116]. However, in a more recent systematic review and meta-analysis, leptin was positively associated with asthma regardless of age [121]. Some studies have found leptin to be positively associated with atopy or allergy [124 128], but these findings are also inconsistent [125]. Leptin seems to be negatively associated with lung function both in children and adults [129 130].

Resistin

Resistin is a pro-inflammatory acting adipokine originally named for its ability to resist insulin action [117 119]. Resistin has been suggested to have potential to impact on a wide range of diseases, including asthma [117 131]. However, little data exists addressing a possible relationship between resistin and asthma, and the results are contradicting both in children [125 132], and adults [133 134]. Few studies have explored the association between resistin and atopy, but a study in children found that resistin was negatively associated with eosinophil blood cell count and serum IgE [125]. The same study did not find any associations between resistin and different lung function variables.

Ghrelin

Ghrelin has an anti-inflammatory effect, and has been found in lower levels in subjects with asthma compared to control subjects, suggesting an anti-inflammatory role of ghrelin in the pathogenesis of asthma [118 120]. A study in female adults also reported lower ghrelin concentrations in subjects with asthma compared with controls . Very little is currently known about the association between ghrelin and atopy, but a letter to editor showing a strong inverse correlation between ghrelin and serum IgE

suggests that ghrelin may act protectively [135]. The association between ghrelin and lung function is not known.

2.7 Summary of introduction

Bronchiolitis is a viral lower respiratory tract infection commonly seen in children less than 1 year of age, and the most common cause of admission to hospital during infancy. Hence, a substantial group of people in all ages will bear this as a part of their medical history. Children hospitalized for bronchiolitis have an increased risk of subsequent asthma and impaired lung function, but the association with atopy is less consistent. Bronchiolitis is heterogeneous, and the associations to subsequent outcomes seems to be related to the virus involved, with a higher risk of asthma in childhood after bronchiolitis caused by rhinovirus compared to respiratory syncytial virus. There is less knowledge about the long-term prognosis after bronchiolitis, but results from other studies indicate that this risk persists towards adulthood. BHR seems to be a predictor of subsequent asthma. Lung function trajectories throughout a life course vary between individuals and abnormal lung function trajectories may originate in early life. It has therefore been hypothesized that COPD may begin in childhood, precipitated by early respiratory insults such as bronchiolitis among other factors. However, the development of respiratory patterns for lung function and BHR during puberty in a post-bronchiolitis population is poorly characterized.

The underlying mechanisms of the associations between bronchiolitis and subsequent respiratory morbidity are not fully understood, but factors related to viral etiology, sex, inflammation, and different premorbid and life-style factors are likely to be involved. Eosinophil inflammation is essential in the pathophysiology of asthma and allergy, but the predictive role of eosinophil blood cells on respiratory morbidity after bronchiolitis is less known. Asthma after bronchiolitis seems heterogeneous, and may also be related to other factors than atopy or eosinophilic inflammation. BMI is associated with respiratory morbidity, and inflammatory markers of fat metabolism including different adipokines are factors potentially involved in these associations. As previous bronchiolitis, elevated BMI, and adipokines all have been associated with

asthma and low lung function, it is relevant to study possible interaction effects between these variables.

These topics have been subjects for my research aiming to increase the knowledge of long-term outcomes after bronchiolitis and some possible pathophysiological factors associated with these outcomes.

3. AIMS OF THE STUDY

Our main aim was to provide new knowledge on long-term outcomes after bronchiolitis. Specifically, we aimed to study associations between bronchiolitis in infancy and subsequent asthma, atopy, and lung function in young adult age, particularly addressing potential modifying factors including sex and viral etiology. Further, we aimed to study trajectories of lung function after bronchiolitis, and potential underlying mechanisms of the long-term outcomes including eosinophil inflammation, growth, and fat metabolism.

3.1 Paper I – Asthma, atopy, and lung function after bronchiolitis

The objectives for paper 1 were to study:

a. The prevalence of asthma and atopy at 17-20 years of age in subjects hospitalized for bronchiolitis in infancy compared to control subjects.
b. Lung function variables at 17-20 years of age in subjects hospitalized for bronchiolitis in infancy compared to control subjects.
c. If the prevalence of asthma and atopy, and lung function variables at age 17-20 years were different after RSV-bronchiolitis compared to non-RSV bronchiolitis.
d. If associations between bronchiolitis in infancy and the outcomes asthma, atopy, and lung function variables at age 17-20 years differed between males and females.

The corresponding null-hypotheses for paper 1 were:

$H_{0\,a}$: The prevalence of asthma and atopy at age 17-20 years do not differ between subjects hospitalized for bronchiolitis in infancy and control subjects.

$H_{0\,b}$: Lung function variables at age 17-20 years do not differ between subjects hospitalized for bronchiolitis in infancy and control subjects.

$H_{0\,c}$: The prevalence of asthma and atopy, and lung function variables at age 17-20 years are not different after RSV-bronchiolitis compared to non-RSV bronchiolitis.

$H_{0\,d}$: Associations between bronchiolitis in infancy and the outcomes asthma, atopy, and lung function at age 17-20 years do not differ between males and females.

3.2 Paper II – Lung function trajectories after bronchiolitis

The objectives for paper 2 were to study:

a. If BHR and z-scores for lung function changed from 11 to 17-20 years of age in subjects hospitalized for bronchiolitis in infancy.
b. If trajectories of lung function and BHR from 11 to 17-20 years of age after bronchiolitis in infancy differed from those of control subjects.

The corresponding null-hypotheses for paper 2 were:

$H_{0\,a}$: BHR and z-scores for lung function do not change from 11 to 17-20 years of age in subjects hospitalized for bronchiolitis in infancy.

$H_{0\,b}$: Trajectories of lung function and BHR from 11 to 17-20 years of age after bronchiolitis in infancy do not differ from those of control subjects.

3.3 Paper III – Eosinophils during bronchiolitis and outcomes

The objectives for paper 3 were to study:

a. If the level of blood eosinophils during bronchiolitis in infancy was associated with asthma, atopy, and lung function at 17-20 years of age.
b. If associations between the level of blood eosinophils during bronchiolitis in infancy and asthma, atopy, and lung function at 17-20 years of age were different after RSV bronchiolitis compared to non-RSV bronchiolitis.

The corresponding null-hypotheses for paper 3 were:

$H_{0\,a}$: The level of blood eosinophils during bronchiolitis in infancy is not associated with asthma, atopy, and lung function at 17-20 years of age.

$H_{0\,b}$: Associations between the level of blood eosinophils during bronchiolitis in infancy and asthma, atopy, and lung function at 17-20 years of age are not different after RSV bronchiolitis compared to non-RSV bronchiolitis.

3.4 Paper IV - BMI and adipokines in young adults after bronchiolitis

The objectives for paper 4 were to study:

a. If associations between BMI and the outcomes asthma, atopy, and lung function at 17-20 years of age differed between young adults previously hospitalized for bronchiolitis and control subjects.
b. If associations between adipokines and the outcomes asthma, atopy, and lung function at 17-20 years of age differed between young adults previously hospitalized for bronchiolitis and control subjects.

The corresponding null-hypotheses for paper 4 were:

$H_{0\,a}$: At 17-20 years of age, associations between BMI and the outcomes asthma, atopy, and lung function do not differ between young adults previously hospitalized for bronchiolitis and control subjects.

$H_{0\,b}$: At 17-20 years of age, associations between adipokines and the outcomes asthma, atopy, and lung function do not differ between young adults previously hospitalized for bronchiolitis and control subjects.

4. MATERIALS AND METHODS

4.1 Study design

This is an observational study enrolling young adults hospitalized for bronchiolitis in infancy. A subgroup of the subjects had participated in a follow-up at 11 years of age [8], and the rest were recruited to the follow-up at 17-20 years of age. The study included two different control groups as described below. A brief overview of the study design is given in Figure 6.

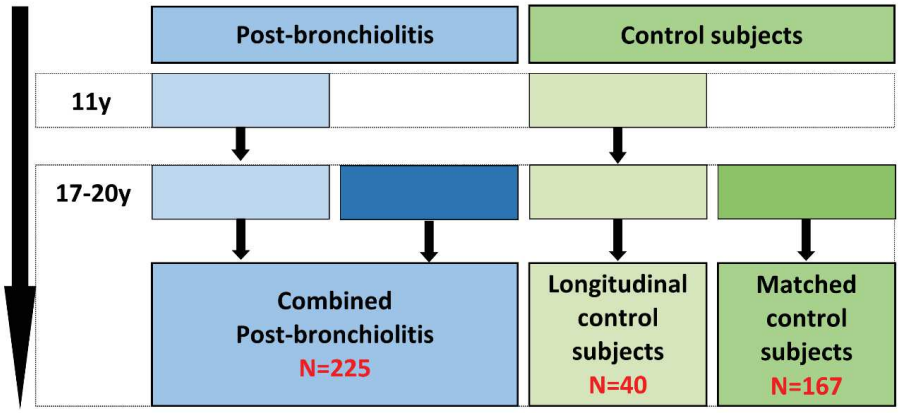

Figure 6. Overview of the study population.
Abbreviations: 11y, First follow-up at age 11 years; 17-20y, Second follow-up at age 17-20 years.

Designating the sub-studies to specific study designs is challenging, and not always straight forward. Paper 1 and 3 are designed as historical cohort studies with exposures in infancy and outcomes measured in young adult age, and with a control group included in Paper 1. Paper 2 is a longitudinal cohort study with outcomes measured both at 11 and 17-20 years of age. In paper 4, both the exposures and the outcomes are measured at the 17-20 year follow-up consistent with a cross-sectional design. However, taking into account that our main aim was to study differences between subjects who have suffered an insult of being hospitalized for bronchiolitis in infancy and control subjects, this study can also be considered as a historical cohort study. Clinical examinations for the 17-20-year follow-up were performed from April 2015 through March 2020 at the University Hospitals in Stavanger and Bergen.

4.2 Subjects

4.2.1 Post-bronchiolitis group

Originally, 131 infants hospitalized for bronchiolitis at the University Hospitals in Stavanger and Bergen, Norway, during the winter seasons 1997 and 1998 were included in a follow-up study [8]. Of these, 121 subjects participated in a longitudinal follow-up at 11 years of age [8]. All 131 were regarded as eligible for invitation to the 17-20 year follow-up. To increase the study population, additional subjects hospitalized for bronchiolitis at the same hospitals between October 1996 and May 2001 were recruited. Eligible subjects were found by searching the hospitals' medical records for hospital stays with a primary International Classification of Diseases (ICD) 10th Revision diagnose of J21 Acute bronchiolitis, and thereafter assessing if the criterion for inclusion was met and that no exclusion criteria were present. Inclusion criterion was hospitalization for bronchiolitis during the first year of life, and exclusion criteria were use of inhaled or systemic corticosteroids prior to the hospitalization, previous hospitalization for bronchiolitis, severe neonatal or other pre-existing chronic lung disease, and prematurity < 32 weeks of gestation. Only subjects with a registered address in or near the hospitals' catchment areas were invited, until the sample size determined by power calculations was reached. Hence, not all eligible subjects were invited.

4.2.2 Control groups

Longitudinal control group

The longitudinal control group was included to be able to study the trajectories of lung function and BHR from 11 years to young adulthood, and consisted of subjects from the control group at the follow up at 11 years with acceptable results from spirometry. These subjects were age-matched by recruiting children from three schools in Stavanger born in the same year (1997) as the children in the post-bronchiolitis group [8]. Exclusion criterion was hospitalization for bronchiolitis during the first year of life. Eighty-nine subjects were eligible for invitation to participate at 17-20 years of age.

Matched control group

The matched control group was recruited to the 17-20-year follow-up. Exclusion criterion was hospitalization for bronchiolitis during the first year of life, and the matching variables were date of birth, sex, and gestational age at birth. This group was established by searching the hospital's birth protocols. The next-born eligible person (i.e. with the same sex and gestational age at birth) to each individual index post-bronchiolitis participant was invited. If the first invited subject declined, the next was invited, and so on until one control was recruited for each index or a maximum of ten invitations were sent.

4.2.3 Paper-specific study population

Paper 1 and 4

To study differences between young adults hospitalized for bronchiolitis and control subjects, we included the combined post-bronchiolitis group consisting of both subjects from the 11-year follow-up and subjects recruited directly to the 17-20-year follow-up. Only the matched control group was included (Figure 7). All subjects were included in paper 1 (N=225 in the post-bronchiolitis group and N=167 in the control group). Whereas for paper 4, only subjects with results from analyses of serum adipokines were included (N=185 in the post-bronchiolitis group and N=146 in the control group).

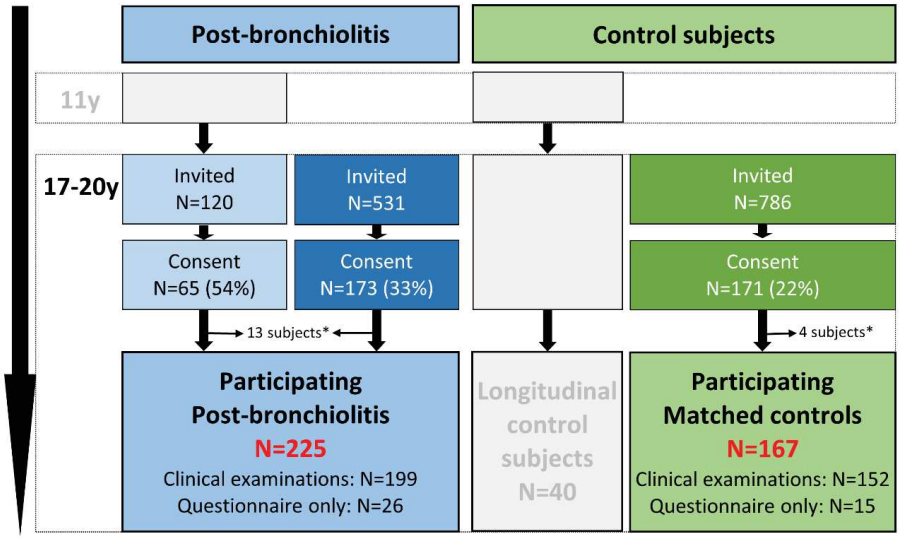

Figure 7. Overview of the study population for paper 1 and 4. *In the post-bronchiolitis group, 2 subjects did not return the questionnaire and 11 subjects did not show up for the clinical examinations. In the matched control group, 4 subjects did not show up for the clinical examinations. Abbreviations: 11y, First follow-up at age 11 years; 17-20y, Second follow-up at age 17-20 years.

Paper 2

To study the trajectories of lung function and BHR, we included only subjects with acceptable results from spirometry at the 11-year follow-up in the post-bronchiolitis group and the longitudinal control group (Figure 8). 108 subjects in the post-bronchiolitis group and 89 control subjects had acceptable results for lung function measured at 11 years of age [8], and were thereby eligible for inclusion in this sub-study.

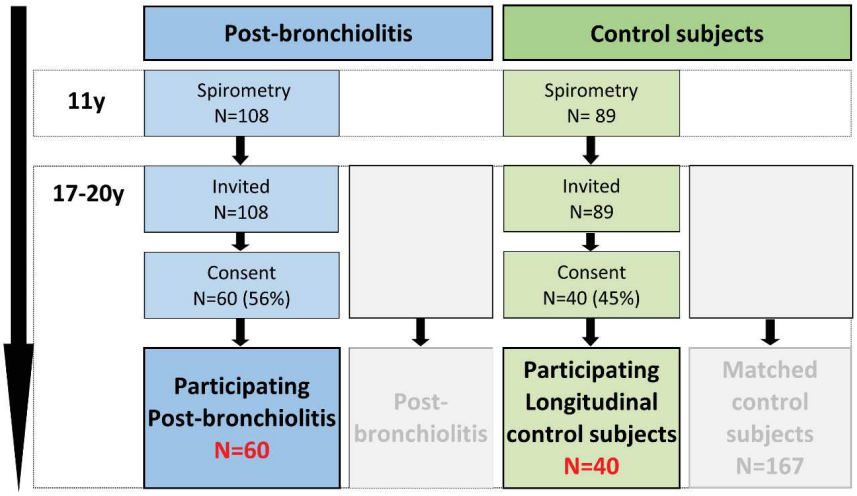

Figure 8. Overview of the study population for paper 2.
Abbreviations: 11y, First follow-up at age 11 years; 17-20y, Second follow-up at age 17-20 years.

Paper 3

In paper 3, we wanted to study associations between the level of eosinophil blood cells during the hospitalization for bronchiolitis and outcomes in young adult age. Hence, we included the combined post-bronchiolitis group consisting of both subjects from the 11-year follow-up and subjects recruited to the 17-20 year follow-up, but none of the control subjects (Figure 9).

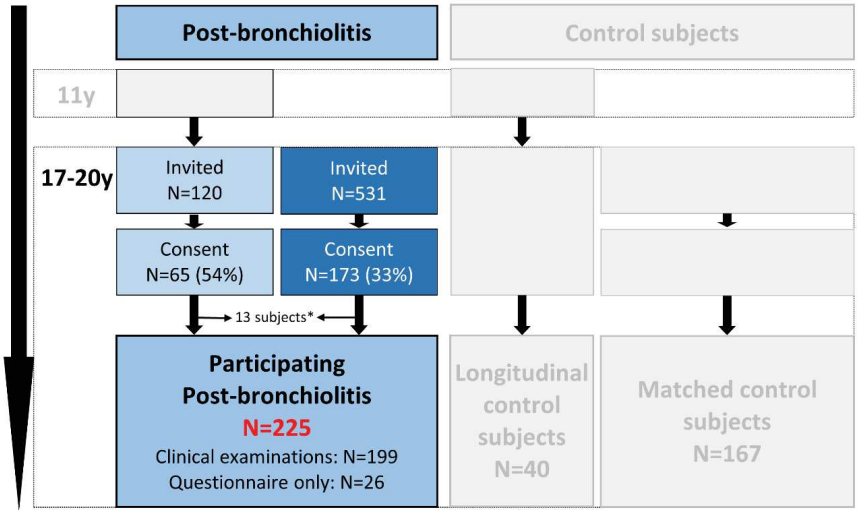

Figure 9. Overview of the study population for paper 3. *2 subjects did not return the questionnaire and 11 subjects did not show up for the clinical examinations.
Abbreviations: 11y, First follow-up at age 11 years; 17-20y, Second follow-up at age 17-20 years.

4.3 Exposures

4.3.1 Bronchiolitis *(Paper 1 and 2)*

Bronchiolitis was defined based on European guidelines as an acute viral respiratory tract infection during the first year of life with fever, tachypnea, dyspnea, prolonged expiration and wheeze on auscultation [15]. During the hospitalization for bronchiolitis, nasopharyngeal mucus was examined for RSV by direct immunofluorescence (*BioMèrieux, Marcy-l'Ètoile, France*). Other viruses were not systematically tested for. Infants testing positive for RSV were defined as having RSV bronchiolitis and infants testing negative as having non-RSV bronchiolitis. All subjects from the original cohort from 1997/98 had results for RSV, but as testing for RSV was not routinely performed in all infants hospitalized for bronchiolitis at the time, viral etiology is missing for some subjects recruited to the expanded cohort in adult age.

4.3.2 Blood eosinophils *(Paper 3)*

The level of eosinophils was analyzed in peripheral blood samples drawn from the infants as a part of the routine tests during the hospitalization for bronchiolitis using Technicon H*2/H*3 or Coulter STKS as previously described [136]. These data were collected in 2020 by consulting medical records from the relevant hospital stays.

4.3.3 Growth and fat metabolism *(Paper 4)*

At the 17-20-year follow-up, height and weight were measured by study nurses or collected from questionnaires for those not participating in the clinical examinations. BMI was calculated as weight divided by the square root of height (kg/m^2).

We analyzed serum levels of four different adipokines; adiponectin (μg/ml), leptin (ng/ml), resistin (ng/ml), and ghrelin (pg/ml). Blood samples were drawn from the subjects by the study nurses at the 17-20-year follow-up, centrifuged and aliquoted, and sera were stored at -80°C. Serum adipokines were measured by electrochemi-

luminiscence on Meso® QuickPlex SQ 120 Imager (*Meso Scale Diagnostics, Rockville, MD, USA)* using the following assay-kits: R-PLEX® Human Adiponectin, R-PLEX® Human Resistin, and U-PLEX® Human Ghrelin and Leptin *(Meso Scale Diagnostics, Rockville, MD, USA).* The samples were diluted using provided sample diluent to 1:8000 for adiponectin, 1:50 for resistin, and 1:4 or 1:10 for ghrelin and leptin. Analyses were carried out in accordance with the procedures from the manufacturer from August 2021 through February 2022. Two independent in-house control samples were analyzed to assess the different assay's coefficient of variation (CV). For adiponectin, the intra- and inter-assay CV were <9.2% and <10.1%, for leptin <5.4% and < 12.7%, for resistin <13.1% and <11.6%, and for ghrelin <8.5% and <15.3%, respectively.

4.4 Outcomes at follow-up

4.4.1 Asthma *(Paper 1 and 3-4)*

Asthma symptoms were recorded by questionnaires based on those used in the ISAAC study [137] (Appendix 1). Asthma ever was defined as a positive answer to *have you ever been diagnosed with asthma by a doctor?* Current asthma was defined as asthma ever and a positive answer to at least one of the two questions: *(1) Have you during the last 12 months had heavy breathing or wheezing/chest-tightness, or (2) Have you during the last 12 months used any asthma medications* (ICS, long-acting or short-acting beta-2-agonists, montelukast, ipratropium bromide, or any combination).

4.4.2 Atopy *(Paper 1 and 3-4)*

Atopy was defined as either a positive SPT defined as a wheal diameter ≥ 3mm larger than the negative control (Soluprick® allergens (*ALK Albello, Hørsholm, Denmark*)) [58], and/or a positive allergen panel or specific IgE > 0.35 kU/L for one of the following allergens: *Dermatophagoides pteronyssinus*, dog, cat, *Cladosporium herbarium*, birch, timothy, egg white, milk, peanut, hazelnut, and codfish. Sera were analyzed for the allergen panels Phadiatop® and fx5E®, and ImmunoCAP hazelnut®

(Thermo Fisher Scientific, Phadia AB, Uppsala, Sweden). Specific IgE was analyzed if positive panels. Three subjects in the post-bronchiolitis group and one control subject had positive allergen panels, but no tested specific IgE >0.35kU/L. These were defined as atopic subjects.

In Paper 3, the subjects were divided into four phenotypes based on the occurrence of atopy and current asthma at the 17-20-year follow-up: 1) Healthy: No asthma, no atopy. 2) Atopic non-asthmatic: Atopy, no asthma. 3) Non-atopic asthma: Asthma, no atopy. 4) Atopic asthma: Asthma and atopy.

4.4.3 Lung function *(Paper 1-4)*

Lung function was measured by spirometry according to established guidelines [138], using V_{max} Encore 229D spirometer (*Sensor Medics Inc., Anaheim, USA*). Variables recorded were FVC, FEV_1, FEV_1/FVC-ratio, and FEF_{25-75}. All variables were standardized for age, height, and sex based on the reference values provided by the GLI [62], and presented as z-scores (Paper 1-4) and percentages of predicted (Paper 1-2).

4.4.4 Bronchial hyperresponsiveness *(Paper 2)*

BHR was measured by methacholine provocation tests using an inhalation-synchronized dosimetry nebulizer in subjects who did not have any contraindications to the test [139 140]. The test continued until a fall in FEV_1 exceeding 20% compared with baseline FEV_1, or until a maximal cumulative dose of 11.5 µmol methacholine had been administered. A dose–response slope (DRS) was calculated as the ratio of percentage decline in FEV_1 from baseline to methacholine dose (%/µmol) in accordance with the method suggested by O'Connor et al. [141]. Percentage decline in FEV_1 was defined as the decline in FEV_1 from the post-saline value after the final methacholine dose was administered, and dose was defined as the final cumulative methacholine dose administered.

4.5 Covariates and confounders

Data from the hospital stay for bronchiolitis were obtained by review of medical records. Clinical characteristics from the follow-up at 11 years were obtained from a stored database containing de-identified information. Birthweight and gestational age at birth were collected from birth protocols. Data regarding atopic dermatitis, smoking, and personal and family history of atopy were collected from questionnaires at follow-up at 17-20 years (Appendix 1). Missing values regarding smoking were interpreted as negative answers.

4.5.1 Special considerations Paper 3

In this sub-study, all included subjects have been hospitalized for bronchiolitis as there is no control group involved. Hence, information from medical records from the hospital stay is available for all subjects, and can be utilized. For this paper, data regarding atopic dermatitis, smoking, and family history of asthma and atopy were obtained from review of medical records at the hospitalization for bronchiolitis, and supplemented by information from the questionnaires at follow-up at 17-20 years of age. Early life exposure for household smoking was defined as positive if described in the medical record, or if positive answer to *do/did anyone smoke in your home* combined with the given number of years with smoking in the household being equal to the subjects' age. Family history of atopy was defined as positive if reported in the medical record, or if positive answer to *do you know if your mother, father or siblings have or have had atopic dermatitis, asthma or positive allergy tests.* Atopic dermatitis ever was defined as positive if described in the medical record, or if positive answer to *have you ever had atopic dermatitis*. Subjects with no information of prematurity in neither birth protocols nor medical records were defined as having an age of gestation at birth > 36 weeks.

4.6 Statistical analyses

4.6.1 General aspects

SPSS V.24.0 and V.26.0 (*IBM Corp. Armonk, N.Y., USA*) and Stata V.15.1, V.16.1, and V.17.0 *(StataCorp LLC, Texas, USA)* were used for the analyses. P-values <0.05 were considered statistically significant.

4.6.2 Descriptive statistics

Continuous data were presented as means with standard deviations (SD) and compared by Student's t-tests if normally distributed, or as medians with interquartile ranges and compared by Mann–Whitney U tests if not normally distributed. Categorical data were presented as counts and percentages and compared by Pearson chi-square tests.

4.6.3 Power

Power analyses were performed prior to study start using SPSS Sample Power 3 (*IBM Corp. Armonk, N.Y., USA*) with power set to 80% and a significance level (α) at 0.05 for the main outcomes asthma, atopy, and lung function. To detect an absolute difference of 10 percentage points in the occurrence of asthma or atopy in the post-bronchiolitis group compared to the control group, 199 subjects were needed in each group. We assumed this to be clinically relevant and reasonable considering the results from other studies [5 54]. To detect a clinically relevant absolute difference of 5% in lung function (calculations performed for FEV_1), 64 subjects needed to be included in each group [82]. For regression analyses, power was mostly assessed based on acknowledged rules of thumb. To detect medium-size relationships in linear regression with $\alpha=0.05$ and power=80%, one would need $N>104 + m$ observations, where m is the number of predictors [142]. To detect a small effect size (f^2 at 0.02 as suggested by Cohen [143]), we calculated that one would need a total sample size of 395 cases assuming one variable of interest, four adjusting variables, $\alpha=0.05$, and power=80%. As for logistic

regressions, a widely used rule of thumb is that 10 cases per variable are needed to be able to discover the relationship between explanatory and dependent variables, even though some argue that this might be too conservative [144].

4.6.4 Paper specific statistics

Paper 1

Regression analyses were performed to study differences between the groups (i.e. post-bronchiolitis vs. control). Categorical outcomes were analyzed by mixed effects logistic regressions and presented as odds ratios (OR) with 95% confidence intervals (CI) and predicted proportions with 95% CI. Continuous outcomes were analyzed by mixed effects linear regressions and presented as regression coefficients (β) with 95% CI and predicted means with 95% CI. P-values from Wald tests were given for OR and β. Potential correlations between matched individuals were allowed for by including a random intercept term in the models. All effect estimates were adjusted for age at follow-up and potential confounders (Figure 10). Differences between sexes were assessed by including an interaction term between sex and group in the models, whereas the RSV group and non-RSV group were directly compared to each other.

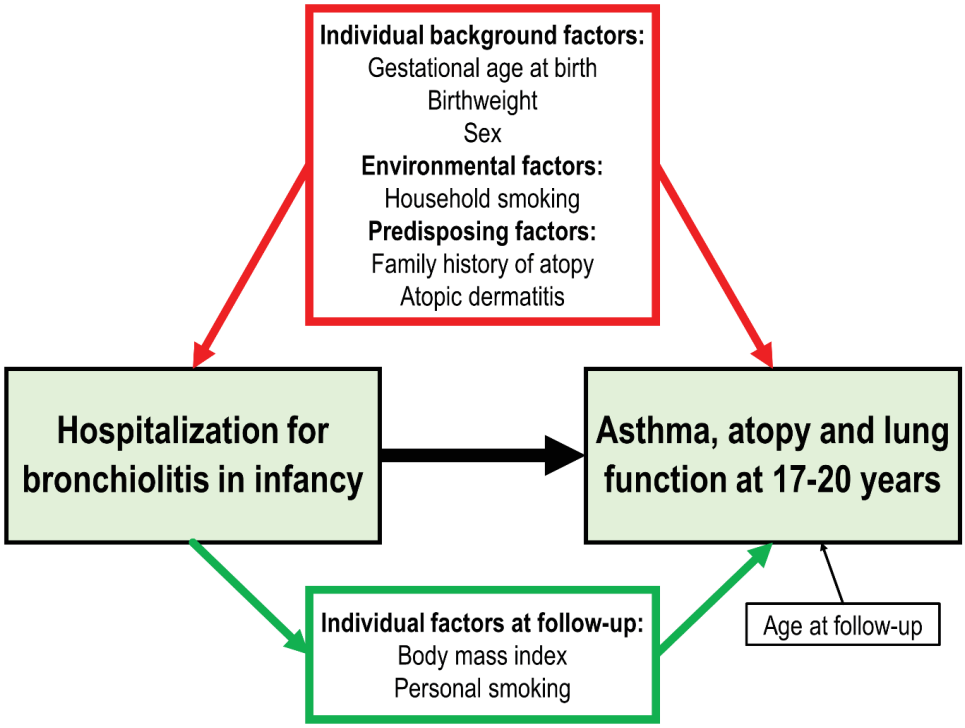

Figure 10. Simplified directed acyclic graph with confounders in the red box and mediators in the green box.

Paper 2

Changes in lung function and DRS to methacholine between the 11-year follow-up and the 17-20-year follow-up in the post-bronchiolitis group and the longitudinal control group were assessed by generalized estimating equations adjusted for atopic sensitization and asthma at 11 years of age, and family history of asthma or atopy. Results were presented as mean changes with 95% CI. Interaction terms between group and time (i.e. 11-year follow-up vs. 17-20 year follow-up) were applied to test for dissimilar trajectories of lung function and DRS between the post-bronchiolitis group and the control group. The distribution of DRS was highly skewed, and therefore transformed using the natural logarithm after negative values were set to zero and 0.1 was added to all values. A Cox regression analysis allowing for correlation between repeated tests of the same individuals was used to analyze the proportion of non-responders at each cumulative dose of methacholine. The

association between BHR at 11-years and current asthma at 17-20-years was analyzed by a multivariable logistic regression analysis adjusted for the following covariates measured at 11 years: group, sex, FEV_1 z-score, and current asthma.

Paper 3

Linear, logistic, and multinomial logistic regression analyses were performed to study associations between the level of eosinophils and different outcomes in young adult age in subjects hospitalized for bronchiolitis. The distribution of the levels of eosinophils was highly skewed and therefore transformed using the natural logarithm after adding 0.1 to all values. Unadjusted and adjusted OR, relative risk ratios (RRR), or β with 95% CI were calculated. We adjusted for the following pre-specified variables: age at follow-up, sex, family history of atopy, RSV-status, and age at hospitalization for bronchiolitis. The potential confounders: atopic dermatitis, gestational age at birth, birthweight, and household smoking, were handled by sensitivity analyses. The impact of viral etiology was assessed by including an interaction term between the level of eosinophils and RSV-status.

Multiple imputation by iterative chained equations resulting in 100 completed datasets was performed to handle missing data on virus (RSV vs. non-RSV), weight, and the level of eosinophils during hospitalization for bronchiolitis, birthweight, atopic dermatitis, household smoking, BMI, asthma, atopy, and lung function. Included auxiliary variables were sex, age at hospitalization for bronchiolitis, gestational age at birth < 36 weeks, family history of atopy, age at follow-up, and personal smoking. When analyzing phenotypes of combinations of asthma and atopy, an otherwise equal separate multiple imputation was performed including a four-category phenotype variable instead of separate variables for asthma and atopy.

Paper 4

Associations between BMI and outcomes were analyzed by mixed effects logistic and linear regression analyses with p-values from Wald tests. BMI had a non-linear relationship to outcomes, and was therefore included in the analyses as a 3-knot restricted cubic spline to allow for flexibility. The patterns of the associations were

described by predicted proportions and means of the outcomes with 95% CI for given BMI levels, and with all covariates at their respective mean values. Differences in the mentioned associations between the post-bronchiolitis group and the control group were assessed by including an interaction term between BMI and group, and illustrated in plots of predicted proportions and means.

Associations between the different adipokines and outcomes were analyzed by mixed effects logistic and linear regression analyses and presented as OR or β with 95% CI, and corresponding p-values from Wald tests. The distribution of all four adipokines were skewed, and therefore transformed using the natural logarithm before entering the regression analyses. Differences in the associations between the post-bronchiolitis group and the control group were assessed by including an interaction term between the different adipokines and group. The interactions are presented as OR or β with 95% CI. In a scenario with no difference in associations between the post-bronchiolitis group and the control group, OR would be equal to 1, and β would be equal to 0. BMI was handled as a confounder, and adjusted for in the main analyses. As BMI also can be considered as a mediator, analyses without adjusting for BMI were in addition presented in the supplemental Table A.1 of Paper 4.

Potential correlations between matched individuals were allowed for by including a random intercept term in the models. All effect estimates were adjusted for potential confounders. Differences between groups were considered significant if the p-values of the interaction terms were <0.05, and analyses stratified by group were subsequently performed.

4.7 Ethical considerations

The study was approved by the Norwegian Regional Committee on Medical Research Ethics (2014/1930/REK west). This approval included permission to use previously recorded data from the 11-year follow-up. Signed statements of informed consent were obtained from all subjects and also from parents if the subjects were younger than 18 years of age. Peripheral blood sampling, and the testing of atopy by SPT, lung function by spirometry, and BHR by methacholine provocation tests, are all

in daily use in outpatient clinics for allergology and pulmonary diseases throughout Norway, and are not considered representing any risk for the subjects.

5. SUMMARY OF RESULTS

In this section, the main results from the four papers of which this thesis is built on are presented.

5.1 Paper 1

5.1.1 Asthma and atopy

We found a higher prevalence of both asthma ever and current asthma in the post-bronchiolitis group compared to the control group, but the prevalence of atopy did not differ between the two groups (Figure 11). Among the subjects with asthma ever, a lower proportion in the post-bronchiolitis group than in the control group had atopy (46% vs. 70%, p=0.027). A similar tendency was found for current asthma (50% vs. 74%, p=0.076).

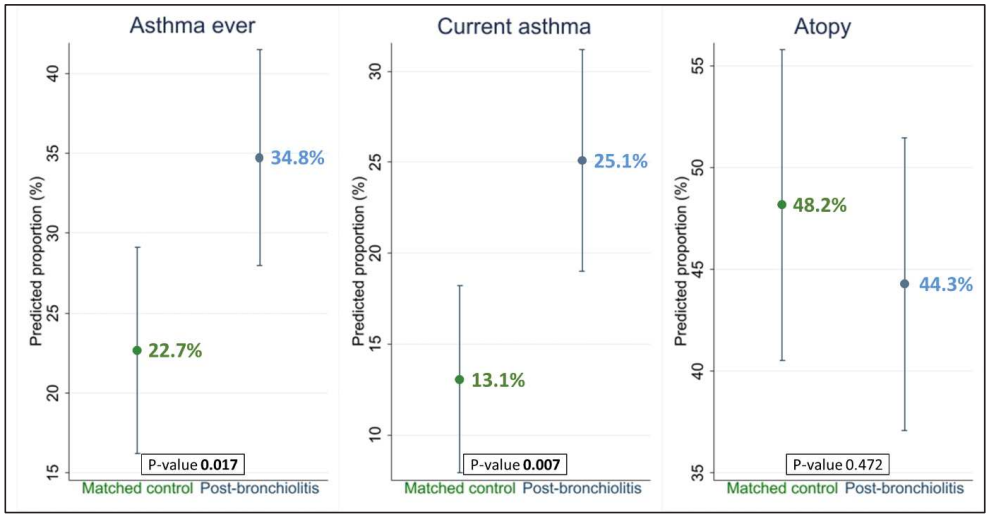

Figure 11. Asthma and atopy at the 17-20 year follow-up in the post-bronchiolitis group and the control group. Figures are predicted proportions with 95% confidence intervals from mixed effects logistic regression analyses adjusted for sex, age, birth weight, gestational age at birth, household smoking ever, atopic dermatitis, and family history of atopy. P-values for odds ratio from Wald tests.

The asthma prevalence was high after both RSV-bronchiolitis and non-RSV bronchiolitis with no differences between the virus groups, neither for asthma ever; 32.8 (24.0 to 41.7) vs. 36.0 (23.5 to 48.5), p=0.685, nor for current asthma; 24.0 (16.1

to 32.0) vs. 23.8 (12.8 to 34.7), p=0.971. The RSV group had lower prevalence of atopy compared to the non-RSV group; 31.4 (22.2 to 40.7) vs. 53.8 (40.6 to 67.0), p=0.007. Figures are adjusted predicted proportions in percentages (95% CI), p-values. We did not find any significant interactions between sex and group for asthma or atopy, meaning that the impact of having bronchiolitis on subsequent asthma or atopy did not differ between sexes.

5.1.2 Lung function

Subjects in the post-bronchiolitis group had a more obstructive lung function pattern with lower mean z-scores for FEV_1, FEV_1/FVC-ratio and FEF_{25-75} compared to control subjects (Figure 12).

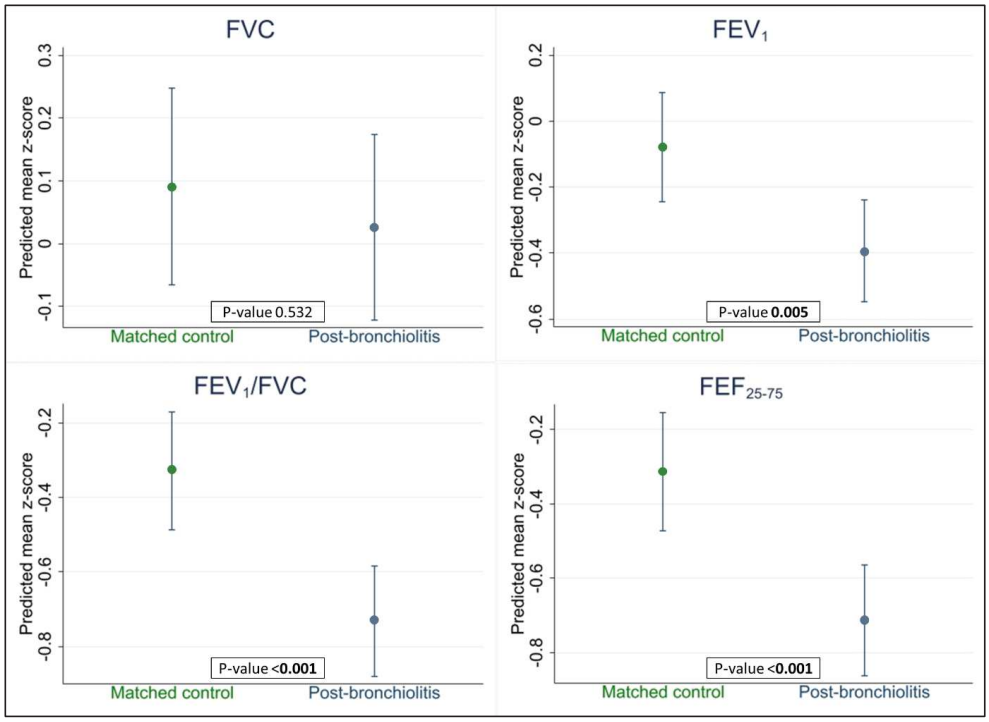

Figure 12. Lung function at the 17-20 year follow-up in the post-bronchiolitis group and the control group. Figures are predicted mean z-scores with 95% confidence intervals from mixed effects linear regression analyses adjusted for birth weight, gestational age at birth, household smoking ever, atopic dermatitis, and family history of atopy. P-values for regression coefficient from Wald tests. Abbreviations: FVC, forced vital capacity; FEV_1, forced expiratory volume in first second; FEF_{25-75}, forced expiratory flow between 25% and 75% of the forced vital capacity.

The non-RSV group had lower mean FEV_1/FVC-ratio compared to the RSV group; -0.97 (-1.24 to -0.70) vs. -0.59 (-0.79 to -0.39), p=0.026. Figures are adjusted predicted mean z-scores (95% CI), p-value. The other lung function variables did not differ between the virus groups. We found a significant interaction between sex and group for FVC (β -0.42; 95% CI -0.82 to -0.02; p=0.039), but not for the other lung function variables. This means that bronchiolitis is differently associated with FVC in males and females, and analyses for FVC stratified by sex showed lower mean FVC in the post-bronchiolitis group compared with control subjects in males (β -0.32; 95% CI -0.58 to -0.06; p=0.017), but not in females (β 0.15; 95% CI -0.14 to 0.44; p=0.313).

5.2 Paper 2

Lung function and BHR were stable from 11 to 17-20 years of age. In both the post-bronchiolitis group and the control group, z-scores for FVC, FEV_1, FEV_1/FVC-ratio, and DRS did not differ between 11 and 17-20 years of age (Figure 13, Table 4). The lung function variables FEV_1, FEV_1/FVC, and FEF_{25-75} were lower in the post-bronchiolitis group compared to the control group (Figure 13), but we found no significant interaction between time and group for any of the outcomes (Table 4). This means that the trajectories of lung function and BHR from 11 to 17-20 years of age did not differ between the post-bronchiolitis group and the control group. Neither did we find any significant interactions between age, group, and sex, meaning that there were no differences between sexes in how bronchiolitis in infancy affected the trajectories through adolescence and puberty. Hence, the trajectories of lung function and BHR from 11 to 17-20 years of age in subjects hospitalized for bronchiolitis were lower, but parallel to those of the control subjects in both males and females. In a multivariable logistic regression analysis, BHR at age 11 was independently associated with current asthma at the follow-up at 17-20 years of age (OR 1.88; 95% CI 1.22 to 2.89; p=0.004).

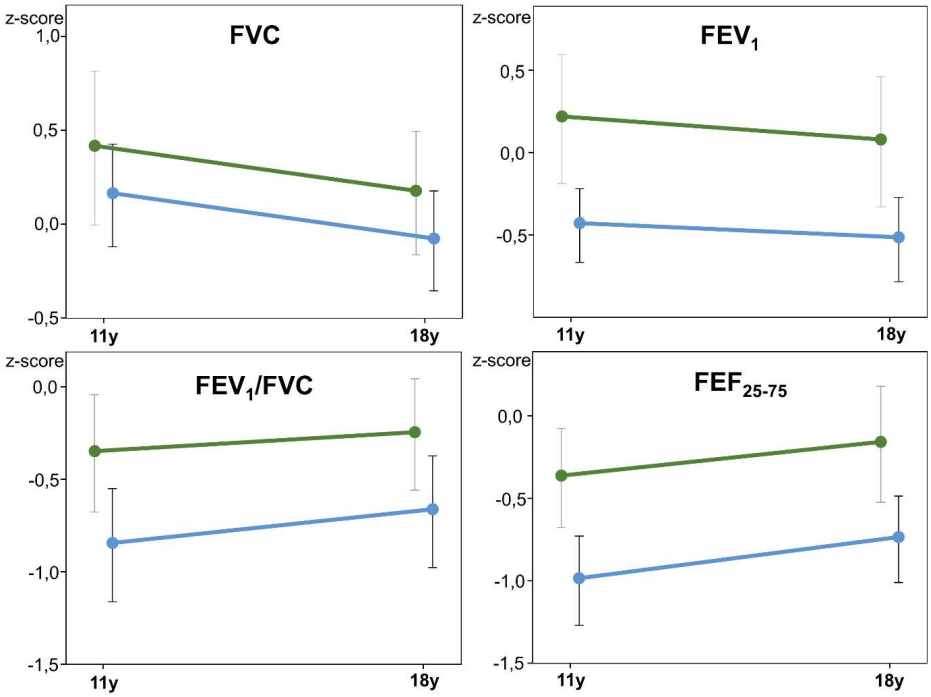

Figure 13. Lung function at 11 and 18 years of age in subjects hospitalized for bronchiolitis (blue lines) and control subjects (green lines). Results from generalized estimating equation analyses. The x-axis depicts age, and the y-axis depicts lung function variables as estimated marginal mean z-scores with 95% confidence intervals. Reworked from Figure 2, Paper 2.
Abbreviations: 11 y, First follow-up at median age 11 years; 18 y, Second follow-up at median age 18 years; FVC, Forced vital capacity; FEV_1, Forced expiratory volume in first second; FEF_{25-75}, Forced expiratory flow between 25% and 75% of the forced vital capacity.

Table 4. Change in lung function variables and BHR from 11 to 17-20 years of age in subjects hospitalized for bronchiolitis in infancy and age-matched control subjects.

	Post-bronchiolitis		**Control subjects**		**Interaction**
	Mean change (95% CI)	P-value	Mean change (95% CI)	P-value	P-value
FVC, z-score	-0.23 (-0.49, 0.04)	0.089	-0.26 (-0.63, 0.12)	0.182	0.911
FEV_1, z-score	-0.08 (-0.30, 0.14)	0.454	-0.15 (-0.55, 0.24)	0.451	0.765
FEV_1/FVC, z-score	0.17 (-0.15, 0.49)	0.301	0.12 (-0.20, 0.43)	0.471	0.819
FEF_{25-75}, z-score	0.24 (-0.00, 0.49)	0.054	0.21 (-0.13, 0.55)	0.230	0.873
LnDRS	-0.30 (-0.77, 0.17)	0.210	-0.22 (-0.73, 0.29)	0.394	0.825

Results from generalized estimating equation models including interaction terms group*time to test for unequal trajectories in the control group and the post-bronchiolitis group. A positive mean change indicates that z-scores were higher at age 18 than 11 years. Analyses are adjusted for family history of asthma or atopy, and atopic sensitization and asthma at 11 years of age. P-values are from Wald tests. DRS (%/µmol) is the ratio of maximum percentage decline in FEV_1 from baseline to cumulative administered dose (µmol) of methacholine. Due to highly skewed distribution, DRS was transformed using the natural logarithm. Reworked from Table 3, Paper 2.
Abbreviations: CI, Confidence interval; FVC, Forced vital capacity; FEV1, Forced expiratory volume in first second; FEF25-75, Forced expiratory flow between 25% and 75% of the forced vital capacity; DRS, Dose response slope.

5.3 Paper 3

The adjusted associations between the level of eosinophils during bronchiolitis and different outcomes in young adult age are shown in Table 5. The level of eosinophils was not associated with neither asthma ever nor current asthma at 17-20 years of age. A positive association between the level of eosinophils and subsequent atopy was found in the unadjusted analysis (OR 1.27; 95% CI 1.03 to 1.56; p=0.026), but not after adjusting for age, sex, family history of atopy, RSV-status, and age at hospitalization for bronchiolitis. However, when studying different phenotypes of asthma, we found a negative association between the level of eosinophils and the non-atopic asthmatic phenotype. The level of eosinophils was negatively associated with the lung function variables FVC and FEV_1, but there was no association between the level of eosinophils and FEV_1/FVC.

Table 5. Associations between the natural logarithm of the level of eosinophils during bronchiolitis in infancy and asthma, atopy, and lung function in 225 young adults. Missing data handled by multiple imputation.

	OR (95% CI)	***P*-value**
Asthma ever, adjusted [a]	0.99 (0.83, 1.18)	0.934
Current asthma, adjusted [a]	0.96 (0.79, 1.16)	0.657
Atopy, adjusted [a]	1.18 (0.94, 1.47)	0.144
Phenotypes	**RRR (95% CI)**	***P*-value**
Healthy	Reference	
Atopic non-asthmatic, adjusted [a]	1.01 (0.77, 1.31)	0.964
Non-atopic asthma, adjusted [a]	0.76 (0.60, 0.98)	**0.031**
Atopic asthma, adjusted [a]	1.37 (0.88, 2.11)	0.162
Lung function	**β (95% CI)**	***P*-value**
FVC z- score, adjusted [b]	-0.11 (-0.19, -0.02)	**0.014**
FEV_1 z- score, adjusted [b]	-0.12 (-0.21, -0.03)	**0.010**
FEV_1/FVC z- score, adjusted [b]	-0.01 (-0.10, 0.08)	0.808
FEF_{25-75} z- score, adjusted [b]	-0.07 (-0.16, 0.01)	0.098

Results from adjusted logistic, multinomial logistic, and linear regression analyses. P-values from Wald tests. Bold values denote statistical significance at the $p < 0.05$ level. Reworked from Table 3, Paper 3.
[a] Adjusted for age, sex, family history of atopy, RSV-status, and age at hospitalization for bronchiolitis.
[b] Adjusted for family history of atopy, RSV-status, and age at hospitalization for bronchiolitis.
Abbreviations: OR = odds ratio; RRR = relative risk ratio; β = regression coefficient; CI = confidence interval; FVC = forced vital capacity; FEV_1 = forced expiratory volume in first second; FEF_{25-75} = forced expiratory flow between 25-75% of the forced vital capacity; RSV = respiratory syncytial virus.

Subjects in the non-RSV group were older at hospitalization; median 4.5 months (quartiles 2.4, 7.8) vs. 3.8 months (2.0, 5.8), p=0.041, and had higher levels of eosinophils during bronchiolitis than the RSV-group (Figure 14). At follow-up at 17-

20 years of age, the non-RSV-group had higher prevalence of the atopic non-asthmatic phenotype (45.5% vs. 20.0%, p=0.001), and lower prevalence of the healthy phenotype compared to the RSV-group (27.3% vs. 56.4%, p<0.001). However, there were no significant interactions between the level of eosinophils and RSV-status for neither atopy, asthma, different phenotypes, nor lung function variables. This means that even though both the exposures and some of the outcomes differed between the two virus groups, the associations between the level of eosinophils and the various outcomes did not differ between the RSV-group and the non-RSV group.

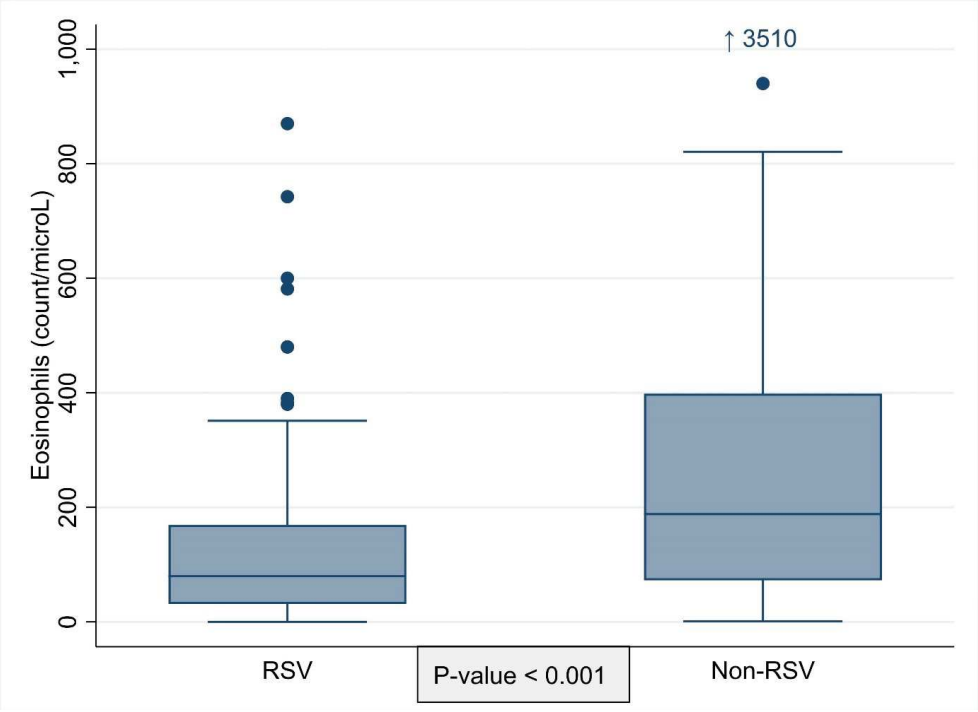

Figure 14 (Paper 3, Figure 2). Box plot depicting levels of eosinophils during RSV-bronchiolitis and non-RSV bronchiolitis. Within each box, the horizontal lines denote median values; boxes extend from the 25th to the 75th percentile; whiskers denote adjacent values (i.e. values within the 1.5 interquartile range of the 25th and 75th percentile of each group); dots denote observations outside the range of adjacent values. Differences are tested by Mann–Whitney U test.
Abbreviations: RSV, respiratory syncytial virus.

5.4 Paper 4

5.4.1 BMI

In the regression analyses including all subjects, BMI was associated with asthma ever, current asthma, and the lung function variables FVC, FEV_1, and FEV_1/FVC, but not with atopy (Figure 15). The directions of the associations between BMI and the various outcomes were non-linear, but with an overall tendency for a positive association with asthma, FVC, and FEV_1, and a negative association with FEV_1/FVC (Figure 15). Figure 16 illustrates the associations between BMI and the outcomes asthma, atopy, and lung function in young adults previously hospitalized for bronchiolitis and control subjects. We found no statistically significant interactions between BMI and group for any of the outcomes, but a tendency for a more pronounced inverted U-shaped curve for FVC, FEV_1, and FEF_{25-75} in the post-bronchiolitis group.

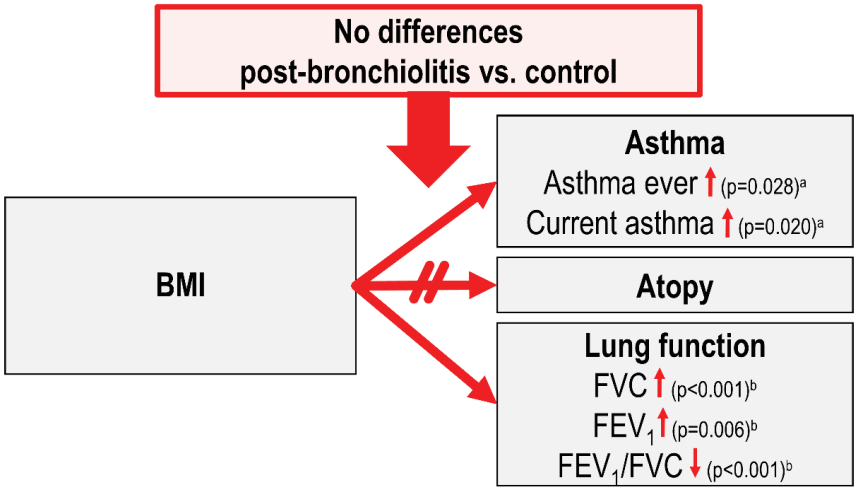

Figure 15. Overview of the associations between BMI and asthma, atopy, and lung function in young adults hospitalized for bronchiolitis and control subjects. Results from adjusted mixed effects logistic and linear regression analyses with p-values from Wald tests. BMI had a non-linear relationship to outcomes, and was included in the analyses as a 3-knot restricted cubic spline.
[a] Adjusted for sex, age of gestation at birth, birthweight, hospitalization for bronchiolitis, exposure for smoking, age at follow-up, and atopy at follow-up.
[b] Adjusted for variables mentioned under [a] except for sex and age at follow-up.
Abbreviations: BMI = body mass index; FVC, Forced vital capacity; FEV_1, Forced expiratory volume in first second; FEF_{25-75}, Forced expiratory flow between 25% and 75% of the forced vital capacity.

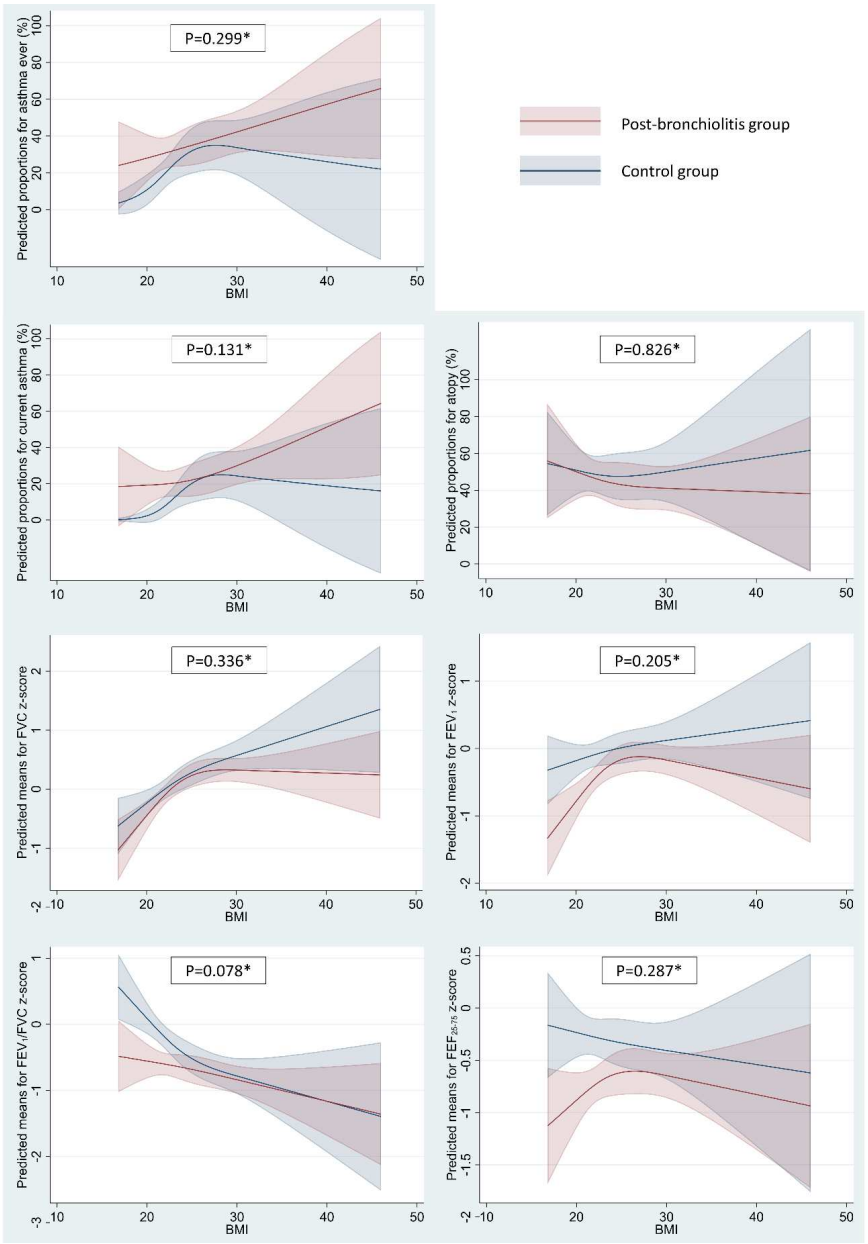

Figure 16 (Paper 4, Figure 2). Associations between BMI and asthma, atopy, and lung function in the post-bronchiolitis group and the control group. Plots showing predicted proportions and means with 95% confidence intervals for each of the two groups, with all other covariates set to sample means. The x-axis depicts BMI, and the y-axis depicts the predicted proportions (%) or means (z-scores) for each outcome. *P-values for the interaction term between BMI and group (i.e. post-bronchiolitis vs. control) from Wald tests.
Abbreviations: BMI = body mass index; FVC = forced vital capacity; FEV1 = forced expiratory volume in first second; FEF25-75 = forced expiratory flow between 25-75% of the forced vital capacity.

5.4.2 Adipokines

In the regression analyses including all subjects, leptin was negatively associated with the lung function variables FVC, FEV_1, and FEF_{25-75}, and tended to be negatively associated with FEV_1/FVC, but we found no other associations between the various adipokines and outcomes (Figure 17). When analyzing interaction effects, only two associations between adipokines and outcomes were found to differ between the post-bronchiolitis group and the control group; adiponectin was differently associated with FVC, and resistin was differently associated with current asthma (Figure 17). For adiponectin there was a tendency for a positive association with FVC in the post-bronchiolitis group (β 0.31; 95% CI -0.07 to 0.69; p=0.115), and the opposite tendency in the control group (β -0.25; 95% CI -0.67 to 0.16; p=0.232). Resistin was positively associated with current asthma in the control group (OR 8.22; 95% CI 1.16 to 58.08; p=0.035), but not in the post-bronchiolitis group (OR 0.71; 95% CI 0.22 to 2.33; p=0.572).

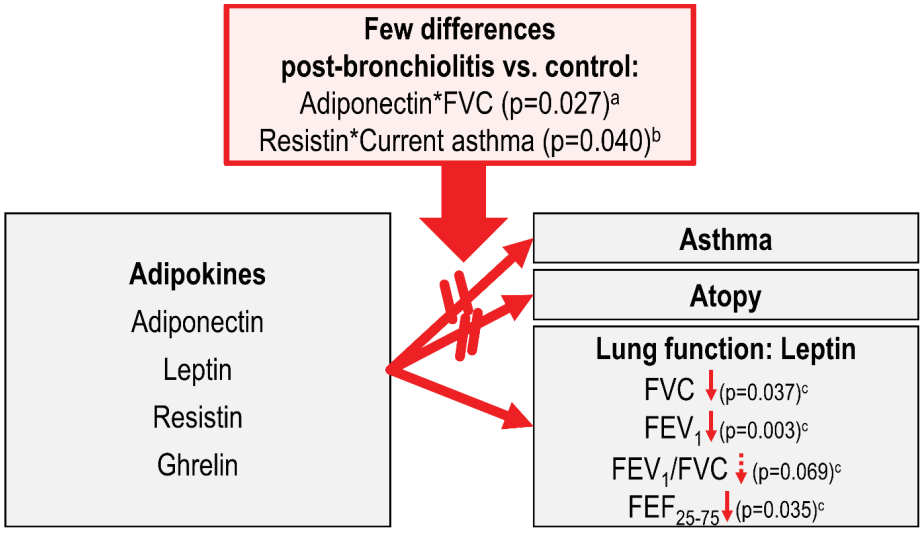

Figure 17. Overview of the associations between adipokines and asthma, atopy, and lung function in young adults hospitalized for bronchiolitis and control subjects. Results from adjusted mixed effects logistic and linear regression analyses. P-values from Wald tests.
[a] P-value for interaction term. Analyses adjusted for BMI, age of gestation at birth, birthweight, hospitalization for bronchiolitis, exposure for smoking, and atopy at follow-up.
[b] P-value for interaction term. Analyses adjusted for variables mentioned under [a] in addition to sex and age at follow-up.
[c] P-value for main analyses not including an interaction term and adjusted as mentioned under [a].
Abbreviations: FVC, Forced vital capacity; FEV_1, Forced expiratory volume in first second; FEF_{25-75}, Forced expiratory flow between 25% and 75% of the forced vital capacity.

6. DISCUSSION

6.1 Methodological considerations

This was an observational study with four sub-studies based on a cohort enrolling subjects hospitalized for bronchiolitis during their first year of life. Further, we consider our study to be a causal inference study in which examinations of causal associations are performed to estimate the causal effect of an exposure on an outcome [145]. Causal inference studies cannot by itself claim causality, only associations, and require careful attention to minimize selection and information bias, and a plan to control for confounding [145]. In the following section, I will reflect on the strengths and weaknesses in relation to the study design, potential biases and confounding, the choice of definitions and examinations, and statistical methods.

6.1.1 Study design and subjects

Post-bronchiolitis group

External validity describes the extent to which the results of a study can be generalized to others with the same condition; i.e., if the results from the relatively small group who was examined in this study can be generalized to the background population of all individuals hospitalized for bronchiolitis during their first year of life. Recruitment to the post-bronchiolitis cohort was done in two different processes. The subjects from the original cohort were recruited during the initial hospitalization for bronchiolitis in infancy, whereas additional subjects were recruited directly to the follow-up at 17-20 years of age. Increasing the number of study subjects was important as it provided our study sufficient power to be able to answer our research questions also for the categorical outcomes asthma and atopy. However, enrollment of the additional subjects also entailed a risk of differences within the overall cohort, as the recruitment process differed. In order to prevent such differences, the medical records of all participants were subject to the same thorough assessment to ensure a homogenous evaluation of the criteria for inclusion and exclusion. All subjects from the original cohort were hospitalized for bronchiolitis during 1997/98, and hence also

included in the subjects eligible for invitation directly to the 17-20-year-follow up who were hospitalized during 1996-2001. Eligible subjects were not in any case selected according to specific risk factors, and both Stavanger and Haukeland University Hospitals serve a rather large geographical area including both urban and rural areas. Hence, we consider the study cohort to be representative for children hospitalized for bronchiolitis during their first year of life in a high-income-setting.

Longitudinal control group

This group (recruited at age 11 years) was included in paper 2 to be able to study trajectories of lung function and BHR from 11 to 17-20 years of age. By recruiting subjects from schools in Stavanger born in the same year as the subjects in the post-bronchiolitis group, this group was matched on age, but not on sex or other factors. There is no free choice of public schools for this age group in Norway, and the geographical area in which you live defines which school to attend to. Hence, the subjects in this group were all living in the same urban environment, and may constitute a more homogeneous group than the post-bronchiolitis group when it comes to background factors. Review of medical records ensured that these subjects had not been hospitalized for bronchiolitis.

Matched control group

At the 17-20-year follow-up, a new matched control group was established, aiming to improve the basis of comparisons with the post-bronchiolitis group. A perfect control group should be identical to the exposed group for all variables except for the exposure variable. This is difficult to achieve in practice, but by matching on sex, date of birth, and age of gestation at birth, we consider the matched control group to be reasonable comparable to the post-bronchiolitis group as regards to background factors.

The process of recruiting subjects to the matched control group was rather comprehensive, and we did not achieve one matched control for each of the subjects in the post-bronchiolitis group. This issue was handled by the selection of statistical methods. Rather than simple paired analyses, we performed mixed effects regressions

to be able to include all subjects. Potential correlations between matched individuals were allowed for by including a random intercept term in the models. This provides an optimized statistical power based on the data available, and it is also important ethically to use data from all subjects who have been willing to contribute to our study through their participation.

An ideal recruitment process for participants in a clinical study is difficult to achieve in real life. In this cohort study, one could have wished for a post-bronchiolitis group in which all subjects had been recruited during the hospitalization for bronchiolitis, and a matched control group recruited at the same time, with enough subjects consenting to participate in young adult age to ensure sufficient power. It would be valuable if both groups underwent regular follow-ups during childhood and adolescence including examinations resembling the ones we did at the 17-20 year follow-up. However, even though our study design carries some limitations, our strengths are the relatively large study groups and careful attention to the handling of our limitations in order to achieve as valid results as possible, among others by the choices of statistical methods.

6.1.2 Biases and confounding

Selection bias

A selection bias may be introduced if the selection of subjects, groups, or data is systematically different between groups. If the selection of study subjects leads to overrepresentation or underrepresentation of certain subgroups, this will result in a sample that is not representative of the population intended to be analyzed. Selection biases are to varying degrees present in every study, and may in our case have been caused by a low participation rate, or by missing data if these were systematically unequally distributed between the groups compared.

We consider the modest participation rate to be one of the main weaknesses of our study. Control subjects had lower participation rates compared to subjects hospitalized for bronchiolitis. This may be due to a greater perceived personal benefit for participation in the post-bronchiolitis group, as the subjects before consenting were

informed that the risk of subsequent respiratory morbidity was increased after bronchiolitis. Further, one could suspect subjects with allergies or respiratory symptoms to be more prone to consent to participation than asymptomatic subjects, but the prevalence of neither asthma nor atopy in our study differed notably from what has been observed in other studies (described in sections 2.2.2 and 2.3 for the general population, and in sections 2.5.1 and 2.5.2 for post-bronchiolitis populations).

It would be of interest to compare the characteristics of subjects who did consent with those who did not consent to participation. For paper 2, data from the 11 year follow-up are published for the 121 post-bronchiolitis subjects and 141 control subjects participating at that time [8], and hence available for comparison with the data from the subgroup that also participated at 17-20 years of age. The prevalence of asthma at the 11-year follow-up including all children in the post-bronchiolitis group was 20.7% vs. 15.0% for the subgroup also participating at the 17-20 year follow-up. For the longitudinal control group, the prevalence of asthma at the 11-year follow-up including all children was 9.2 % vs. 12.5% for the subgroup also participating at the 17-20 year follow-up. This suggests a stronger tendency for selection of those with respiratory symptoms in the longitudinal control group compared to the post-bronchiolitis group.

In retrospect, I have reflected on possible explanations for the modest participation rate. Firstly, I do not believe that our recruiting process was the best suited for our target group. In accordance with our ethical approval, we sent an information letter by post. The subjects then had to fill in an attached consent-form, put it in an enclosed envelope, and send it back to us. I believe that an electronic approach would be better suited. Secondly, in today's society, we are to a much greater extent than before exposed to repeated requests to evaluate or answer questions from various quarters. Hence, requests useful to the society including invitations to clinical studies might be at risk of disappearing in the crowd.

Information bias

An information bias is a flaw in measuring or recording of the exposure, covariate, or outcome variables that results in different quality of

information between groups. A certain degree of information bias is inevitable, but nevertheless important to minimize, especially systematical differences between groups. In our study, we used different strategies to diminish the risk of information biases. Dedicated study nurses performed the clinical examinations after specific training for the study, and all biochemical analyses were performed by biomedical laboratory scientists. I created my database, and plotted all data. Original reports from lung function testing were reviewed to ensure valid results, and the database was double checked. We also included additional control questions in our questionnaires to minimize categorization errors due to misunderstandings of the questionnaire by the study subjects.

Recall bias is a systematic error that occurs when participants do not remember previous events accurately or omit details, if this tendency differs between groups. Recall bias is a problem in every study that uses self-reporting, especially when asking for events backwards in time. It is possible that the memory works differently in individuals with a history of hospitalization for bronchiolitis than in individuals with no such history, in that events relating to respiratory symptoms or problems are perceived in different ways later in life. In addition, features such as smoking or high weight that are considered undesirable, might be underreported. However, in our study this is not likely to be present in one group more than the other, and hence should not result in systematical differences.

Missing data

This study has missing data for several variables. A bias occurs if the missing data is differently distributed between groups. We have missing data due to lack of information in medical records or birth protocols, non-completion of clinical examinations either due to the subjects own choice or due to contraindications or technical issues, and incomplete questionnaires. Although we cannot exclude the possibility, we do not suspect the missing data to be systematically differently distributed between groups. In paper 3, missing data were handled by multiple imputation. For the post-bronchiolitis group only, we have medical records from the hospital stay for bronchiolitis that might include more detailed information than the

study subjects can recall in young adult age. To avoid differences between groups, we retained from using this information in paper 1, 2, and 4, and used only the information from the questionnaires at 17-20 years available for both groups.

Confounding

A variable is confounding for the association between an exposure and an outcome if it affects both the exposure and the outcome. Potential confounding factors should be chosen a priori based on previous knowledge and clinical judgement. We evaluated potential confounding factors using directed acyclic graphs, a simplified example of which is given in Figure 10. However, there are always potential confounding factors that we are not yet aware of, or that we do not have data on. Older siblings reduce the risk of asthma and allergy in childhood [146 147], and one could think that infants with older siblings would be at increased risk of being hospitalized for bronchiolitis due to increased exposure to viruses in young age. Hence, older siblings could be a potential confounder in our study. In addition, both socioeconomic status and exposure to air pollution could be factors that may affect both the exposure and the outcome. However, we do not have data for these factors, and in practice a certain degree of residual confounding can never be ruled out.

Confounding can be controlled for in the study design, and by means of the methods used to analyze the data. By including a matched control group, we hoped to diminish systematical differences between groups on background factors other than the matching factors, which are not controlled for by the matching in itself. For the analyses, we used directed acyclic graphs to evaluate potential confounders, and adjusted for the chosen confounders in multivariable regression analyses. However, it is not applicable to adjust for more than a certain number of selected covariates. The choice of adjustment variables remains a balancing act between the wish to present the most correct and "un-confounded" result possible, and retaining a stable model.

6.1.3 Definitions and clinical examinations

In recent years, it has been emphasized that the "bronchiolitis" diagnosis comprises more than one condition, with viral etiology as a key element to distinguish between different entities [24]. RSV was tested for by direct immunofluorescence in most subjects in the post-bronchiolitis group. This is a test in which antibodies detect RSV antigen in epithelial cells in respiratory secretions [148]. Antigen detection kits in pediatric specimens have acceptable sensitivities of 72 to 94% and specificities of 95 to 100% as compared to cell cultures, dependent of the quality of the specimen [148]. These methods are less specific and far less sensitive than nucleic acid amplification assays such as polymerase chain reaction (PCR)-tests [148]. PCR-tests were not routinely performed, and no other viruses were systematically tested for. Specific viral etiology by PCR-testing including RV would have enhanced our study considerably, but was unfortunately neither available from the original cohort from 1997/1998 nor by review of medical records, as extensive testing for different viruses by PCR was not part of the clinical routine at that time. Based on knowledge about the different viruses involved in bronchiolitis [24] (Figure 1), it is reasonable to assume that RV occurred most frequently in our non-RSV-group, but also other viruses, bearing other characteristics than RV, such as human bocavirus and metapneumovirus are likely to be present [149 150]. The lack of viral etiology in the non-RSV group is one of the main weaknesses of this study, resulting in a non-RSV group that is heterogeneous and not directly comparable to a RV-group as it consists of an unknown variation of viruses.

We chose to define bronchiolitis based on the European guidelines [15], and to only include infants hospitalized during their first year of life. Further, the fact that we only included individuals who had been in need of hospitalization implied that we have only included infants in the severe end of the spectrum of bronchiolitis. This gives us a more homogenous study group, but makes it challenging to compare our results to others who have used 2 or 3 years of age as cut-off and/or included all degrees of severity of bronchiolitis. By choosing a higher age limit for inclusion, one is probably at risk of including more subjects in whom the episode of bronchiolitis represents a first episode of recurrent viral-induced wheezing or asthma.

Asthma should ideally be defined by a combination of symptoms, clinical assessment, spirometry, and tests of BHR and/or reversible airflow obstruction. However, this would be time- and resource-consuming and was not feasible given our available resources. We therefore chose to define asthma based on questionnaires, a method that is commonly used in epidemiological studies, but entails challenges. The heterogeneity of definitions in use make comparisons between studies challenging, and careful consideration of the prevalence of asthma in relation to the corresponding control group is warranted. In our study, the prevalence of current asthma was rather high at 13.1% in the control group, but this does not deviate notably from that of the general population in high-income countries found in other questionnaire-based studies [38 151]. The prevalence is nevertheless higher than the 5-6% that has been reported based on number of collected prescriptions of asthma medication [43]. This might partly be explained by over-estimation of the true prevalence of asthma by self-reporting in questionnaires, as some subjects might misread normal health related complaints as symptoms. However, 5.4% in our control group used ICS, a figure that is consistent with the reported prevalence of asthma based on number of collected prescriptions. This may either indicate suboptimal adherence to recommended treatment, i.e. that the "prescription-based prevalence" is under-estimated, or that the questionnaire-based prevalence is over-estimated due to factors described above. Perhaps lies the true asthma prevalence somewhere in between the two. This illuminates the difficulties in interpretation of asthma prevalence by different methods.

After publishing paper 2 on the longitudinal data for lung function and BHR, a debate arose on whether the focus should rather have been on assessment of reversible versus irreversible airflow obstruction instead of BHR [152 153]. Examinations of reversibility of lung function variables after bronchodilation would certainly have been a valuable contribution, but as testing of reversibility cannot reliably be performed at the same day as a methacholine provocation test, we chose to prioritize tests of BHR. Results for BHR were originally planned to be assessed also for comparison of the total post-bronchiolitis group and the matched control group, but due to methodological deviations as described in more details in the errata for Paper 2, these data have not yet been analyzed.

We chose which clinical examinations to prioritize in 2014 when planning this study. In retrospect, we could have drawn whole blood samples. This would neither constitute any increased burden to the subjects nor be more time consuming, as serum samples were already drawn. Eosinophil blood cells measured in healthy state during the 17-20 year follow-up would be of interest to obtain a closer approach to the type of inflammation. Another possibility to assess type of inflammation in young adult age is to measure FeNO. FeNO has been shown to correlate with eosinophilic airway inflammation, and provide a non-invasive marker of Th2-type inflammation in asthma [154]. This examination was actually performed in our 17-20-year follow-up, but unfortunately with a substantial number of missing data due to invalid results and a measuring device that was out of order in periods. We therefore chose not to include FeNO in our publications.

6.1.4 Statistical considerations

Power

The power analyses described in materials and methods (Section 4.6.3) were performed in the planning phase of the project for the main outcomes based on simple comparisons between two independent groups, and acknowledged rules of thumb as regards to regression analyses. According to this, our sample size to assess differences between the post-bronchiolitis group and the control group was large for the continuous variables assessing lung function, and acceptable for the categorical outcomes asthma and atopy.

For Paper 1, we consider the sample size sufficient to detect main effects. We have used mixed effects regression analyses and included random intercept terms, a choice that tends to reduce the power. However, this also allowed us to include more subjects in the analyses resulting in increased power, and hence we still consider the power to be acceptable. For Paper 2, we also consider the sample size to be acceptable for assessment of the continuous variables for lung function. A sample size of 53 subjects in one group and 31 subjects in the other group, enables us to detect an absolute difference of 6.5% in FEV_1 which we consider to be reasonable in terms of

clinical relevance. For Paper 3, we adhere to the acknowledged rules of thumb for regression analyses.

Paper 4 presents mainly negative results, with few differences between the post-bronchiolitis group and the control group regarding associations between BMI and adipokines and outcomes in young adult age. In this case, critical consideration of the power is crucial, as low power results in a high risk of these negative results being false (type-2-errors). Differences between groups are in this study assessed by interaction analyses. Power calculations based on regression analyses are not directly transferable to the power to detect interaction effects, as this can be lower compared to the power to detect main effects. We therefore chose to include effect estimates (OR or β) with 95% CI for the interaction terms in the result section of paper 4 to make it possible to evaluate the degree of imprecision of the estimate. For the categorical outcomes, OR with values of 1.46, 2.50, and 4.14 may be interpreted as small, medium, and large effect sizes, respectively (given a rate of the outcome of interest in the non-exposed group at 10%) [155]. Evaluating if the 95% CI for OR for the interaction terms include either these figures or the corresponding inverse figures ($\frac{1}{OR}$), reveals that medium effect sizes are not included for neither leptin, resistin, nor ghrelin. Hence, is seems that we can interpret that there are no medium and large interaction effects for these adipokines. For the interaction between adiponectin and the categorical outcomes asthma and atopy, the confidence intervals were rather wide including both zero effect and the OR for large effects, and hence, these results in particular must be interpreted with caution. For the continuous outcomes, we approximated the power a posteriori by performing analyses for linear regression models including interaction terms, and found that we would be able to detect small-to-medium effects for the interaction term (Cohen's f of 0.18) [143], with a total sample size of N=292, power=0.8, and α=0.05.

Although acknowledging that our sample size results in some limitations for analyses of categorical outcomes in particular, our study is to date the largest cohort study of young adults after hospitalisation for bronchiolitis during infancy, also including a control group.

Multiple testing

Multiple testing refers to situations in where a dataset is subjected to multiple statistical tests. This amplifies the probability of false-positive results (type-1-errors), and may be a problem when testing for a large number of associations as we do for adipokines vs. outcomes in paper 4. Multiple testing can be corrected for by various statistical methods, but we have not made any correction for multiple tests in our study. By choosing not to do this, the risk of type-1-errors will increase with increased number of analyses. Correction for multiple tests, on the other hand, would increase the risk of false negative results (type-2-errors). In paper 4, we conclude that the increased risk of asthma and impaired lung function observed in young adults after bronchiolitis seems unrelated to growth and fat metabolism, i.e. we conclude that there are no important interaction effects. Hence, it is not likely that correction for multiple tests would change the conclusion.

Fitting the models for Paper 4

Finding the model which was best fitted to analyze associations between BMI and adipokines and the various outcomes was challenging. Based on clinical knowledge, we suspected that BMI had a non-linear relationship to outcomes. This was confirmed by statistical testing, i.e. we tested a linear model against restricted cubic spline models with different number of knots, and found that the overall better fitted model was the one in which BMI was included as a 3-knot restricted cubic spline. This allows for increased flexibility, but bares the cost of an extra degree of freedom, which has to be taken into account when evaluating the total number of variables included versus the sample size needed to detect relationships between the explanatory and dependent variables.

The adipokines were not normally distributed, and were considered for transformation using the natural logarithm. We tested multivariable fractional polynomial models for both the original variables and for the log transformed variables for adipokines, and concluded that no further transformations were required and that the overall preferred predictors were the log transformed adipokines. Assessments of

dfbeta confirmed that the use of log transformed variables for adipokines was the better choice considering influence.

6.2 Discussion of the main results

6.2.1 Asthma

The higher prevalence of both asthma ever and current asthma in the post-bronchiolitis group compared to the control group are in line with both previous post-bronchiolitis cohort-studies [5 11 77 80 81 83 84 156 157], and a meta-analysis [158]. Whereas others have found higher asthma prevalence after non-RSV-bronchiolitis compared to RSV-bronchiolitis [11 78], we found an equally high prevalence in both virus groups. Our finding is surprising, but not unique, as also other post-bronchiolitis-studies in young adult age have found high asthma prevalence irrespective of the virus involved [83 84]. In childhood however, the finding of a higher prevalence of asthma after non-RSV bronchiolitis seems more consistent. A recent meta-analysis in children found up to 4 times increased risk of asthma or recurrent wheezing after RV-bronchiolitis compared to RSV-bronchiolitis [159]. Increased asthma risk after non-RSV bronchiolitis was also found in our previous follow-up at 11 years of age [8].

Previous studies have suggested a U-shaped prevalence-curve for asthma after RSV bronchiolitis from early childhood to young adulthood [72]. Results from both the Tucson birth cohort study and a meta-analysis from 2013 suggest an association between RSV-infection and asthma that decreases with age [12 160]. However, we and others show high prevalence of asthma after RSV also in young adult age [5]. The reason for this tendency of asthma-symptoms to relapse in early adulthood after RSV-bronchiolitis is not known. A study of trajectories of asthma and allergy symptoms from childhood to adulthood in a general population found that some classes of trajectories showed increasing probability of wheeze in adolescence and young adulthood [161]. These trajectories showed the strongest associations with environmental exposures, especially smoking, but also mould, dog ownership, and occupational exposures [161]. Perhaps one can imagine that these subjects possess a vulnerability that make them more susceptible to environmental exposures. Further, one could suspect

that asthma after RSV-bronchiolitis may be an expression of a similar vulnerability related to either a persistent structural airway damage from the insult of the viral bronchiolitis or to a premorbid condition leading to both a severe clinical course of bronchiolitis and subsequent respiratory morbidity, potentially accelerated by environmental exposures.

We found that the impact of having bronchiolitis on subsequent asthma did not differ between sexes. Based on this, we suggest that bronchiolitis in infancy is probably not included in the mechanisms leading to the switch between sexes during puberty resulting in a higher adult asthma prevalence in females [44-46]. Our results contrasts those of a Swedish post-bronchiolitis study which found female sex to be a significant risk factor for asthma at age 17-20 years [83].

6.2.2 Atopy

We found no difference in atopy between the post-bronchiolitis group and the control group. This is broadly in line with other cohort studies in young adult age [77 81 83], but contrasts the Swedish study by Sigurs [5]. Even though the prevalence of atopy approached 50% in both the post-bronchiolitis group and the control group, this is similar to what has been reported in the general population, as described in section 2.3 [54 59 60]. The results by Sigurs stand out in the literature as different, and are not directly comparable to ours, but the prevalence of atopy is higher in their all-RSV-population at around 50%, and lower in the control group at around 30% [5]. As further discussed in section 6.2.7, our RSV-group had a lower prevalence of atopy at around 30%.

Among subjects with asthma, however, we found atopy to be less common in the post-bronchiolitis group than in the control group. This finding corroborates that non-atopic asthma is common after bronchiolitis, a notion that is further discussed in the following.

6.2.3 Lung function

The post-bronchiolitis group had impaired lung function with a more obstructive lung function pattern compared to the control group. These results are in

line with findings in other post-bronchiolitis cohorts in young adult age [5 10 77 82]. We did not measure lung function after bronchodilation, but this has been done by others who report a sustained reduced FEV_1/FVC-ratio in the post-bronchiolitis group compared to the control group also after administration of a bronchodilator [5 10 82]. We found lower FEV_1/FVC-ratio after non-RSV bronchiolitis compared to RSV-bronchiolitis. This is consistent with a follow-up at 12 years of age of the Finnish cohort from 1992/93 [162], and may support a notion that damage to the airways during bronchiolitis may occur in different ways depending on the virus involved. However, this is not consistent in the literature, as neither the Finnish cohort from 1981/82 nor the Swedish cohort from 1984/85 found that lung function in adult age was affected by viral etiology [10 78].

We found that the association between bronchiolitis in infancy and FVC in adult age differed between sexes with a negative association present in males only. This may indicate that the impact of a history of bronchiolitis is more pronounced in males than in females. Few post-bronchiolitis studies have examined the impact of sex, but a study from Sweden reported lower mid-expiratory flow rate compared to control subjects in males only [10]. Boys are in general more vulnerable to respiratory events in early life, partly due to differences between sexes in anatomy and physiology such as airway size, airway muscle bulk, airway reactivity, airway tone and cough reflexes [163]. This may make boys more susceptible to damage to airways from the insult of having an acute episode of bronchiolitis, resulting in a sustained impaired lung function in adult age.

Low lung function (FEV_1 < 80% predicted) in young adulthood has been found to be associated with increased prevalence and earlier incidence of respiratory, cardiovascular, and metabolic abnormalities, and also a higher all-cause mortality compared to individuals with normal lung function [164]. In our study, lung function after bronchiolitis was lower than that of the control group, but within the normal range. Nevertheless, this is clinically relevant, as even mild to moderate impairment of lung function (FEV_1) has been shown to be a predictor of later cardiorespiratory morbidity and mortality [165].

6.2.4 Trajectories of lung function

We found that BHR and z-scores for lung function were stable from 11 to 17-20 years of age in children hospitalized for bronchiolitis, with trajectories lower than, but parallel to those of the control group. In line with our results, a study combining data from three population-based birth cohort studies from the UK and Australia found wheezing to be an early life predictor of a persistently low trajectory for lung function [66]. No other post-bronchiolitis cohorts have studied trajectories of lung function longitudinally using the same method as us, but reduced lung function compared to the control group seems persistent in follow-ups throughout childhood and adolescence [5] [73].

Children who follow lung function trajectories below normal, may show catch-up towards the normal range either during later childhood or during the excessive lung growth in puberty, and by this still reach the top plateau for expected lung function in young adult age [64]. However, our study show no signs of catch-up effect in lung function after bronchiolitis. Similar persistently low lung function trajectories have also been shown after other early respiratory insults such as extreme prematurity [166] [167], and in non-selected birth cohorts such as the Tucson cohort which found reduced lung function present shortly after birth to be a risk factor for airflow obstruction at 22 years of age [67]. These results corroborate that abnormal lung function trajectories may originate in childhood precipitated by early respiratory insults among other factors, and potentially culminate in COPD as also described by others [65].

We found BHR at age 11 to be independently associated with asthma at 17-20 years of age, even after adjustment for asthma at 11 years of age. This is in line with results from general population cohort studies [55] [56], and a Norwegian unselected birth cohort study which found BHR at 10 years of age to be a predictor of current asthma 6 years later [54]. BHR is a hallmark of asthma. These findings indicate that BHR is a clinical characteristic that precedes the development of asthma, either as an inborn feature present from the very beginning [168], or as a feature that represents the first presentation of a pathological process eventually leading to symptomatic asthma. Results from the Danish Copenhagen Prospective Studies on Asthma in Childhood (COPSAC) at-risk birth cohort of children born to mothers with asthma showed that

children with asthma at some point at age 1 month to 13 years had airway obstruction and BHR before symptom debut, which did not worsen with increased asthma symptom duration or attenuate with remission [169]. This implies stable trajectories of airway obstruction and BHR during childhood, and that asthma may in part be a consequence of these features rather than their cause.

6.2.5 Eosinophil inflammation

We did not find the level of eosinophils during bronchiolitis to be associated with asthma in adult age. This is in line with results from the Finnish 1981/82 cohort reporting no association between the level of eosinophils during the acute infection and asthma up to 18-20 years of age [108]. However, at the 28-31 year follow-up of the same cohort, eosinopenia during bronchiolitis predicted low asthma risk, and eosinophilia in healthy state predicted elevated asthma risk [170]. Recently, a study from the Finnish 1992/93 cohort also found eosinophil blood cells during bronchiolitis to be a risk factor of asthma in young adults [157]. Hence, our results are unexpected, and have also been subjects for a prolonged discussion after paper 3 was published [171 172].

The timing of sampling of eosinophils plays a role as measurements obtained in a healthy state differ from those obtained during an acute viral infection, and it seems reasonable to think that the predictive values of these measurements are neither equivalent nor directly comparable. Both Finnish cohorts include children hospitalized before 2 years of age. As previously described, this may result in a more heterogeneous study group potentially including some subjects with early onset asthma and thereby increased risk of both eosinophilic inflammation during the viral infection and subsequent eosinophilic asthma. Hence, the association between the two may be affected. Atopic predisposition, age at hospitalization, and non-RSV bronchiolitis, especially RV-bronchiolitis, are all factors that are associated with eosinophil inflammation [23 24]. As these factors may act as confounders in the association between bronchiolitis and later asthma, they are adjusted for in our analyses. Differences in the choice of adjustment variables between studies may also contribute to different results.

An association between eosinophil blood cells and subsequent asthma has also been found in childhood after bronchiolitis [104 105 136 173]. Our results may deviate from these partly because non-eosinophil inflammation is more common in adult asthma whereas childhood asthma is dominated by eosinophil inflammation [48]. As time passes by after bronchiolitis, the group of subjects with asthma is likely to gradually become more heterogeneous by progressively including more subjects with non-eosinophilic inflammation. Hence, an association between eosinophils and asthma may be diluted over time.

Consistent with the results from our 11-year follow-up [105], we did not find the level of eosinophils during bronchiolitis to be associated with atopy in young adult age. However, when analyzing phenotypes based on both atopy and asthma, we found a negative association between the level of eosinophils and non-atopic asthma. This may corroborate that both bronchiolitis and asthma after bronchiolitis are heterogeneous conditions, with different types of immune responses in play. Bearing in mind that bronchiolitis in general increases the risk of subsequent asthma, this finding should probably not be interpreted as that eosinophilia during bronchiolitis protects against subsequent asthma, rather that this may predict the type of asthma, i.e. Th2-high vs. Th2-low.

We found that the level of eosinophils during bronchiolitis was associated with lower lung function in young adult age. This was found for both FVC and FEV_1, but not FEV_1/FVC ratio, suggesting a tendency towards a more restrictive than obstructive lung function pattern. The Finnish 1981/82 cohort, however, found high levels of eosinophils during bronchiolitis to be associated with irreversible airway obstruction at 28-31 years of age [170]. This may also be partly explained by their inclusion of children up to 2 years of age, in that subjects in whom the bronchiolitis represent early onset asthma have a higher risk of long-term obstructive lung function.

6.2.6 Growth and fat metabolism

A recent post-bronchiolitis multicenter cohort study found that different growth trajectories gave differential risks for developing asthma by 5 years of age, with the

highest probability in children with persistent obesity [174]. This suggests that BMI may impact on asthma risk not only in the general population, but also in the aftermath of bronchiolitis. Our paper 4 is the first study to explore if the increased respiratory morbidity after bronchiolitis is related to fat metabolism.

When studying the post-bronchiolitis group and the control group all together, we found BMI to be associated with lung function and asthma, but not with atopy. This is in line with studies from the general population [113-115]. However, consistent with a post-bronchiolitis study showing no association between weight status and asthma or allergy in adolescents [85], the associations between BMI and the various outcomes did not differ between the post-bronchiolitis group and the control group.

When assessing adipokines in the post-bronchiolitis group and the control group all together, we found that neither adiponectin, leptin, resistin, nor ghrelin were associated with the outcomes asthma or atopy. In line with others [129 130 175], we found leptin to be negatively associated with lung function, but this was not the case for any of the other adipokines. Only two associations between adipokines and outcomes differed between the post-bronchiolitis group and the control group. Firstly, the association between adiponectin and FVC differed, with a tendency towards a positive association in the post-bronchiolitis group in line with other studies in general populations [126], and the opposite tendency in the control group. This may indicate a possible anti-inflammatory protective effect of adiponectin on FVC only present in the post-bronchiolitis-group. However, we have assessed multiple associations, and the effect estimates are imprecise in both groups. Hence, these results must be interpreted with caution. In addition, previous research on this field is not consistent. Two studies in children as well as a Norwegian population-based study in adults found no associations between adiponectin and FVC [175-177]. Secondly, we found the pro-inflammatory adipokine resistin to be differently associated with asthma, with resistin being positively associated with asthma in the control group, but not in the post-bronchiolitis group. This may indicate a pro-inflammatory effect of resistin on asthma only in the control group.

To summarize, associations between BMI and adipokines versus the outcomes asthma, atopy, and lung function in young adults previously hospitalized for

bronchiolitis do not seem to differ notably from those of the general population. The interpretation of this will be that neither growth nor inflammation linked to fat metabolism seem to be major explanatory factors for the increased risk of respiratory morbidity observed after bronchiolitis.

6.2.7 RSV vs. non-RSV

Our study supports that there are clinical and pathophysiological differences between RSV bronchiolitis and non-RSV bronchiolitis, as also emphasized by others [24]. We found a high prevalence of asthma and impaired lung function in adult age in both virus groups, but age at hospitalization, eosinophil inflammation, and the prevalence of atopy differed between the two groups. Children with RSV bronchiolitis were younger and had lower levels of eosinophils during bronchiolitis, and lower prevalence of atopy in adult age. Children with non-RSV bronchiolitis on the other hand, were older and had higher levels of eosinophils during bronchiolitis, and more atopy and a more obstructive lung function pattern with lower FEV_1/FVC-ratio in young adult age. We suspected RSV-status to impact on the associations between the level of eosinophils and different outcomes in young adult age, but found no interactions between the level of eosinophils and virus group for neither asthma, atopy, nor lung function. This finding may partly be related to the time at which the outcomes were measured. As described above, the impact of an eosinophil inflammation present during an acute viral infection in infancy might dilute during the transition from childhood to adulthood.

The differences between the virus groups found in our study corroborates a substantial heterogeneity both in bronchiolitis and the outcomes in adult age depending on the virus involved, with atopy and a Th2-high eosinophilic inflammation being more pronounced in non-RSV bronchiolitis. There has been a growing body of literature focusing on RV as the main actor of this association, and especially RV subtype C which binds to the CDHR3-receptor has been connected to increased risk of asthma [24 178 179] . A study from Finland found that a RV-induced first wheezing episode predicted atopic, but not non-atopic asthma at school age [180]. Even though we did not

have results for RV, we assume RV to be frequent in our non-RSV-group, a notion supported by the increased prevalence of atopy and eosinophils inflammation of this group.

Taken together, the pathway leading to adult respiratory morbidity after bronchiolitis seems to be closely dependent on the virus involved. On one side, morbidity after non-RSV-bronchiolitis seems to rely on an interaction between the virus and host, in which different factors including genetics, atopic predisposition, and an overall vulnerability to develop a Th-2 type immune response including eosinophil inflammation play important roles. Infants hospitalized for RSV-bronchiolitis, on the other hand, seems to have a normal inflammatory response during the acute viral infection with suppressed eosinophils, and also less propensity for Th2-type immune response and atopy later in life. Their affected airways may either be damaged by the insult of the acute infection during bronchiolitis, or they may possess an early or even inborn vulnerability, that make them susceptible both for a severe course of bronchiolitis, and also subsequent respiratory morbidity.

6.2.8 General considerations

Both RSV and RV-infections are common, and the majority of children are infected with these viruses during the first few years of life, often multiple times. However, only a small proportion develops clinical bronchiolitis, and even fewer will have symptoms severe enough to be in need of hospitalization. Further, of infants hospitalized for bronchiolitis, approximately one third will develop asthma (adjusted predicted proportion for asthma ever at 17-20 years of age was in our study 34.8%). What distinguishes these individuals from those who suffer no consequences from the same exposures? Although the knowledge of mechanisms underlying chronic respiratory outcomes after bronchiolitis is increasing, there are still many unanswered questions remaining [181].

A Norwegian birth cohort study of healthy infants found reduced lung function at birth to be associated with increased risk of asthma by 10 years of age [182]. It is not yet known whether the respiratory morbidity observed in young adult age after

bronchiolitis results from the infection itself, or reflects a premorbid susceptibility or abnormal lung function. An unselected birth cohort study found that decreased lung function at birth predisposed to severe RSV disease, and to post-RSV wheeze [183], indicating a premorbid affected lung function. However, another study did not find impaired lung function before RSV bronchiolitis [184]. We do not have data on lung function before the bronchiolitis in our cohort, and can therefore not exclude that premorbid impaired lung function or inborn narrow airways preceded both the bronchiolitis and the outcomes in young adult age. BHR was found to precede acute severe bronchiolitis in response to infections with respiratory tract virus in 1-month-old neonates from the COPSAC at-risk birth cohort [185]. This suggest BHR to be an independent and possibly inborn feature that may predispose to both a severe course of bronchiolitis and subsequent respiratory morbidity for some subjects. For RV, there are indications suggesting that an individual genetic predisposition, deficiency in the antiviral IFN-response, and an inappropriate activation of a Th2-inflammation in response to the infection seem to be important [178]. Much uncertainty still exists in this field, and hence the chicken or the egg paradox of viral bronchiolitis and respiratory morbidity remains unsolved.

7. CLINICAL IMPLICATIONS AND FUTURE PERSPECTIVE

This thesis corroborates that bronchiolitis in infancy is associated with long-term impairment of respiratory health persisting into young adulthood, and contributes to increased understanding of the underlying mechanisms of these associations. Eosinophil inflammation during the acute viral bronchiolitis is associated with subsequent impaired lung function, but does not seem to be predictive of subsequent asthma or atopy in young adulthood. Growth and fat metabolism do not seem to explain the increased respiratory morbidity after bronchiolitis.

The importance of bronchiolitis as a risk factor for subsequent morbidity was recently underlined by a birth cohort study from the Great Britain which found that LRTI during early childhood was associated with almost a two times increased risk of premature adult deaths from respiratory disease [186]. Hence, increased knowledge of the long-term outcomes after bronchiolitis, as this thesis contributes to, will certainly be valuable. Increased knowledge may form the basis for developing more personalized guidelines for follow-up, which in turn may provide an opportunity to initiate preventive measures.

Currently, we have no measures that are effective in preventing the long-term consequences after bronchiolitis, even though various treatments have been assessed. For RSV-bronchiolitis, treatment with the monoclonal Ig palivizumab in a randomized controlled trial resulted in a significant reduction in the number of wheezing days during the first year of life [187], but had no major effect on current asthma or lung function at age 6 years [188]. Anti-inflammatory treatment with corticosteroids has also not proved useful after RSV-bronchiolitis [189]. As for RV-bronchiolitis, the ongoing Innovative Steroid Treatment to Reduce Asthma Development in Children After First-time Rhinovirus Induced Wheezing (INSTAR) study aims to assess the efficacy of corticosteroids in preventing recurrent wheezing and asthma in high-risk, first-time severe wheezing children with rhinovirus infection [190]. In addition, targeting the Th2-inflammation by biological agents, augmenting antiviral pathways with antiviral agents, vaccines, or the macrolide azithromycin, as well as modifying the airway microbiome with probiotics or antibiotics, are all assessed as possible preventive

measures, but so far without resulting in any treatment available for use in clinical practice [178].

Future studies need to take into consideration the challenges that relate to the diagnostic heterogeneity of both bronchiolitis and the subsequent respiratory outcomes. Age at the hospitalization, viral etiology, and atopic predisposition seem to be important factors to take into account. In addition, more advanced elements such as different immune responses, genetics, and the microbiome are of interest. A recent commentary in Chest emphasized the value of precision epidemiology approaches [181]. In these approaches, various biological samples, clinical data, and environmental and socioeconomic data are combined in a so-called multiomics data generation and integration process which gives rise to a more individualized base for risk-stratification screening, treatment, and prevention. Studies outgoing from the Multicenter Airway Research Collaboration multicenter prospective bronchiolitis cohorts, have investigated different approaches to subtyping bronchiolitis in Finland and the USA. Different profiles for severe bronchiolitis identified by a clustering approach were found to be differentially associated with asthma in childhood with the highest risk in a profile characterized by history of wheezing and eczema, wheezing during acute illness, and RV infection [191]. Integrated clustering of clinical, virus, and proteome data were able to identify biologically distinct endotypes of bronchiolitis that had differential risks of asthma development [192], and clustering analysis of nasopharyngeal airway metabolome data identified biologically distinct metabotypes [193]. As for the subgroup of bronchiolitis with RV, an integrated omics analysis identified four different biologically meaningful RV bronchiolitis endotypes [194].

I believe that the future lies in differentiating the bronchiolitis diagnosis into more homogeneous subgroups in order to study these more specifically, rather than to study this heterogeneous group as a whole. I hope for the future that we will be able to subtype bronchiolitis based on a set of markers, and further establish guidelines for follow up to better suit each subtype, hopefully accompanied by a toolbox of effective preventive measures at least for some subtypes. In turn, this can help us to move forward in the process towards being able to modify the development of disabling respiratory morbidity and premature death after bronchiolitis.

8. CONCLUSIONS

The overall conclusion of this thesis is that bronchiolitis in infancy is associated with impaired respiratory health persisting into young adulthood, but the underlying mechanisms of these associations remain unclear and in need of further investigations.

Based on the results from the four papers and considering the methodological limitations, the following conclusions can be given to the specific objectives of this thesis, listed in Chapter 3 under the heading ”Aims of the study”:

- The prevalence of asthma at 17-20 years of age was higher in subjects hospitalized for bronchiolitis in infancy compared to control subjects, but the prevalence of atopy did not differ between the two groups.
- Subjects hospitalized for bronchiolitis in infancy had a more obstructive lung function pattern with lower mean z-scores for FEV_1, FEV_1/FVC-ratio, and FEF_{25-75} at 17-20 years of age compared to control subjects.
- The asthma prevalence at 17-20 years of age was high after both RSV bronchiolitis and non-RSV bronchiolitis with no difference between the virus groups, but the non-RSV-group had higher prevalence of atopy and lower mean FEV_1/FVC-ratio compared to the RSV-group.
- Associations between bronchiolitis in infancy and asthma and atopy at age 17-20 years did not differ between males and females, but bronchiolitis was differently associated with FVC, with lower mean FVC in the post-bronchiolitis group compared with control subjects in males, but not in females.
- Z-scores for lung function and BHR did not change from 11 to 17-20 years of age in subjects hospitalized for bronchiolitis in infancy.
- Trajectories of lung function and BHR from 11 to 17-20 years of age after bronchiolitis in infancy did not differ from those of control subjects.
- The level of blood eosinophils during bronchiolitis in infancy was not associated with asthma or atopy at 17-20 years of age, but was negatively associated with the lung function variables FVC and FEV_1.

- Associations between the level of blood eosinophils during bronchiolitis in infancy and asthma, atopy, and lung function at 17-20 years of age were not different after RSV bronchiolitis compared to non-RSV bronchiolitis.
- At 17-20 years of age, associations between BMI and the outcomes asthma, atopy, and lung function did not differ between young adults previously hospitalized for bronchiolitis and control subjects.
- At 17-20 years of age, only associations between adiponectin and FVC, and between resistin and current asthma differed between young adults previously hospitalized for bronchiolitis and control subjects.

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10. ERRATA

Paper I

Methods: In the section describing the post-bronchiolitis group, we have written that "Among eligible subjects, 131 have previously participated in a longitudinal prospective follow-up study at 11 years of age". Whereas 131 is the number of subjects in the original cohort from 1997/98, 121 is the correct number of participants in the 11-year follow-up [8].

Paper II

After publishing this article, we uncovered two methodological deviations concerning the data for BHR at the 17-20-year follow-up.

An inconsistency in the protocol set-up for the methacholine provocation test was uncovered for some subjects. During the time-period for clinical examinations, both Stavanger University Hospital and Haukeland University Hospital replaced their spirometers. Some of the subjects in the 17-20-year follow-up completed the examinations on the old spirometers, and some on the new ones. Whereas the old spirometers were set up to use the post-saline values as baseline for decline in FEV_1 as recommended [139], the new ones were set up to use pre-saline values as baseline. Affected results were re-recorded based on post-saline values for all subjects, and results in which a different baseline led to a premature termination of the methacholine provocation test were excluded (five subjects in the post-bronchiolitis group and four control subjects).

Further, DRS was calculated as the ratio of *maximum* percentage decline in FEV_1 from baseline to cumulative administered dose of methacholine. This deviates from the 11-year follow-up, where the decline in FEV_1 after the *final* methacholine dose was used. The latter is consistent with the method suggested by O'Connor et al. [141] This results in an overestimation of DRS for subjects who had lower results for FEV_1 during the provocation test than after the final dose of Methacholine was administered.

Corrected results are given in the revised Tables and Figures in the published erratum (included in Chapter 12 under the heading "Reprint of paper number I-IV").

Subjects in the post-bronchiolitis group had higher DRS than controls at 11 years, but only a tendency to the same at 18 years (revised Table 2, erratum Paper 2). However, this issue was not the focus of the study; rather our aim was to study the trajectories of BHR during the important pubertal growth spurt. The effect sizes changed, but this did not influence the conclusion that BHR after hospitalization for bronchiolitis in infancy remained stable from 11 to 17-20 years of age.

11. APPENDIX

Appendix I - Questionnaire from follow-up at 17-20 years of age

Spørreskjema LAMA studien

Generelle spørsmål om deg:

1. **Hvor mange bor det i husstanden din utenom deg (der du bor mest)? _____**

2. **Hvor mange søsken har du?_____**

3. **Har du vært innlagt på sykehus med luftveisinfeksjon i første leveår, for eksempel infeksjon forårsaket av RSV virus?**
   ☐ ja
   ☐ nei
   ☐ vet ikke

4. **Har du vært innlagt på sykehus med lungebetennelse eller annen infeksjon i nedre luftveier (for eksempel bronkitt) etter 1 års alder?**
   ☐ ja
   ☐ nei
   ☐ vet ikke

   Hvis ja, hvor mange ganger?_____

5. **Har du vært innlagt på sykehus med astma etter 1 års alder?**
   ☐ ja
   ☐ nei
   ☐ vet ikke

6. **Har du noen gang hatt barneeksem (atopisk eksem)?**
   ☐ ja
   ☐ nei

   Hvis ja, ved hvilken alder ble du kvitt plagene?_____

7. **Har du <u>noen gang</u> hatt kløe/renning fra øyne og/eller kløende/rennende/tett nese utenom forkjølelse?**
   ☐ ja
   ☐ nei

   Hvis ja, har du hatt dette de siste 12 mnd? ☐

   I tilfelle, hva utløste plagene?
   ☐ Pollen fra trær
   ☐ Pollen fra gress

☐ Hund
☐ Katt
☐ Andre pelsdyr
☐ Husstøvmidd
☐ Muggsopp
☐ Vet ikke

8. Har du <u>noen gang</u> hatt allergi for matvarer?
☐ ja
☐ nei

Hvis ja, har du hatt dette de siste 12 mnd? ☐

Hvis ja, hvilken matvare har du reagert på eller reagerer du på?
☐ Egg
☐ Melk
☐ Hasselnøtt
☐ Peanøtt
☐ Andre nøtter
☐ Fisk
☐ Skalldyr
☐ Annet-skriv navnet_____

9. Hva er din vekt: _____

10. Hva er din lengde? _____

11. Ved hvilken alder fikk du menstruasjon? Angi alder i år og mnd så nær du husker. _____

12. Røyker du?
☐ ja
☐ nei

Hvis ja, hvor mange år har du røyket? _____

Antall sigaretter pr dag? _____

13. Bruker du noen faste medisiner?
☐ ja
☐ nei
Navn på medisin:

_____

_____

_____

Spørsmål om din familie:

1. **Kjenner du til om din mor, far eller søsken har/har hatt astma?**
   ☐ ja
   ☐ nei
   ☐ vet ikke

   Hvis ja, hvem?_____

2. **Kjenner du til om din mor, far eller søsken har tatt allergitest?**
   ☐ ja
   ☐ nei
   ☐ vet ikke

   Hvis ja, hos hvem, og hva ble det påvist allergi for?

   Mor:_____

   Far:_____

   Bror:_____

   Søster:_____

3. **Kjenner du til om din mor, far eller søsken har/har hatt barneeksem (atopisk eksem)?**
   ☐ ja
   ☐ nei
   ☐ vet ikke

   Hvis ja, hvem?_____

4. **Er det noen som røyker hjemme hos deg?**
   ☐ ja
   ☐ nei

   Hvis ja, innendørs?
   ☐ ja
   ☐ nei

   Antall sigaretter pr dag_____

   Hvis ja, hvor mange år har det vært røyket hjemme hos deg?_____

Spørsmål om luftveissykdom og symptomer hos deg:

1. **Har du <u>noen gang</u> hatt tung pust eller piping/surkling/tetthet i brystet?**
   ☐ ja
   ☐ nei
   Hvis du har svart nei, gå til spørsmål 6

2. **Har du hatt tung pust eller piping/surkling/tetthet i brystet i løpet av de siste 12 måneder ?**
   ☐ ja
   ☐ nei
   Hvis du har svart nei, gå til spørsmål 6

3. **Hvor mange anfall av tung pust eller piping/surkling/tetthet i brystet har du hatt i løpet av de siste 12 måneder?**
   ☐ ingen
   ☐ 1 til 3
   ☐ 4 til 12
   ☐ mer enn 12

4. **Hvor ofte har din søvn i gjennomsnitt blitt forstyrret på grunn av tung pust eller piping/surkling/tetthet i brystet de siste 12 måneder ?**
   ☐ aldri våknet
   ☐ mindre enn 1 natt pr. uke
   ☐ 1 eller flere netter pr. uke

5. **Har piping/surkling/tetthet i brystet eller tung pust vært så alvorlig de siste 12 måneder at du har hatt problemer med å snakke, slik at du bare kunne si ett eller to ord mellom hvert pust?**
   ☐ ja
   ☐ nei

---

6. **Har du noen gang fått diagnosen astma av lege?**
   ☐ ja
   ☐ nei

7. **Har du i løpet av de siste 12 måneder hatt tung pust eller piping/surkling/tetthet i brystet under eller etter fysisk trening, aktiv lek eller mosjonering?**
   ☐ ja
   ☐ nei

8. **Har du i løpet av de siste 12 måneder hatt tørr hoste om natten, utenom hoste i forbindelse med en forkjølelse eller andre luftveisinfeksjoner?**
   ☐ ja
   ☐ nei

9. **Har du i løpet av de siste 12 måneder noen gang brukt noen av disse astma-medisinene?**
   ☐ Ventoline, Bricanyl, Oxis, Salbutamol, Atrovent
   ☐ Pulmicort, Flutide, Becotide, Symbicort, Seretide
   ☐ Singulair
   ☐ Andre (skriv navnet): _____

12. REPRINT OF PAPER NUMBER I-IV

I

BMJ Open Respiratory Research

Asthma, atopy and lung function in young adults after hospitalisation for bronchiolitis in infancy: impact of virus and sex

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ABSTRACT

Background Hospitalisation for bronchiolitis is a risk factor for asthma and impaired lung function during childhood, but outcomes in young adults are poorly described. Our primary aim was to study the prevalence of asthma and atopy, and lung function at 17–20 years of age after bronchiolitis in infancy and, secondarily, the impact of viral aetiology (respiratory syncytial virus (RSV) vs non-RSV) and sex on these outcomes.

Methods This Norwegian cohort study enrolled 225 young adults hospitalised for bronchiolitis in infancy during 1996–2001 and 167 matched control subjects. The follow-up included questionnaires for asthma and examinations of lung function and atopy. Outcomes were analysed by mixed effects regressions.

Results Current asthma was more frequent in the postbronchiolitis group versus the control group: 25.1% (95% CI 19.0% to 31.2%) vs 13.1% (95% CI 7.9% to 18.2%), but not atopy: 44.3% (95% CI 37.1% to 51.5%) vs 48.2% (95% CI 40.5% to 55.8%), adjusted predicted proportions (95% CIs). Asthma prevalence did not differ between the RSV group and the non-RSV group: 24.0% (95% CI 16.1% to 32.0%) vs 23.8% (95% CI 12.8% to 34.7%) nor between sexes. Forced expiratory volume in 1 s (FEV_1), the ratio FEV_1/forced vital capacity (FVC), and forced expiratory flow between 25% and 75% of FVC, were lower in the postbronchiolitis group.

Conclusion Young adults hospitalised for bronchiolitis had higher prevalence of asthma, but not atopy, and a more obstructive lung function pattern than control subjects. The asthma prevalence was high after both RSV bronchiolitis and non-RSV bronchiolitis, and there was no difference between sexes. Bronchiolitis in infancy is associated with respiratory morbidity persisting into young adulthood.

Key messages

- Key question: What are the long-term outcomes of bronchiolitis in infancy regarding the prevalence of asthma and atopy, and lung function at 17–20 years of age, and how do viral aetiology (respiratory syncytial virus (RSV) vs non-RSV) and sex impact on these outcomes?
- Young adults hospitalised for bronchiolitis had more asthma and a more obstructive lung function pattern, but similar prevalence of atopy compared with control subjects. The asthma prevalence was high after both RSV bronchiolitis and non-RSV bronchiolitis, and there was no difference between sexes.
- This is the largest cohort study of respiratory outcomes in young adults after hospitalisation for bronchiolitis in infancy, and shows that bronchiolitis is associated with respiratory morbidity persisting into young adulthood. This is important as even mild lung function impairment may be a predictor of later cardiorespiratory morbidity and mortality.

INTRODUCTION

Bronchiolitis is a viral lower respiratory tract infection commonly seen in children less than 1 year of age.[1 2] Bronchiolitis constitutes a substantial health burden worldwide, and is the most common reason for admission to hospital during infancy in high-income countries.[3 4] Children hospitalised for bronchiolitis have increased risk of subsequent asthma and impaired lung function later in childhood.[2 5–8]

The risk of asthma after bronchiolitis is related to the virus involved.[2 7] The highest risk of asthma has been observed in children with bronchiolitis caused by other viruses than respiratory syncytial virus (RSV),[7] and particularly by human rhinovirus (HRV).[9 10] Whereas asthma after RSV bronchiolitis seems to be linked to a T-helper cell (Th)1 dominated inflammatory response and structural airway damage, asthma after non-RSV bronchiolitis such as HRV bronchiolitis, is probably more related to atopy and a Th2 dominated eosinophilic inflammation.[11–13]

Few studies have evaluated the impact of sex on respiratory outcomes in young adults with a previous history of bronchiolitis, but in general the risk of asthma is related to sex. During childhood the prevalence of asthma is higher in males, but after a switch during

puberty females have a higher prevalence in adulthood.[14 15]

Knowledge regarding long-term respiratory morbidity in adults with former bronchiolitis is limited, but a few small studies have reported a sustained increased risk of asthma and lower lung function.[6 16 17] A Finnish study reported irreversible airway obstruction at 30 years of age after severe bronchiolitis in infancy,[18] which may suggest permanent structural alterations in the airways, in line with studies indicating that bronchiolitis predisposes to the development of chronic obstructive pulmonary disease (COPD).[19–21] COPD is a major public health problem,[22] and improved insights into how early life respiratory tract infections influence subsequent development of respiratory morbidity is therefore of great importance.

We hypothesised that young adults hospitalised for bronchiolitis in infancy have a higher risk of asthma and lower lung function, but similar prevalence of atopy compared with control subjects. Our primary aim was to study the prevalence of asthma and atopy, and lung function at 17–20 years of age after bronchiolitis in infancy and, secondarily, the impact of viral aetiology (RSV vs non-RSV) and sex on these outcomes.

METHODS

Study design

This is a historical cohort study of young adults hospitalised for bronchiolitis in infancy and a matched control group.

Postbronchiolitis group

Between October 1996 and May 2001, 1168 children under 1 year of age were discharged from the University Hospitals in Stavanger and Bergen, Norway with a diagnosis of acute bronchiolitis, and were potentially eligible for invitation to this study (figure 1). Exclusion criteria were use of inhaled or systemic corticosteroids prior to the hospitalisation, previous hospitalisation for bronchiolitis, severe neonatal or other pre-existing chronic lung disease and prematurity <32 weeks of gestation. Among eligible subjects, 131 have previously participated in a longitudinal prospective follow-up study at 11 years of age.[7 23] Information regarding eligibility and data from the hospital stay for bronchiolitis were obtained retrospectively by review of medical records.

Control group

A control group not hospitalised for bronchiolitis, but matched on date of birth, sex and gestational age at birth was established by searching the hospital's birth protocols. The next-born eligible person to each individual index postbronchiolitis participant was invited. If the first invited person declined, the next was invited and so on until one control was recruited for each index or a maximum of ten invitations were sent.

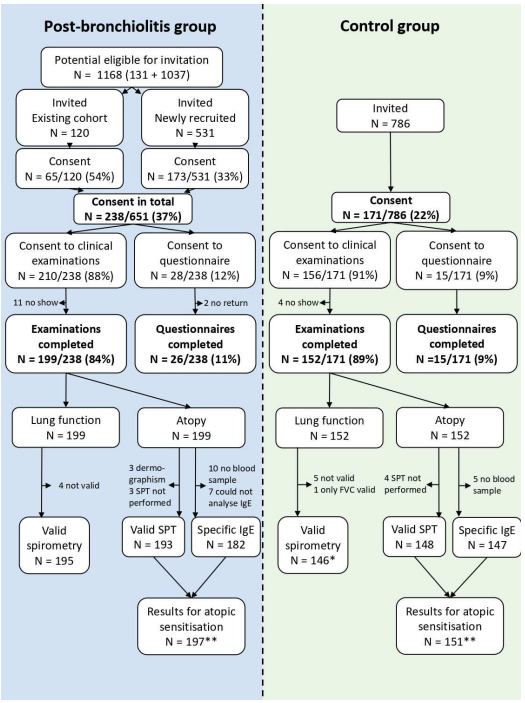

Figure 1 Overview of participants in the postbronchiolitis group and the control group. *N=147 for FVC. **Three subjects in the postbronchiolitis group and one control subject had positive allergen panels (Phadiatop and/or fx5E), but no tested specific IgEs>0.35 kU/L. These were defined as atopic subjects. FVC, forced vital capacity; SPT, skin prick test.

Exposures

Bronchiolitis was defined as an acute viral respiratory tract infection during the first year of life with fever, tachypnoea, dyspnoea, prolonged expiration and wheeze on auscultation.[1] During hospitalisation for bronchiolitis, nasopharyngeal mucus was examined for RSV by direct immunofluorescence (*BioMèrieux, Marcy-l'Ètoile, France*). Other viruses were not systematically tested for. Infants testing positive for RSV were defined as having RSV bronchiolitis and infants testing negative as having non-RSV bronchiolitis.

Outcomes

Asthma symptoms were recorded by a questionnaire based on the International Study of Asthma and Allergies in Childhood.[24] Asthma ever was defined as a positive answer to have you ever been diagnosed with asthma by a doctor? Current asthma was defined as asthma ever and a positive answer to at least one of the two questions: (1) Have you during the last 12 months had heavy breathing or wheezing/chest-tightness (2) Have you during the last 12 months used any asthma medications (inhaled

corticosteroids (ICS), long-acting or short-acting beta-2-agonists, montelukast, ipratropium bromide; any combination).

Atopy was defined as either a positive skin prick test defined as a weal diameter ≥3 mm larger than the negative control (Soluprick allergens (ALK Albello, Hørsholm, Denmark)),[25] and/or a positive allergen panel or specific immunoglobuline E (IgE) >0.35 kU/L for one of the following allergens: Dermatophagoides pteronyssinus, dog and cat dander, Cladosporium herbarium, birch, timothy, egg white, milk, peanut, hazelnut and codfish. Serum were analysed for ImmunoCAP hazelnut and the allergen panels Phadiatop and fx5E (Thermo Fisher Scientific, Phadia AB, Uppsala, Sweden). If positive panels, specific IgE was analysed.

Lung function was measured by spirometry according to established guidelines,[26] using V_{max} Encore 229D spirometer (Sensor Medics, Anaheim, USA). Variables recorded were forced vital capacity (FVC), forced expiratory volume in 1 s (FEV_1), FEV_1/FVC-ratio and forced expiratory flow between 25% and 75% of the FVC (FEF_{25-75}), all standardised for age, height and sex[27] and presented as z-score and percentage of predicted.

Clinical examinations were performed from April 2015 to March 2020.

Covariates/confounders

Prior to the analyses, factors possibly influencing both the exposure and outcomes were identified as potential confounders as illustrated in a directed acyclic graph (online supplemental figure 1).

Birth weight and gestational age at birth were collected retrospectively from birth protocols. Anthropometry was measured by study nurses or collected from questionnaires for those not participating in the clinical examinations. Use of asthma medication, personal and family history of atopy, and smoking were collected through questionnaires. Personal smoking was defined as a positive answer to do you smoke. Ever household smoking was defined as a positive answer to do/did anyone smoke in your home. Missing values were interpreted as negative answer. Atopic dermatitis was defined as a positive answer to have you ever had atopic dermatitis, and family history of atopy as a positive answer to do you know if your mother, father or siblings have or have had atopic dermatitis, asthma or positive allergy tests.

Statistical analysis

Continuous data were presented as mean with SD and compared by Student's t-test if normally distributed, or as median and IQR and compared by Mann-Whitney U-test if not normally distributed. Categorical data were presented as count and percentage, and compared by Pearson χ^2 test. Categorical outcomes were analysed by mixed effects logistic regression and presented as OR with 95% CI and predicted proportion with 95% CI. Continuous outcomes were analysed by mixed effects linear regression and presented as regression coefficient (β) with 95% CI and predictive margin with 95% CI. P values from Wald test are given for OR and β. Potential correlations between matched individuals were allowed for by including a random intercept term in the models. All effect estimates were adjusted for age and potential confounders. The impact of sex was assessed by including an interaction term between sex and group (ie, postbronchiolitis vs control), whereas the RSV group and non-RSV group were directly compared with each other.

To investigate possible confounding and mediating effects of various variables on the association between bronchiolitis and subsequent asthma, one by one of these variables were added to the model. Changes in OR ≥10% were considered clinically important.[28]

SPSS V.26.0 (IBM) and Stata V.16.1 (StataCorp) were used for analyses. Values of $p<0.05$ were considered statistically significant.

Power

Statistical power analyses were performed prior to study start using SPSS Sample Power V.3 (IBM) with power set to 80% and significance level to 0.05. To detect an absolute difference of 10% in the occurrence of asthma or atopy in the postbronchiolitis group compared with the control group, 199 subjects were needed in each group. We assumed this to be clinically relevant and reasonable considering the results from other studies.[16 29] To detect a clinically relevant absolute difference of 5% in FEV_1, 64 subjects needed to be included in each group.[18]

Ethics

Signed statements of informed consent were obtained from all participants and from parents if the participants were younger than 18 years of age.

RESULTS

Participants

A detailed overview of the inclusion process is given in figure 1. Of 651 invited participants to the postbronchiolitis group, 238 (37%) consented, 199 completed the clinical examinations, and 26 returned the questionnaire only. Of 786 invited control subjects, 171 (22%) consented, 152 completed the clinical examinations, and 15 returned the questionnaire only.

Background and clinical characteristics

Baseline characteristics of the postbronchiolitis group and control group are presented in table 1A. Except lower birth weight and more use of ICS at follow-up in the postbronchiolitis group, there were no baseline differences between the two groups.

Clinical characteristics during the hospitalisation for bronchiolitis are given in table 1B. Subjects in the non-RSV group were older at hospitalisation. The RSV group had longer length of hospital stay.

Table 1 Background and clinical characteristics in the postbronchiolitis group and the control group.

(A) Background and clinical characteristics of young adults hospitalised for bronchiolitis in infancy compared with control subjects.

	Postbronchiolitis		Control		P value*
	N		N		
Males, n (%)	225	117 (52.0)	167	82 (49.1)	0.57
Gestational age at birth <36 weeks, n (%)	204	4 (2.0)	164	1 (0.6)	0.266
Birth weight, grams, mean (SD)	197	3526 (619)	164	3661 (515)	**0.024**
At follow-up					
Age, years, median (quartiles)	225	19.4 (18.6, 20.3)	167	19.2 (18.6, 20.6)	0.241
BMI, kg/m^2, median (quartiles)	223	23.5 (21.0, 27.4)	166	22.7 (21.2, 25.5)	0.076
Height, cm, median (quartiles)	224	172.5 (167.0, 181.8)	166	175.0 (167.6, 182.0)	0.218
Weight, kg, median (quartiles)	224	70.0 (63.1, 83.4)	166	71.0 (63.0, 80.1)	0.651
Use of inhaled corticosteroids the last 12 months, n (%)	223	25 (11.2)	166	9 (5.4)	**0.046**
Personal smoking, n (%)	225	20 (8.9)	167	8 (4.8)	0.119
Household smoking ever, n (%)	225	74 (32.9)	167	41 (24.6)	0.073
Atopic dermatitis, n (%)	215	50 (23.3)	164	35 (21.3)	0.658
Family history of atopy, n (%)	225	155 (68.9)	167	107 (64.1)	0.316

(B) Clinical variables at hospitalisation for bronchiolitis for all postbronchiolitis subjects and for the RSV group compared with the non-RSV group.

	All postbronchiolitis		RSV		Non-RSV		P value†
	N		N		N		
Age at hospitalisation, months, median (quartiles)	225	4.2 (2.3, 6.8)	128	3.8 (2.0, 5.8)	64	4.5 (2.4, 7.8)	**0.041**
Weight at hospitalisation, grams, mean (SD)	197	6911 (1905)	112	6539 (1823)	54	7107 (1818)	0.062
Previous history of BPO, n (%)	225	32 (14.2)	128	13 (10.2)	64	12 (18.8)	0.095
Length of hospital stay, days, median (quartiles)	225	3.0 (1.0, 4.0)	128	3.0 (2.0, 5.5)	64	2.0 (1.0, 3.0)	**0.001**
Corticosteroids (inhaled/systemically) given during admission, n (%)	225	15 (6.7)	128	6 (4.7)	64	6 (9.4)	0.206

P values are from Student's t-test for normally distributed variables given as means (SDs), Mann-Whitney U test for continuous variables not normally distributed given as medians (quartiles) and Pearson χ^2 test for dichotomous variables. Bold values denote statistical significance at the p<0.05 level.
*P values comparing the postbronchiolitis group and the control group.
†P values comparing the RSV group and the non-RSV group.
BMI, body mass index; BPO, broncopulmonal obstruction; n, number of participants with the characteristic described; N, number of participants with available data; RSV, respiratory syncytial virus.

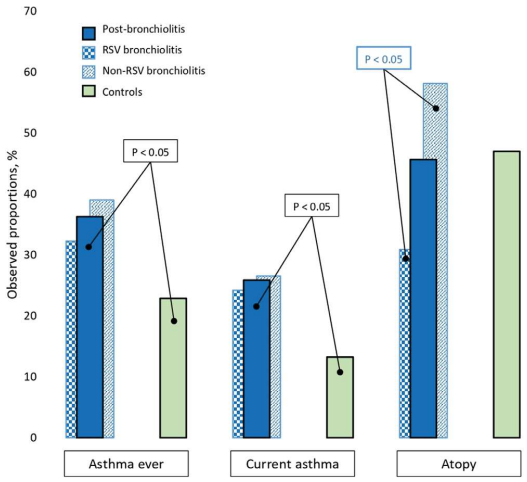

Figure 2 Asthma and atopy in the postbronchiolitis group and the control group including separate columns for RSV bronchiolitis and non-RSV bronchiolitis. Figures are observed proportions. Differences are tested by Pearson χ^2 test and p<0.05 are marked. RSV, respiratory syncytial virus.

Asthma

Directly observed proportions of asthma and atopy are presented in figure 2, whereas table 2A,B presents unadjusted and adjusted results from regression analyses.

At follow-up, the postbronchiolitis group had higher prevalence of both asthma ever and current asthma compared with the control group (figure 2, table 2A). There was no significant interaction between sex and group (ie, postbronchiolitis vs control) regarding asthma (table 2A), and the prevalence of asthma did not differ between the RSV group and the non-RSV group (figure 2, table 2B).

When adding age and potential confounders (sex, family history of atopy, atopic dermatitis, household smoking, birth weight, gestational age at birth) and mediators (atopy, body mass index, personal smoking) one by one to a regression analysis studying the association between group (ie, postbronchiolitis vs control) and current asthma, none of the variables individually changed the OR more than 10% (data not shown).

Atopy

In the postbronchiolitis group, 90 (45.7%) subjects were atopic. Of these 55 (61.1%) were sensitised to two or more allergens, 51 (56.7%) were sensitised to airborne allergens only, 5 (5.6%) to food allergens only, and 34 (37.8%) were sensitised to both airborne and food allergens. In the control group 71 (47.0%) subjects were atopic. Of these 44 (62.0%) were sensitised to two or more allergens, 42 (59.2%) were sensitised to airborne allergens only, 2 (2.8%) to food allergens only, and 27 (38.0%) were sensitised to both.

There was no difference in the prevalence of atopy between the postbronchiolitis and control group (figure 2, table 2A). We found no significant interaction between sex and group (ie, postbronchiolitis vs control) regarding atopy (table 2A). The RSV group had lower prevalence of atopy compared with the non-RSV group (figure 2, table 2B).

Among subjects with asthma ever, a lower proportion in the postbronchiolitis group than in the control group were atopic (46% vs 70%; p=0.027). The same tendency was seen for current asthma (50% vs 74%; p=0.076).

Lung function

Lung function is presented as z-scores in table 3A,B with the corresponding % predicted in the online supplemental table 1 A,B. Participants in the postbronchiolitis group had a more obstructive lung function pattern with lower FEV_1, FEV_1/FVC ratio and FEF_{25-75} compared with control subjects.

We found a significant interaction between sex and group (ie, postbronchiolitis vs control) for FVC (β −0.42; 95% CI −0.82 to −0.02; p=0.039), but not for other lung function variables (table 3A). Analyses for FVC stratified by sex showed lower FVC in the postbronchiolitis group compared with control subjects in males (β −0.32; 95% CI −0.58 to −0.06, p=0.017), but no difference between the two groups in females (β 0.15; 95% CI −0.14 to 0.44; p=0.313).

The non-RSV group had lower FEV_1/FVC-ratio compared with the RSV group, otherwise lung function did not differ between these two groups (table 3B, online supplemental table 1B).

DISCUSSION

This is to date the largest cohort study of respiratory outcomes in young adults after hospitalisation for bronchiolitis during infancy, also including a large control group. We found a higher prevalence of asthma in the postbronchiolitis group, with no difference between the RSV group and the non-RSV group nor between sexes. We found no difference in atopy between the postbronchiolitis group and the control group, but the prevalence of atopy was lower in subjects with former RSV bronchiolitis compared with subjects with former non-RSV bronchiolitis. A lower proportion of children with asthma were atopic in the postbronchiolitis group than in the control group. The postbronchiolitis group had a more obstructive lung function pattern than the control group.

Strengths and limitations

The main strengths of this study were the high number of participants with clinical data on lung function and atopy, as well as inclusion of children hospitalised for both RSV bronchiolitis and non-RSV bronchiolitis. Only

Table 2 Asthma and atopy at follow-up in the postbronchiolitis group and the control group with separate results for RSV bronchiolitis and non-RSV bronchiolitis.

(A) Asthma and atopy at 19 years in the postbronchiolitis group compared with the control group						
	Effect estimate			Predicted proportion, % (95% CI)		Interaction sex*group§
	N	OR (95% CI)	P value*	Postbronchiolitis	Control	P value*
Asthma ever						
Asthma ever†	389	1.92 (1.22 to 3.02)	**0.005**	36.3 (30.0 to 42.6)	22.9 (16.5 to 29.3)	
Asthma ever‡	346	1.89 (1.12 to 3.21)	**0.017**	34.8 (28.0 to 41.5)	22.7 (16.2 to 29.1)	0.925
Current asthma						
Current asthma†	390	2.61 (1.41 to 4.82)	**0.002**	25.8 (20.1 to 31.5)	13.3 (8.1 to 18.4)	
Current asthma‡	347	2.75 (1.32 to 5.73)	**0.007**	25.1 (19.0 to 31.2)	13.1 (7.9 to 18.2)	0.972
Atopy						
Atopy†	348	0.95 (0.62 to 1.45)	0.805	45.7 (38.7 to 52.6)	47.0 (39.1 to 55.0)	
Atopy‡	310	0.84 (0.52 to 1.36)	0.472	44.3 (37.1 to 51.5)	48.2 (40.5 to 55.8)	0.776
(B) Asthma and atopy at 19 years after RSV bronchiolitis compared with non-RSV bronchiolitis.						
	Effect estimate			Predicted proportion to % (95% CI)		
	N	OR (95% CI)	P value*	RSV	Non-RSV	
Asthma ever						
Asthma ever†	191	0.74 (0.40 to 1.39)	0.353	32.3 (24.2 to 40.4)	39.1 (27.1 to 51.0)	
Asthma ever‡	162	0.86 (0.42 to 1.77)	0.685	32.8 (24.0 to 41.7)	36.0 (23.5 to 48.5)	
Current asthma						
Current asthma†	192	0.88 (0.44 to 1.76)	0.724	24.2 (16.8 to 31.6)	26.6 (15.7 to 37.4)	
Current asthma‡	163	1.02 (0.45 to 2.29)	0.971	24.0 (16.1 to 32.0)	23.8 (12.8 to 34.7)	
Atopy						
Atopy†	165	0.32 (0.16 to 0.63)	**0.001**	30.9 (22.3 to 39.5)	58.2 (45.1 to 71.2)	
Atopy‡	139	0.35 (0.16 to 0.75)	**0.007**	31.4 (22.2 to 40.7)	53.8 (40.6, 67.0)	

Results from mixed effects logistic regression analyses including calculations of predicted proportions.
The predicted proportions are products of the regression analyses and correspond to the expected proportions of the outcomes if everyone had a previous history of bronchiolitis (A) or RSV bronchioltis (B), or if everyone had no history of bronchiolitis (A) or a history of non-RSV bronchiolitis (B), with all other covariates kept at their original value.
*P values for OR from Wald test. Bold values denote statistical significance at the p<0.05 level.
† Unadjusted model.
‡Adjusted for sex, age, birth weight, gestational age at birth, household smoking ever, atopic dermatitis, family history of atopy.
§Interaction term between sex and group (ie, postbronchiolitis vs control).
RSV, respiratory syncytial virus.

children hospitalised during their first year of life were included, ensuring a homogeneous study population.[4] The main weaknesses were the modest participation rate potentially increasing the risk of selection bias, and lack of specific viral aetiologies in the non-RSV group. Nevertheless, the study population was drawn from all children hospitalised for bronchiolitis in the two participating hospitals during the inclusion period, and we, therefore, hold that the results are generalisable for children hospitalised for bronchiolitis under 1 year of age in comparable high-income countries.

Interpretation

Asthma

The higher prevalence of asthma in the postbronchiolitis group compared with the control group is in line with previous research.[6 16–18 30 31] In an earlier publication including a subgroup from this study, 21% in the postbronchiolitis group had current asthma at 11 years of age,[7] a figure also in line with this study. This underlines that bronchiolitis in infancy is associated with long-term respiratory morbidity not only during childhood, but also persisting into young adult age.

Surprisingly, the prevalence of asthma in young adults did not differ between the RSV group and the non-RSV group, but were high in both groups. These results are in line with a Swedish postbronchiolitis study reporting asthma prevalence at 17–20 years of age of 48% after RSV bronchiolitis and 41% after non-RSV bronchiolitis (p=0.53),[17] but differ from two Finnish studies which found a tendency for higher prevalence in adults after non-RSV bronchiolitis compared with RSV bronchiolitis.[30 32] Most follow-up studies reporting outcomes during childhood find a higher risk of subsequent asthma after bronchiolitis with HRV or other non-RSV viruses compared with RSV bronchiolitis.[7 9 10 30] In our previous publication from the 11-year follow-up, 36% of children with former non-RSV bronchiolitis vs 16% with former RSV bronchiolitis reported current asthma.[7]

Table 3 Lung function at follow-up in the postbronchiolitis group and the control group with separate results for RSV bronchiolitis and non-RSV bronchiolitis.

(A) Lung function at 19 years in the postbronchiolitis group compared with the control group.						
	Effect estimate			Predictive margin (95% CI)		Interaction sex*group§
	N	β (95% CI)	P value*	Postbronchiolitis	Control	P value*
FVC						
z-score†	342	−0.10 (−0.30 to 0.09)	0.313	0.02 (−0.12 to 0.15)	0.12 (−0.04 to 0.27)	
z-score‡	306	−0.07 (−0.27 to 0.14)	0.532	0.03 (−0.12 to 0.17)	0.09 (−0.07 to 0.25)	**0.039**
FEV1						
z-score†	341	−0.31 (−0.52 to −0.10)	**0.004**	−0.38 (−0.52 to −0.24)	−0.07 (−0.23 to 0.10)	
z-score‡	305	−0.32 (−0.54 to −0.09)	**0.005**	−0.39 (−0.55 to −0.24)	−0.08 (−0.24 to 0.09)	0.174
FEV1/FVC						
z-score†	341	−0.33 (−0.55 to −0.12)	**0.002**	−0.67 (−0.81 to −0.53)	−0.33 (−0.49 to −0.17)	
z-score‡	305	−0.40 (−0.62 to −0.18)	**<0.001**	−0.71 (−0.86 to −0.56)	−0.31 (−0.47 to −0.15)	0.32
FEF_{25-75}						
z-score†	341	−0.37 (−0.57 to −0.16)	**0.001**	−0.70 (−0.83 to −0.56)	−0.33 (−0.48 to −0.17)	
z-score‡	305	−0.40 (−0.62 to −0.19)	**<0.001**	−0.73 (−0.88 to −0.58)	−0.33 (−0.49 to −0.17)	0.78
(B) Lung function at 19 years after RSV bronchiolitis compared with non-RSV bronchiolitis.						
	Effect estimate			Predictive margin (95% CI)		
	N	β (95% CI)	P–value*	RSV	Non–RSV	
FVC						
z-score†	164	−0.18 (−0.49 to 0.13)	0.256	−0.08 (−0.25 to 0.10)	0.10 (−0.15 to 0.36)	
z-score‡	139	−0.27 (−0.61 to 0.07)	0.12	−0.12 (−0.32 to 0.07)	0.14 (−0.13 to 0.42)	
FEV1						
z-score†	164	−0.02 (−0.36 to 0.32)	0.912	−0.44 (−0.63 to −0.25)	−0.42 (−0.70 to −0.14)	
z-score‡	139	−0.03 (−0.40 to 0.35)	0.888	−0.47 (−0.69 to −0.25)	−0.44 (−0.75 to −0.14)	
FEV1/FVC						
z-score†	164	0.26 (−0.05 to 0.57)	0.103	−0.62 (−0.80 to −0.44)	−0.88 (−1.13 to −0.62)	
z-score‡	139	0.38 (0.04 to 0.72)	**0.026**	−0.59 (−0.79 to −0.39)	−0.97 (−1.24 to −0.70)	
FEF_{25-75}						
z-score†	164	0.17 (−0.16 to 0.50)	0.311	−0.68 (−0.87 to −0.49)	−0.85 (−1.12 to −0.58)	
z-score‡	139	0.24 (−0.11 to 0.59)	0.18	−0.68 (−0.89 to −0.48)	−0.92 (−1.21 to −0.64)	

Results from mixed effects linear regression analyses including calculations of predictive margins.
The predictive margins are products of the regression analyses and correspond to the predicted mean values of the outcome if everyone had a previous history of bronchiolitis (A) or RSV bronchioltis (B), or if everyone had no history of bronchiolitis (A) or a history of non-RSV bronchiolitis (B), with all other covariates kept at their original value.
*P values for β from Wald test. Bold values denote statistical significance at the p<0.05 level.
†Unadjusted model.
‡Adjusted for birth weight, gestational age at birth, household smoking ever, atopic dermatitis, family history of atopy.
§Interaction term between sex and group (ie, postbronchiolitis vs control).
FEF_{25-75}, forced expiratory flow between 25% and 75% of the forced vital capacity; FEV_1, forced expiratory volume in 1 s; FVC, forced vital capacity; RSV, respiratory syncytial virus.

The Tucson Children's Respiratory Study reported increased risk of asthma during the first 10 years of life after RSV infection before 3 years of age, but the increased risk rapidly subsided by age and was not present after the age of 13 years.[8] A similar decrease in asthma prevalence by age after hospitalisation for RSV infection was reported in a meta-analysis.[33] However, in this study of young adults, we found no difference between the RSV-group and the non-RSV-group, the prevalence of asthma was high also after RSV bronchiolitis. Our results are in line with the follow-up study at 18 years of age by Sigurs *et al*[16] and some other postbronchiolitis studies suggesting a U-shaped prevalence curve for asthma after RSV bronchiolitis from early childhood to young adult age.[6]

We found no interaction between sex and group (ie, postbronchiolitis vs control) in the adjusted models, meaning that the impact of having bronchiolitis on subsequent asthma did not differ between sexes. Thus, in contrast to the results from a Swedish study reporting increased risk of asthma in female young adults with former wheezing bronchitis under the age of 2 years,[17] we observed no switch to a higher prevalence of asthma in females during adolescence.

Differences in asthma prevalence between studies could partly be explained by variations in age criteria at inclusion and the definitions of asthma. We included only infants hospitalised for bronchiolitis during their first year of life, whereas others used 2 or 3 years of age as cut-off. Increased age limit for inclusion increases the heterogeneity of the study population by including more participants in whom the bronchiolitis may represent a first episode of asthma. Definitions of asthma used in epidemiological studies are highly inconsistent and make comparisons between studies challenging.[34] Prevalence of asthma must, therefore, be considered in relation to the prevalence in the corresponding control group, which in this study was high, but in line with studies of the general population.[35]

The prevalence of use of ICS was lower than one would expect based on the corresponding prevalence of asthma in both groups. This may indicate suboptimal adherence to recommended treatment,[36] a high number of mild asthma cases, or even other aetiologies with symptoms mimicking asthma.

Atopy

We found no difference in atopy between the postbronchiolitis group and control group, but atopy was less frequent in the RSV group compared with the non-RSV group. This is in line with other similar studies including our previous follow-up at 11 years of age,[7 8 37 38] but contrasting the study by Sigurs *et al*.[16] The prevalence of atopy was high in both groups, but a similar prevalence of 49% was found among 16 years in a prospective population-based birth cohort from Oslo, Norway.[29] In subjects with asthma ever, atopy was less common in the postbronchiolitis group, with the same tendency among those with current asthma. This finding corroborates that non-eosinophilic asthma is common after bronchiolitis, a notion formerly reported particularly after RSV bronchiolitis.[11] Our study did not have sufficient power to evaluate if viral aetiology during bronchiolitis is associated with different phenotypes of asthma in young adults.

Lung function

Consistent with previous research,[18] the postbronchiolitis group had a more obstructive lung function pattern with lower FEV_1, FEV_1/FVC and FEF_{25-75}. Although mean FEV_1 was within a clinically normal range, it is important to emphasise that even mild to moderate impairment of FEV_1 may be a predictor of later cardiorespiratory morbidity and mortality.[39] Lung function was not measured before the episode with bronchiolitis, and we can, therefore, not exclude that genetically determined small airways have contributed to these findings.

In line with previous research in younger children, young adults with former non-RSV bronchiolitis had lower FEV_1/FVC and hence a more obstructive lung function pattern compared with those with former RSV bronchiolitis.[40] This may indicate that different viruses during bronchiolitis in infancy affect lung function in different ways later in life.

We found a significant interaction between sex and group (ie, postbronchiolitis vs control) for FVC indicating that the impact on lung function of having a history of hospitalisation for bronchiolitis is more pronounced in males than in females. Lung development differs between sexes, and boys are in general more vulnerable for respiratory events during childhood, partly due to differences in anatomy and physiology such as airway size, airway muscle bulk, airway reactivity, airway tone and cough reflexes.[41] In analyses stratified by sex, we found decreased FVC in males after bronchiolitis, a result that differs from a study from Sweden reporting only mid-expiratory flow rate lower than control subjects after bronchiolitis in males.[42]

CONCLUSION

Young adults hospitalised for bronchiolitis had higher prevalence of asthma, but not atopy, and a more obstructive lung function pattern than control subjects. The asthma prevalence was high after both RSV bronchiolitis and non-RSV bronchiolitis, and there was no difference between sexes. The study confirms that bronchiolitis in infancy is associated with impaired respiratory health persisting into young adulthood. Further follow-up studies in adult age are needed to explore the potential for subsequent respiratory morbidity including early-onset COPD after this prevalent childhood disorder.

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Contributors KGS and IBM had full access to all of the data in the study and takes responsibility for the overall content as guarantors for the integrity of the data and the accuracy of the data analysis, including and especially any adverse effects. KGS, KØ, TH and IBM contributed substantially to the study design. Biostatistician ID supervised the statistical analyses, and all authors contributed substantially to the data analysis and interpretation, and the writing of the manuscript.

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Supplemental Table 1a. Lung function in percent of predicted at 19 years in the post-bronchiolitis group compared to the control group. Results from mixed effects linear regression analyses including calculations of predictive margins.

			Effect estimate		Predictive margin (95% CI)	
		N	β (95% CI)	P-value*	Post-bronchiolitis	Control
FVC	% of predicted [a]	342	-0.01 (-0.03, 0.01)	0.350	100.4 (98.7, 102.0)	101.5 (99.6, 103.3)
	% of predicted [b]	306	-0.01 (-0.03, 0.02)	0.586	100.5 (98.7, 102.2)	101.1 (99.3, 103.0)
FEV_1	% of predicted [a]	341	-0.04 (-0.06, -0.01)	**0.004**	95.5 (93.9, 97.1)	99.2 (97.3, 101.0)
	% of predicted [b]	305	-0.04 (-0.06, -0.01)	**0.005**	95.3 (93.5, 97.1)	99.0 (97.1, 100.9)
FEV_1/FVC	% of predicted [a]	341	-0.03 (-0.04, -0.01)	**0.002**	94.4 (93.4, 95.5)	97.0 (95.8, 98.3)
	% of predicted [b]	305	-0.03 (-0.05, -0.01)	**<0.001**	94.1 (92.9, 95.2)	97.2 (95.9, 98.4)
FEF_{25-75}	% of predicted [a]	341	-0.08 (-0.12, -0.03)	**0.001**	86.0 (83.2, 88.9)	93.6 (90.3, 96.9)
	% of predicted [b]	305	-0.08 (-0.13, -0.04)	**<0.001**	85.3 (82.2, 88.3)	93.6 (90.3, 96.9)

Abbreviations: CI = confidence interval; β = regression coefficient comparing the post-bronchiolitis group to the control group; FVC = forced vital capacity; FEV_1 = forced expiratory volume in first second; FEF_{25-75}: forced expiratory flow between 25-75% of the forced vital capacity.

The predictive margins are products of the regression analyses and correspond to the predicted mean values of the outcomes if everyone had a previous history of bronchiolitis, or if everyone had no history of bronchiolitis, with all other covariates kept at their original value.

* P-values for β from Wald test. Bold values denote statistical significance at the $P < 0.05$ level.

[a] Unadjusted model.

[b] Adjusted for birthweight, gestational age at birth, household smoking ever, atopic dermatitis, family history of atopy.

Supplemental Table 1b. Lung function in percent of predicted at 19 years after RSV bronchiolitis compared to non-RSV bronchiolitis. Results from mixed effects linear regression analyses including calculations of predictive margins.

			Effect estimate		Predictive margin (95% CI)	
		N	β (95% CI)	P-value*	RSV	Non-RSV
FVC	% of predicted [a]	164	-0.02 (-0.06, 0.02)	0.282	99.3 (97.1, 101.4)	101.3 (98.3, 104.4)
	% of predicted [b]	139	-0.03 (-0.07, 0.01)	0.134	98.7 (96.3, 101.1)	101.8 (98.5, 105.1)
FEV_1	% of predicted [a]	164	-0.00 (-0.04, 0.04)	0.923	94.8 (92.5, 97.1)	95.0 (91.8, 98.2)
	% of predicted [b]	139	-0.00 (-0.05, 0.04)	0.898	94.4 (91.9, 97.0)	94.7 (91.2, 98.3)
FEV_1/FVC	% of predicted [a]	164	0.02 (-0.01, 0.04)	0.131	94.8 (93.4, 96.3)	92.9 (90.8, 94.9)
	% of predicted [b]	139	0.03 (0.00, 0.06)	**0.038**	95.0 (93.4, 96.6)	92.1 (90.0, 94.3)
FEF_{25-75}	% of predicted [a]	164	0.03 (-0.03, 0.10)	0.317	86.3 (82.4, 90.2)	82.9 (77.3, 88.4)
	% of predicted [b]	139	0.05 (-0.02, 0.12)	0.179	86.3 (82.0, 90.5)	81.3 (75.4, 87.1)

Abbreviations: RSV = respiratory syncytial virus; CI = confidence interval; β = regression coefficient comparing the RSV group to the non-RSV group; FVC = forced vital capacity; FEV_1 = forced expiratory volume in first second; FEF_{25-75} = forced expiratory flow between 25-75% of the forced vital capacity;.

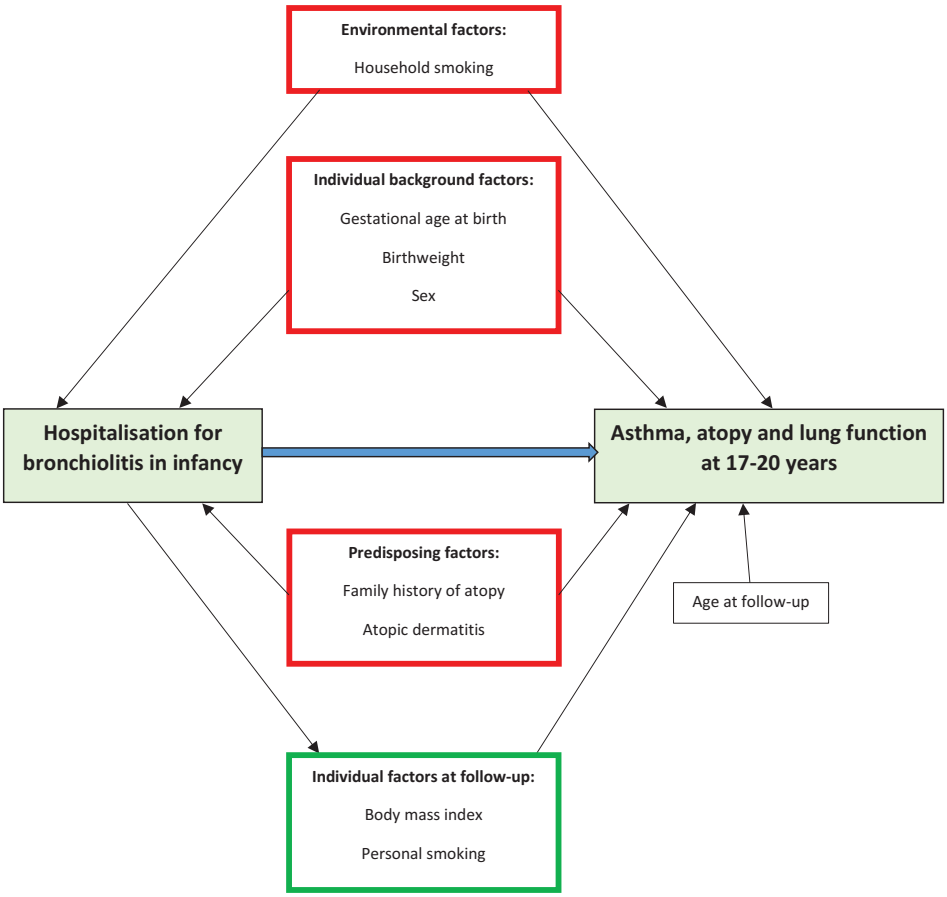
The predictive margins are products of the regression analyses and correspond to the predicted mean values of the outcomes if everyone had a previous history of RSV bronchiolitis, or if everyone had a history of non-RSV bronchiolitis, with all other covariates kept at their original value.

* P-values for β from Wald test. Bold values denote statistical significance at the $P < 0.05$ level.

[a] Unadjusted model.

[b] Adjusted for birthweight, gestational age at birth, household smoking ever, atopic dermatitis, family history of atopy.

Supplemental figure 1. Simplified directed acyclic graph with confounders in red boxes and mediators in green boxes.



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ORIGINAL ARTICLE

WILEY

Lung function and bronchial hyper-reactivity from 11 to 18 years in children with bronchiolitis in infancy

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Abstract

Background: Various trajectories for lung function and bronchial hyper-reactivity (BHR) from early childhood to adulthood are described, including puberty as a period with excessive lung growth. Bronchiolitis in infancy may be associated with increased risk of developing chronic obstructive pulmonary disease, but the development of respiratory patterns during puberty is poorly characterized for these children. We aimed to study the development and trajectories of lung function and BHR from 11 to 18 years of age in children hospitalized for bronchiolitis in infancy.

Methods: Infants hospitalized for bronchiolitis at the University Hospitals in Stavanger and Bergen, Norway, during 1997-1998, and an age-matched control group, were included in a longitudinal follow-up study and examined at 11 and 18 years of age with spirometry and methacholine provocation test (MPT). The MPT data were managed as dose-response slope (DRS) in the statistical analyses. Changes in lung function and DRS from 11 to 18 years of age were analyzed by generalized estimating equations, including interaction terms.

Results: z-scores for forced vital capacity (FVC), forced expiratory volume in first second (FEV_1), FEV_1/FVC ratio, and DRS were not different from 11 to 18 years of age in both the post-bronchiolitis and the control group. The trajectories from 11 to 18 years did not differ between the two groups. BHR at age 11 was independently associated with asthma at age 18.

Conclusion: Children hospitalized for bronchiolitis had stable predicted lung function and BHR from 11 to 18 years of age. The lung function trajectories were not different from controls.

KEYWORDS
adolescent, asthma, bronchial hyper-reactivity, bronchial provocation tests, bronchiolitis, child, methacholine chloride, puberty, spirometry

1 | INTRODUCTION

Longitudinal cohort studies have shown that the lung function trajectories throughout a life course vary between individuals and that abnormal lung function trajectories may originate in early life.[1-3] This has been shown both in unselected populations[2,4] and after infant respiratory disease such as bronchopulmonary dysplasia associated with extreme prematurity.[5] It has therefore been hypothesized that chronic obstructive pulmonary disease (COPD) may begin in childhood, conceivably precipitated by interactions between genetic predispositions, disadvantageous intrauterine environments, or early respiratory insults.[6]

Worldwide, bronchiolitis represents a substantial health burden for infants, and it is the most common cause for hospitalization during infancy in developed countries.[7] These children have increased risk of developing asthma, low lung function, and increased bronchial hyper-reactivity (BHR) both during childhood[8-11] and adulthood,[12-14] and possibly increased risk of developing COPD.[6] However, we do not know the nature of this association, that is, if it is the bronchiolitis per se that alters the pattern of lung development or if both disorders are caused by inherent predispositions or vulnerabilities of genetic or antenatal origin. Puberty is the period of life with the most excessive lung growth,[1,15] but we do not know if bronchiolitis in infancy modulates the development of airway size and hyper-reactivity during the pubertal growth spurt.

We have previously reported lung function data in a cohort of 11-year-old children hospitalized for bronchiolitis in 1997-1998.[10] The present study is based on examinations of the same subjects at age 18 years. We aimed to study if lung function and BHR changed from 11 to 18 years of age in children hospitalized for bronchiolitis in infancy, and whether lung function trajectories during this period was different from an age-matched control group.

Key Message

Children hospitalized for bronchiolitis in infancy had stable predicted lung function and bronchial hyper-reactivity (BHR) from 11 to 18 years of age. Lung function trajectories were significantly lower, but parallel to that of an age-matched control group, suggesting that children with former bronchiolitis follow a lung function trajectory below normal peak values during puberty, but that the development between 11 and 18 years of age is parallel to healthy controls with no catch up nor decline. BHR at age 11 was associated with asthma at age 18. Children with severe bronchiolitis in infancy could benefit from regular clinical follow-ups to monitor lung function and development of asthma.

2 | METHODS

Originally, 131 children hospitalized for bronchiolitis during their first year of life during the winter seasons 1997 and 1998 at the University Hospitals in Stavanger and Bergen, Norway, were included in a longitudinal prospective follow-up study.[16] Bronchiolitis was defined as an acute viral respiratory tract infection during the first year of life with fever, tachypnea, dyspnea, prolonged expiration, and wheeze on auscultation.[17] In order to avoid including children with other conditions such as viral-induced wheezing and asthma, only children below

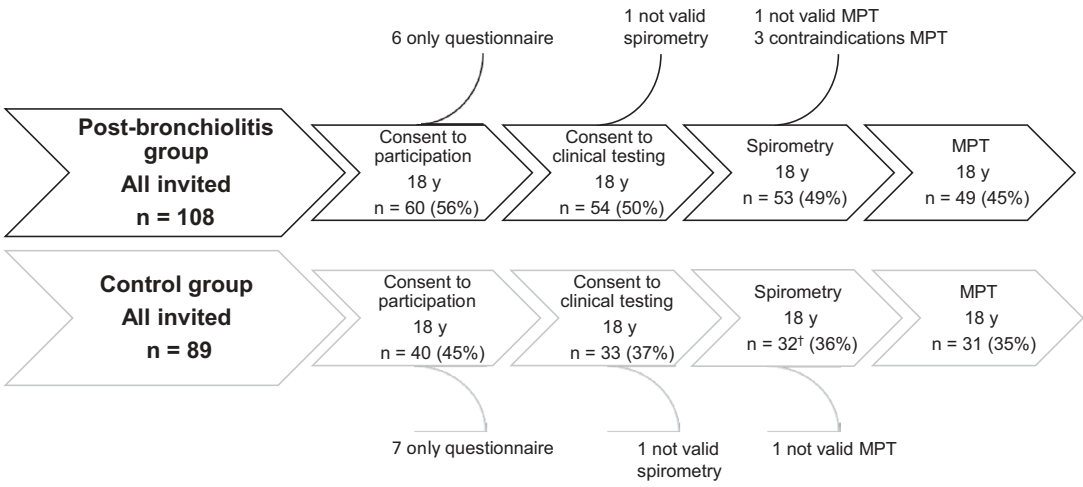

FIGURE 1 Flowchart of the study population, consisting of children hospitalized for bronchiolitis in infancy and an age-matched control group. The invited children participated in the 11-y examination. 18 y, Second follow-up at median 18 years of age; MPT, Methacholine provocation test. †For one participant, only forced vital capacity (FVC) was considered valid, making n = 31 in calculations of other lung function variables

12 months of age were included.[7] At hospitalization, nasopharyngeal mucus was examined for respiratory syncytial virus (RSV) by direct immunofluorescence (bioMèrieux, Marcy-l'Ètoile, France). Children testing positive for RSV were defined as RSV-positive, the others were defined as RSV-negative.

One-hundred and twenty-one children (92%) participated in a first follow-up at 11 years of age, together with an age-matched control group of 141 children. The control group included children born in 1997 with no previous history of hospitalization for bronchiolitis recruited from three different schools in Stavanger, Norway. The 11-year examination included questionnaires and tests for atopic sensitization, lung function, and BHR, as previously reported.[10]

All participants from the original study were invited to a second follow-up at approximately 18 years of age, including questionnaires and clinical tests of lung function, BHR, and atopic sensitization. This study presents results from the children participating at both follow-ups. A total of 108 children from the post-bronchiolitis group and 89 children from the age-matched control group at the 11-year examination were invited to participate in this substudy (Figure 1).

2.1 | Lung function, BHR, and atopic sensitization

At both follow-ups, lung function was measured by spirometry according to established guidelines,[18] using Vmax Encore 229D spirometer (SensorMedics Inc), with data standardized for age, height, and sex.[19] Results were presented as z-scores and % predicted.

Bronchial hyper-reactivity was measured by methacholine provocation test (MPT) performed with an inhalation-synchronized dosimetric nebulizer, providing baseline FEV_1 > 65% predicted.[20,21] The test continued until a fall in FEV_1 of >20% compared with baseline FEV_1, or until a maximal cumulative dose of 11.5 µmol methacholine had been administered. A dose–response slope (DRS) was calculated as the ratio of maximal percentage decline in FEV_1 from baseline to cumulative administered dose of methacholine (%/µmol).[22]

Atopic sensitization was assessed by using skin prick tests according to guidelines[23] and by measuring specific immunoglobulin E (IgE). For details, see Appendix 1.

TABLE 1 Clinical characteristics at 11 and 18 years in children hospitalized for bronchiolitis in infancy and an age-matched control group

	Post-bronchiolitis group N = 60	Control group N = 40	*P*-value*
Boys (%)	29 (48.3)	21 (52.5)	.683
Age at hospitalization, mo	4.0 (1.8, 6.2)		
RSV-positive bronchiolitis, n (%)	50 (83.3)		
Personal smoking, n (%)	4 (6.7)	1 (2.5)	.349
Ever household smoking, n (%)	21 (35.0)	8 (20.0)	.105
Family history of atopy, n (%)	45 (75.0)	22 (55.0)	**.037**
First follow-up at 11 y			
Age, y	11.3 (11.0, 11.7)	11.8 (11.3, 12.1)	**.001**
Height, cm	148.5 (144.0, 153.8)	149.0 (146.0, 155.8)	.459
Weight, kg	38.3 (35.0, 45.4)	40.0 (35.3, 44.9)	.625
Current asthma, n (%)	9 (15.0)	5 (12.5)	.724
Allergic sensitization, n (%)	12 (20.0)	17 (42.5)	**.015**
Second follow-up at 18 y			
Age, y	18.0 (17.0, 18.0)	18.0 (17.0, 18.0)	.100
Height, cm	172.4 (164.4, 180.9)	173.8 (166.5, 181.2)	.635
Weight, kg	65.9 (59.9, 75.9)	63.2 (57.8, 72.4)	.233
Current asthma, n (%)	18 (30.0)	9 (22.5)	.408
Asthma ever, n (%)	23 (38.3)	10 (25.0)	.249
Allergic sensitization, n (%)†	13 (24.1)	20 (60.6)	**.001**
Allergic rhinoconjunctivitis, n (%)	28 (46.7)	24 (60.0)	.191
Atopic dermatitis ever, n %	15 (25.0)	14 (35.0)	.280

Note: [a]Bold values denote statistical significance at the $P < 0.05$ level.
Data are presented as medians (interquartile ranges) unless otherwise stated.
Abbreviations: RSV, Respiratory syncytial virus.
**P*-values from Mann–Whitney *U* test for continuous variables and Pearson's chi-square test for dichotomous variables.
†54 subjects in the post-bronchiolitis group and 33 controls underwent allergy tests at the second follow-up at 18 y.

TABLE 2 Lung function and bronchial hyper-reactivity at 11 and 18 years in children hospitalized for bronchiolitis in infancy and an age-matched control group

		Post-bronchiolitis group		Control group		
		N	Observed mean (95% CI)	N	Observed mean (95% CI)	*P*-value*
FEV_1						
z-score	11 y	60	−0.44 (−0.67, −0.21)	40	0.20 (−0.20, 0.61)	.004
% of predicted			94.9 (92.3, 97.5)		102.2 (97.5, 107.0)	.004
z-score	18 y	53	−0.57 (−0.83, −0.30)	31	0.15 (−0.28, 0.57)	.003
% of predicted			93.3 (90.1, 96.5)		101.6 (96.7, 106.6)	.003
FVC						
z-score	11 y	60	0.15 (−0.13, 0.43)	40	0.41 (−0.02, 0.83)	.302
% of predicted			102.0 (98.7, 105.3)		105.1 (99.9, 110.2)	.293
z-score	18 y	53	−0.13 (−0.41, 0.15)	32	0.23 (−0.12, 0.59)	.107
% of predicted			98.5 (95.2. 101.8)		102.8 (98.6. 107.0)	.112
FEV_1/FVC						
z-score	11 y	60	−0.86 (−1.17, −0.54)	40	-0.36 (−0.69, −0.03)	.037
% of predicted			93.1 (90.7, 95.5)		97.1 (94.8, 99.4)	.023
z-score	18 y	53	−0.66 (−0.98, −0.34)	31	-0.20 (−0.53, 0.13)	.058
% of predicted			94.3 (91.8, 96.8)		98.2 (95.8, 100.6)	.039
FEF 25-75						
z-score	11 y	60	−1.00 (−1.28, −0.72)	40	-0.38 (−0.69, −0.06)	.004
% of predicted			79.8 (74.2, 85.5)		92.1 (85.0, 99.2)	.007
z-score	18 y	53	−0.75 (−1.03, −0.48)	31	-0.09 (−0.46, 0.28)	.004
% of predicted			84.8 (79.2. 90.4)		99.1 (91.0. 107.3)	.003
DRS†						
Geometric mean	11 y	58†	5.86 (3.76, 9.12)	39†	2.28 (1.33, 3.89)	.008
Geometric mean	18 y	49	7.17 (4.31, 11.93)	31	2.58 (1.40, 4.75)	.012

Note: DRS (%/μmol) is the ratio of maximum percentage decline in FEV_1 from baseline to cumulative administered dose (μmol) of methacholine.
Abbreviations: 11 y, First follow-up at median 11 years of age; 18 y, Second follow-up at median 18 years of age; CI, Confidence interval; DRS, Methacholine dose–response slope; FEF_{25-75}, Forced expiratory flow between 25% and 75% of the forced vital capacity; FEV_1, Forced expiratory volume in first second; FVC, Forced vital capacity.
**P*-values from Student's *t* test.
†Due to contraindications in two subjects in the post-bronchiolitis group and one control, methacholine provocation test was only performed in 58 and 39 subjects, respectively, at 11 y.

2.2 | Data collection and definitions

Asthma symptoms and medications during the last year were reported by the parents (11-year examination) and study subjects (18-year examination) from questionnaires based on the International Study of Asthma and Allergies in Childhood (ISAAC).[24] In addition, at 18 years more detailed data regarding personal and family history of asthma and atopy were collected through questionnaires and supplemented with information from medical records at hospitalization. For details, see Appendix 1.

Asthma ever was defined as positive answer to *have you ever been diagnosed with asthma?* Current asthma was defined as asthma ever combined with a positive answer to at least one of the two questions: (a) *Have you during the last 12 months had heavy breathing or wheezing/chest-tightness* and (b) *Have you during the last 12 months used any asthma medications* (inhaled corticosteroids, long- or short-acting beta-2 agonists, montelukast, ipratropium bromide, or any combinations).

2.3 | Ethics

The study was approved by the Regional Committee on Medical Research Ethics. Signed statements of informed consent were obtained from all participants and from parents if the participants were younger than 18 years of age.

2.4 | Statistical analysis

Continuous variables are presented as group means with 95% confidence intervals, and were compared by Student's *t* test or as medians and interquartile range (IQR) and compared by Mann–Whitney *U* test, as appropriate. Categorical variables are presented as counts

TABLE 3 Change in lung function variables from 11 to 18 y of age in children hospitalized for bronchiolitis in infancy and an age-matched control group, presented as mean change with 95% CI

	Post-bronchiolitis		Controls		Interaction
	Mean change (95% CI)	*P*-value*	Mean change (95% CI)	*P*-value*	*P*-value*
FEV_1, z-score	-0.09 (−0.31, 0.13)	.442	-0.14 (−0.53, 0.25)	.484	.816
FEV_1, z-score†	-0.08 (−0.30, 0.14)	.454	-0.15 (−0.55, 0.24)	.451	.765
FVC, z-score	-0.24 (−0.51, 0.03)	.076	-0.24 (−0.61, 0.13)	.203	.997
FVC, z-score†	-0.23 (−0.49, 0.04)	.089	-0.26 (−0.63, 0.12)	.182	.911
FEV_1/FVC, z-score	0.18 (−0.14, 0.50)	.263	0.10 (−0.22, 0.42)	.529	.731
FEV_1/FVC, z-score†	0.17 (−0.15, 0.49)	.301	0.12 (−0.20, 0.43)	.471	.819
FEF_{25-75}, z-score	0.25 (0.00, 0.50)	**.046**	0.21 (−0.13, 0.54)	.236	.834
FEF_{25-75}, z-score†	0.24 (−0.00, 0.49)	.054	0.21 (−0.13, 0.55)	.230	.873
LnDRS	0.17 (−0.29, 0.64)	.465	0.11 (−0.42, 0.64)	.697	.851
LnDRS†	0.17 (−0.29, 0.63)	.471	0.10 (0.43, 0.62)	.721	.835

Note: Bold values denote statistical significance at the $P < 0.05$ level.
DRS (%/μmol) is the ratio of maximum percentage decline in FEV_1 from baseline to cumulative administered dose (μmol) of methacholine. Due to highly skewed distribution, DRS was transformed using the natural logarithm. The group-wise mean changes were estimated in generalized estimating equation (GEE) models including interaction terms group*time to test for unequal trajectories in controls and post-bronchiolitis. A positive mean change indicates that z-scores were higher at 18 than 11 y of age.
Abbreviations: CI, Confidence interval; DRS, Methacholine dose–response slope; FEF_{25-75}, Forced expiratory flow between 25% and 75% of the forced vital capacity; FEV_1, Forced expiratory volume in first second; FVC, Forced vital capacity.
**P*-values from Wald test.
†Adjusted for family history of asthma or atopy, atopic sensitization at 11 y of age, and asthma at 11 y of age

and percentages, and differences were tested by Pearson's chi-square test. Lung function and DRS to methacholine at both follow-ups were compared by generalized estimating equations (GEE). Interaction terms (group*time) were applied to test divergent development of lung function and DRS between the post-bronchiolitis and control group from 11 to18 years of age, and the analyses were adjusted for atopic sensitization and asthma at 11 years of age as well as family history of asthma or atopy. The distribution of the DRS to methacholine was highly skewed and therefore transformed using the natural logarithm, after negative values were set to zero and 0.1 was added to all DRS values. A Cox regression analysis allowing for correlation between repeated tests of the same individuals was used to analyze the proportion of non-responders at each cumulative dose of methacholine.

The association between BHR at 11 years and current asthma at 18 years was analyzed by multivariable logistic regression analysis. LnDRS was included as explanatory variable, and the analyses were adjusted for the following covariates measured at 11 years: group variable, gender, z-score FEV_1, and current asthma.

Analyses were carried out using SPSS version 24.0 (IBM Corp.) and Stata version 15.1 (StataCorp LLC). Generally, *P*-values ≤ .05 were considered statistically significant.

3 | RESULTS

Sixty children (56%) in the post-bronchiolitis group and 40 (45%) in the control group consented to participate, and 54 (50%) in the post-bronchiolitis group and 33 (37%) controls consented to clinical tests at the 18-year examination (Figure 1). One control and one in the post-bronchiolitis group failed to complete spirometry according to standard quality criteria, leaving 53 (49%) and 32 (36%) individuals with results from spirometry, respectively. One control had low peak expiratory flow and only acceptable FVC, and not valid MPT. In the post-bronchiolitis group, MPT was not valid in one subject and not performed in three subjects due to contraindications, hence 49 (45%) participants in the post-bronchiolitis group and 31 (35%) controls had acceptable MPT (Figure 1).

Baseline characteristics of both groups are presented in Table 1. There were no differences regarding, age, gender, weight, and length between the two groups. In the post-bronchiolitis group, 83% had been hospitalized with RSV-positive bronchiolitis. Atopic sensitization was more common in the control group than in the post-bronchiolitis group at both follow-ups.

3.1 | Lung function and bronchial hyper-reactivity

Lung function and DRS to methacholine in both groups and at both ages are presented in Table 2. Children in the post-bronchiolitis group had lower FEV_1, FEV_1/FVC, FEF_{25-75}, and higher DRS than controls at both follow-ups.

For both groups, there were no significant changes in z-scores for forced vital capacity (FVC), forced expiratory volume in first second (FEV_1), or FEV_1/FVC ratio between 11 and 18 years of age (Table 3). In the post-bronchiolitis group, but not in the control group, z-scores for forced expiratory flow between 25% and 75% of FVC (FEF_{25-75}) was higher at 18 than

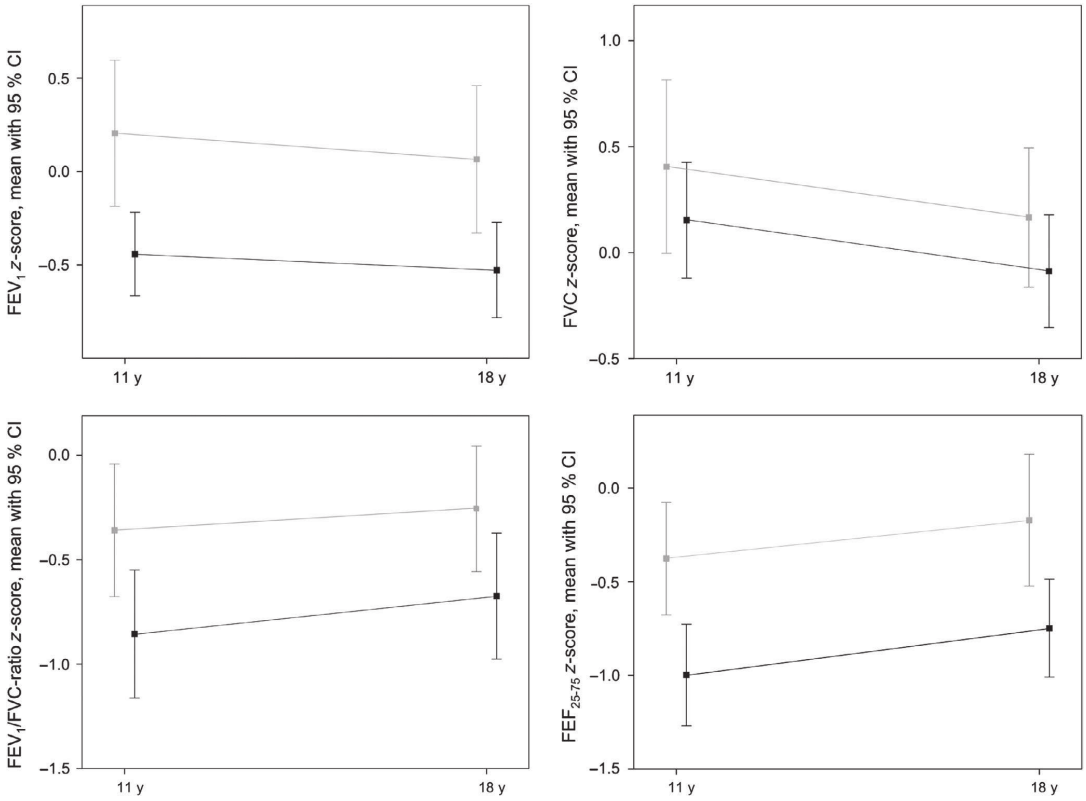

FIGURE 2 z-scores for lung function variables presented as estimated marginal means with 95% confidence intervals at 11 and 18 y of age in subjects hospitalized for bronchiolitis in infancy and controls. Results from generalized estimating equation (GEE) analysis. The *x*-axis depicts age, and the *y*-axis depicts mean *z*-scores. The black lines represent spirometric scores for the post-bronchiolitis group and the gray lines represent spirometric scores for the control group. 11 y: First follow-up at median 11 years of age; 18 y, Second follow-up at median 18 years of age; FEV_1, Forced expiratory volume in first second; FVC, Forced vital capacity; FEF_{25-75}, Forced expiratory flow between 25% and 75% of the forced vital capacity

11 years of age. Change in absolute lung function variables from 11 to 18 years of age is presented in Table S1 and commented in Appendix 2. We found no significant interaction between age and group, meaning that the trajectories of lung function and DRS from 11 to 18 years of age did not differ between the post-bronchiolitis and the control group neither in the unadjusted nor in the adjusted analyses (Table 3, Table S1, Figures 2 and 3). Furthermore, there were no significant interactions between age, group, and gender, that is, we found no differences between boys and girls in how bronchiolitis in infancy affected the trajectories of lung function and BHR from 11 to 18 years. Figure 3 displays the methacholine responsiveness at 11 and 18 years of age. There was no significant interaction effect between group and age by Cox regression (P = .988) or from the GEE analysis (Table 3), and we found no differences in the trajectories for DRS from 11 to 18 years of age between the post-bronchiolitis and control groups.

In the multivariable logistic regression analysis, BHR at age 11 was independently associated with current asthma at age 18 (OR 1.88; 95% CI 1.22-2.89, P = .004).

4 | DISCUSSION

The present study shows that lung function z-scores and BHR were stable from 11 to 18 years of age in children hospitalized for bronchiolitis during their first year of life, and that the trajectories for lung function and BHR from 11 to 18 years of age were not different from the control group. These results applied for both boys and girls. BHR at age 11 was independently associated with asthma at age 18.

In this rather small cohort, lung function was lower and BHR was higher after bronchiolitis in infancy compared to age-matched controls with no such history both at 11 and at 18 years of age. This is in line with previous follow-up studies during childhood,[10,11,25] and a

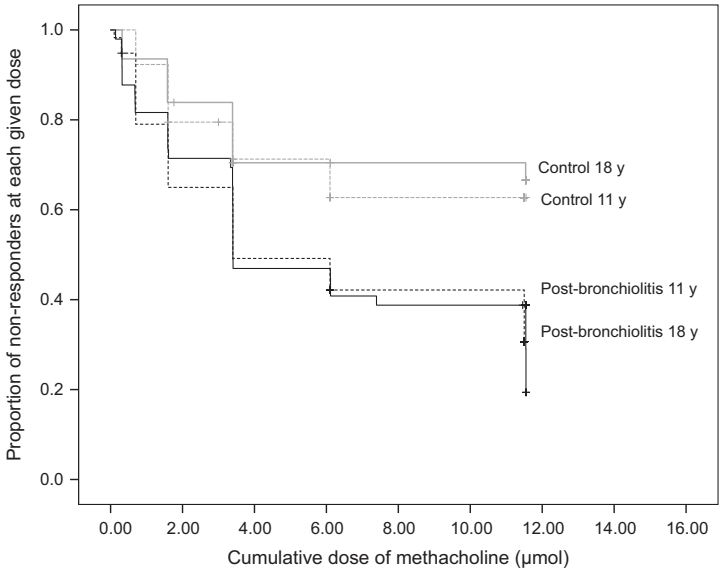

FIGURE 3 Bronchial hyper-reactivity to methacholine at 11 and 18 years of age in subjects hospitalized for bronchiolitis in infancy and controls. The *x*-axis depicts total cumulative dose of methacholine given to each subject, censored at the maximum given dose of 11.5 μmol. The *y*-axis depicts the proportion of non-responders at each given dose. 11 y, First follow-up at median 11 years of age; 18 y, Second follow-up at median 18 years of age

similar pattern has also been shown in adults with a history of bronchiolitis in early childhood.[13,14,26] However, this issue was not the focus of the present study, rather our aim was to study the trajectories of lung function and BHR during the important pubertal growth spurt.

Although there are suggestions that childhood respiratory illnesses, such as viral bronchiolitis, predispose to subsequent asthma and COPD,[6] only a few longitudinal studies include repeated measurements of lung function and/or BHR during childhood and up to young adulthood in children hospitalized for bronchiolitis in infancy.[12-14] As far as we know, none of these previous post-bronchiolitis studies have investigated lung function and/or BHR longitudinally during the important transitional period from childhood to young adulthood.

During childhood and adolescence, the lung function trajectories are characterized by a growth phase reaching a peak after puberty at 18-25 years of age, followed by a plateau phase, and finally a decline linked to physiological aging.[1] Some children following a trajectory below normal may have catch-up of lung function during childhood and adolescence.[1] Unselected population cohort studies have shown that lung function trajectories contributing to COPD include both early and persistent low lung function as well as accelerated decline in adulthood.[3] The results from the present study suggest that children with former bronchiolitis during puberty follow a lung function trajectory below normal peak values, but that the development between 11 and 18 years of age is parallel to healthy controls with no catch-up nor decline. The results support a notion that low lung function and increased BHR, which has been observed at different ages after bronchiolitis, are features that are established in early life, either due to airway damage caused by the respiratory insult during bronchiolitis or that these abnormalities are already present prior to the respiratory event, as also suggested by others.[6] The accelerated growth and pubertal period is also characterized by a shift from male-dominated childhood asthma to female-dominated adult asthma.[15,27] We did not find that gender affected the impact of former bronchiolitis on the lung function trajectories during puberty.

Similar lung function trajectories, as observed in the present study, have been found in young adults/adolescents after other early respiratory insults such as repeated episodes of viral wheeze and extreme prematurity.[4,5] The Tucson cohort included children with viral wheeze up to the age of 3 years, and found that patterns of wheezing prevalence and levels of lung function were established by the age of six and did not change significantly by the age 16 years.[4,28] Similarly, a Norwegian longitudinal cohort study from mid-childhood to adulthood showed that individuals born extremely preterm consistently had lower lung function and increased BHR compared to term-born controls, and that the trajectories from 10 to 25 years of age were parallel and irrespective of the degree of bronchopulmonary dysplasia.[5]

We found a tendency for increased z-scores for FEF_{25-75} from 11 to 18 years in the post-bronchiolitis group, but when tested in interaction terms, this development did not differ from the control group. The finding must be further elaborated in larger longitudinally studies, but may at least support that lung function does not decline in this group during puberty.

Bronchiolitis is associated with subsequent asthma both in children and adults, and BHR is a fundamental characteristic of asthma.[10,13] We found that BHR was stable from 11 to 18 years of age in the post-bronchiolitis group, and the trajectory did not differ from controls. However, we found that BHR at 11 years was associated with current

asthma at 18 years, also when adjusting for asthma at age 11. The association between BHR and subsequent asthma is in line with the results from a Norwegian unselected birth cohort study.[29] The finding supports the speculation that BHR might be an independent and possibly inborn feature that can be causally related to bronchiolitis as well as to subsequent development of asthma.[30,31] The clinical implication could be that children with severe BHR may benefit from regular clinical follow-ups to monitor if asthma develops later in life.

4.1 | Strength and limitations

The main strength of this study is the longitudinal design, and the main weakness is the modest participation rate and power, increasing the risk of selection bias and false-negative results. The lack of lung function data from infancy and early childhood precluded assessment of studying tracking from early life.

5 | CONCLUSION

This longitudinal study shows that lung function z-scores and BHR were stable from 11 to 18 years of age in children hospitalized for bronchiolitis in infancy, following trajectories that were significantly lower, but parallel to those of the control group in both boys and girls. BHR at age 11 was associated with asthma at age 18. Further long-term follow-up studies are needed to study if, and possibly to what extent, children with former bronchiolitis have increased risk of developing COPD.

ACKNOWLEDGMENT

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CONFLICT OF INTEREST

The authors declare that they have no potential conflict of interest related to the manuscript content.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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APPENDIX 1

ATOPIC SENSITIZATION

Atopic sensitization at 11 years of age was defined by a positive skin prick test (SPT) for at least one allergen (wheal diameter ≥3 mm larger than the negative control), and at 18 years of age as a positive SPT or specific immunoglobulin E (IgE) ≥ 0.35 kU/L for at least one allergen. Both tests included the following allergens: *Dermatophagoides pteronyssinus*, dog and cat dander, *Cladosporium herbarium*, birch, timothy, egg white, milk, peanut, hazelnut, and codfish. The SPT was performed with Soluprick® allergens (ALK Albello, Hørsholm, Denmark). Histamine (10 mg/mL) was used as a positive control and a 0.9% saline solution as a negative control. For analysis of specific IgE, blood was drawn and serum stored at −70°C and analyzed by Phadiatop®, fx5E®, and by specific IgE when positive at the Department of Medical Biochemistry, Stavanger University Hospital.

DEFINITIONS AND QUESTIONNAIRES

At the 18-year examination, more detailed data regarding personal and family history of asthma and atopy were collected through questionnaires and supplemented with information from medical records at hospitalization. Atopic dermatitis was defined as a positive answer to *have you ever had atopic dermatitis*. Family history of asthma or allergic diseases was defined as a positive answer to *do you know if your mother, father, or siblings have or have had atopic dermatitis, asthma, or positive allergy tests*. Ever household smoking was defined as a positive answer to *do/did anyone smoke in your home*. Smoking in participants was defined as a positive answer to *do you smoke*. Allergic rhinoconjunctivitis was defined as a positive answer to *have you ever had runny or itching nose and/or eyes apart from colds*.

APPENDIX 2

CHANGE IN ABSOLUTE LUNG FUNCTION

The change in lung function between 11 and 18 years adjusted for gender, age, and height is best expressed by z-scores as given in the article (Table 3). As the changes in absolute lung function may add some clinical information, these are presented in Table S1. The results show that the absolute lung function values apart from the ratio for FEV1/FVC were higher at 18 than 11 years of age. In line with the results from the analyses using z-scores, there were no significant interaction effects between age and group, underlining that the changes in lung function from 11 to 18 years did not differ between the control and post-bronchiolitis group.

Supplemental table 1. Change in absolute lung function variables from 11 to 18 years of age in children hospitalized for bronchiolitis in infancy and an age-matched control group, results from unadjusted analyses, presented as mean change with 95% CI.

	Post-bronchiolitis		Controls		Interaction
	Mean change (95% CI)	**P-value***	**Mean change (95% CI)**	**P-value***	**P-value***
FEV_1, liter	1.50 (1.32, 1.68)	**<0.001**	1.63 (1.37, 1.90)	**<0.001**	0.421
FVC, liter	1.79 (1.56, 2.02)	**<0.001**	1.92 (1.63, 2.20)	**<0.001**	0.508
FEV_1/FVC, ratio	0.016 (-0.00, 0.04)	0.087	-1.85 (-5.43, 1.74)	0.313	0.309
FEF_{25-75}, liter/second	1.57 (1.32, 1.82)	**<0.001**	1.78 (1.40, 2.15)	**<0.001**	0.363

Abbreviations: CI: Confidence interval. FEV_1: Forced expiratory volume in first second. FVC: Forced vital capacity. FEF_{25-75}: Forced expiratory flow between 25-75% of the forced vital capacity. The group-wise mean changes were estimated in generalized estimating equations (GEE) models including interaction terms group*time to test for unequal trajectories in controls and post-bronchiolitis.
*P-values from Wald test.

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ERRATUM

In Sørensen, KG et al[1], the published article contains wrong data for bronchial hyper-reactivity at the 18 year follow-up. The correct results are shown below for Figure 3, Tables 2 and 3. The authors confirm that the effect sizes have changed, but the conclusions of this article remain unchanged

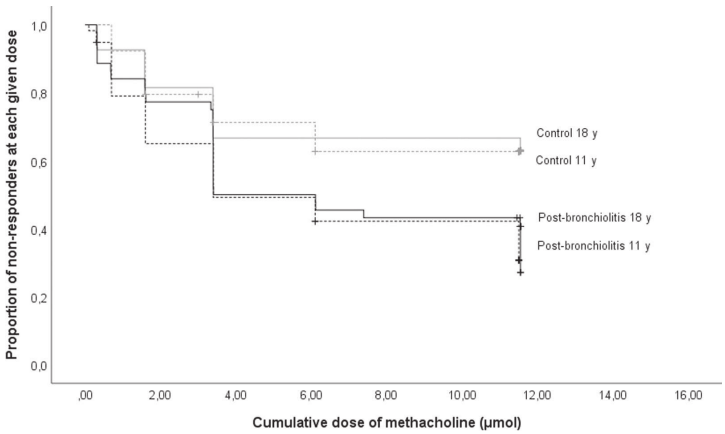

FIGURE 3 Bronchial hyper-reactivity to methacholine at 11 and 18 years of age in subjects hospitalized for bronchiolitis in infancy and controls. The x-axis depicts total cumulative dose of methacholine given to each subject, censored at the maximum given dose of 11.5 µmol. The y-axis depicts the proportion of nonresponders at each given dose. 11 y, first follow-up at median 11 years of age (dotted lines); 18 y, second follow-up at median 18 years of age (solid lines).

TABLE 2 Bronchial hyper-reactivity at 18 years in children hospitalized for bronchiolitis in infancy and an age-matched control group.

			Postbronchiolitis group		Control group		
			N	Observed mean (95% CI)	*N*	Observed mean (95% CI)	*p*-Value*
DRS	Reported geometric mean	18y	49	7.17 (4.31, 11.93)	31	2.58 (1.40, 4.75)	.012
	Revised geometric mean	**18y**	**44**	**4.50 (2.67, 7.60)**	**27**	**2.10 (1.09, 4.04)**	**.070**

Note: Bold values denote revised results.

DRS (%/µmol) is the ratio of percentage decline in forced expiratory volume in first second (FEV_1) from baseline to cumulative administered dose of methacholine.

Abbreviation: 18y, Second follow-up at median 18 years of age; CI, confidence interval; DRS, Methacholine dose–response slope.

*p-Values from Student''s T- test.

TABLE 3 Change in bronchial hyper-reactivity from 11 to 18 years of age in children hospitalized for bronchiolitis in infancy and an age-matched control group, presented as mean change with 95% CI.

	Postbronchiolitis group		Control group		Interaction
	Mean change (95% CI)	*p*-Value*	Mean change (95% CI)	*p*-Value*	*p*-Value*
Reported LnDRS	0.17 (−0.29, 0.64)	.465	0.11 (−0.42, 0.64)	.697	.851
Revised LnDRS	**−0.30 (−0.76, 0.17)**	**.215**	**−0.20 (−0.72, 0.32)**	**.449**	**.791**
Reported LnDRS[a]	0.17 (−0.29, 0.63)	.471	0.10 (−0.43, 0.62)	.721	.835
Revised LnDRS[a]	**−0.30 (−0.77, 0.17)**	**.210**	**−0.22 (−0.73, 0.29)**	**.394**	**.825**

Note: Bold values denote revised results.

DRS (%/μmol) is the ratio of percentage decline in forced expiratory volume in first second (FEV_1) from baseline to cumulative administered dose of methacholine. Due to highly skewed distribution, DRS was transformed using the natural logarithm. The group-wise mean changes were estimated in generalized estimating equation (GEE) models including interaction terms group × time to test for unequal trajectories in controls and post-bronchiolitis. A positive mean change indicates that z-scores were higher at 18 than 11 years of age.

Abbreviation: CI, confidence interval; DRS, Methacholine dose–response slope.

*p-Values from Wald test.

[a]Adjusted for family history of asthma or atopy, atopic sensitization 11 years of age and asthma 11 years of age.

REFERENCE

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ORIGINAL ARTICLE

ACTA PÆDIATRICA NURTURING THE CHILD WILEY

Blood eosinophils during bronchiolitis: Associations with atopy, asthma and lung function in young adults

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Abstract

Aim: To study if blood eosinophils during bronchiolitis were associated with atopy, asthma and lung function in young adults and if these associations differed between respiratory syncytial virus (RSV) bronchiolitis and non-RSV bronchiolitis.

Methods: This historical cohort enrolled 225 subjects. Blood eosinophils were measured during bronchiolitis in infancy, and the subjects were invited to a follow-up at 17–20 years of age including questionnaires for asthma and examinations of lung function and atopy.

Results: The level of eosinophils was positively associated with subsequent atopy in the unadjusted analysis, but not in the adjusted analysis, and not with asthma. There was a negative association between the level of eosinophils and forced vital capacity (FVC) (−0.11; −0.19, −0.02) and forced expiratory volume in first second (FEV_1) (−0.12; −0.21, −0.03) (regression coefficient; 95% confidence interval). The non-RSV group had higher levels of eosinophils during bronchiolitis, but there was no interaction between the level of eosinophils and RSV status for any outcome.

Conclusions: The level of eosinophils during bronchiolitis was negatively associated with lung function in young adult age, but we found no associations with atopy or asthma. These associations were not different after RSV bronchiolitis compared to non-RSV bronchiolitis.

KEYWORDS
asthma, atopy, bronchiolitis, eosinophils, lung function

1 | INTRODUCTION

Bronchiolitis is a common viral lower respiratory tract infection in early childhood.[1,2] Children hospitalised for bronchiolitis have an increased risk of asthma and impaired lung function persisting into young adulthood,[3–6] but the underlying mechanisms of these associations are less understood.

Suppression of blood eosinophils is the expected response to an acute viral infection,[7] but a subset of infants with bronchiolitis have normal or even elevated levels.[8] Recently, it has been indicated that the 'bronchiolitis' diagnosis clinically and pathophysiologically comprises more than one condition,[9] which may be related to the atopic status of the individual, age at hospitalisation and the virus involved. Whereas non-respiratory syncytial virus (RSV) bronchiolitis,

Abbreviations: β, regression coefficient; BMI, body mass index; CI, confidence interval; FEF_{25-75}, forced expiratory flow between 25% and 75% of the forced vital capacity; FEV_1, forced expiratory volume in first second; FVC, forced vital capacity; OR, odds ratio; RRR, relative risk ratio; RSV, respiratory syncytial virus; SD, standard deviation; Th, T-helper cell.

especially bronchiolitis due to human rhinovirus, has been connected to a T-helper cell (Th) 2-driven eosinophilic inflammation, RSV bronchiolitis is more often linked to a type-1 response and a neutrophilic inflammation.[9–11]

Asthma is a heterogeneous disease with various mechanistic pathways (endotypes) and variable clinical presentations (phenotypes).[12,13] Based on endotypes, asthma may be divided into asthma dominated by a Th-2-high eosinophilic airway inflammation, and asthma dominated by a Th-2-low neutrophilic inflammation.[12] Asthma in children and adolescents is most often linked to a concomitant eosinophilic inflammation, whereas up to 25% of asthmatic adults have neutrophilic inflammation.[13]

As both bronchiolitis and asthma show diversity linked to different expressions of eosinophilic inflammation, it is interesting to study if the level of eosinophils during bronchiolitis is associated with subsequent atopy and respiratory morbidity. Studies investigating this association in childhood found associations between the level of eosinophils during bronchiolitis and subsequent asthma.[14–16] In a follow-up at 11 years of age after bronchiolitis in infancy, increasing levels of eosinophils were associated with an increased risk of asthma and lower lung function.[16] There is little published data on associations between the level of eosinophils during bronchiolitis and respiratory outcomes in young adult age. In a Finnish post-bronchiolitis study, the level of eosinophils during bronchiolitis did not predict subsequent asthma at 18–20 years of age,[17] but low levels of eosinophils during bronchiolitis predicted low asthma risk at 28–31 years.[14]

We aimed to study if the level of blood eosinophils during bronchiolitis in infancy was associated with atopy, asthma and lung function in young adults and if these associations differed between RSV bronchiolitis and non-RSV bronchiolitis.

Key Notes

- Children hospitalised for bronchiolitis have an increased risk of asthma and impaired lung function persisting into young adulthood, but the underlying mechanisms including the role of eosinophilic inflammation are less known.
- The level of eosinophils during bronchiolitis in infancy was negatively associated with lung function in young adult age, but not associated with atopy or asthma.
- These associations did not differ between respiratory syncytial virus (RSV) bronchiolitis and non-RSV bronchiolitis.

2 | PATIENTS AND METHODS

2.1 | Study design and subjects

This is a historical cohort study enrolling young adults hospitalised for bronchiolitis below the age of 12 months in Stavanger and Bergen, Norway between October 1996 and May 2001. Eligible subjects were invited to a follow-up at 17–20 years of age as described in more detail previously.[6,18] Bronchiolitis was defined based on European guidelines as an acute viral respiratory tract infection during the first year of life with fever, tachypnoea, dyspnoea, prolonged expiration and wheeze on auscultation.[1] Exclusion criteria were use of inhaled or systemic corticosteroids prior to the hospitalisation, previous hospitalisation for bronchiolitis, severe neonatal or other pre-existing chronic lung diseases, and prematurity with gestational age at birth <32 weeks. During hospitalisation for bronchiolitis, nasopharyngeal mucus was examined for RSV by direct immunofluorescence (*BioMèrieux, Marcy-l'Ètoile, France*). Other viruses were not systematically tested for. Infants testing positive for RSV were defined as having RSV bronchiolitis and infants testing negative as having non-RSV bronchiolitis.

2.2 | Exposures

The level of eosinophils was analysed in blood samples drawn from the infants as a part of the routine tests immediately after admission to the hospital for bronchiolitis as previously described.[19]

2.3 | Outcomes

Atopy was defined as either a positive skin prick test[20] and/or a positive allergen panel or specific immunoglobulin E for at least one common allergen. Asthma ever was defined as a positive answer to the question *have you ever been diagnosed with asthma by a doctor*? Current asthma was defined as asthma ever combined with symptoms of asthma and/or use of asthma medication during the last 12 months. Lung function was measured by spirometry according to established guidelines.[21] Clinical examinations were performed from April 2015 to March 2020, and methods for recording outcomes at follow-up are described in more detail previously.[6]

Subjects were divided into four phenotypes based on the occurrence of atopy and current asthma at follow-up: (1) healthy: no asthma, no atopy; (2) atopic non-asthmatic: atopy, no asthma; (3) non-atopic asthma: asthma, no atopy and (4) atopic asthma: asthma and atopy.

2.4 | Covariates/confounders

Factors possibly influencing both the exposure and outcomes were identified as potential confounders as illustrated in a directed acyclic graph (Figure S1).

Clinical data from the hospital stay for bronchiolitis were obtained retrospectively by review of medical records. Birthweight and gestational age at birth were collected from birth protocols and supplemented by information from medical records. Subjects with no information of prematurity were defined as having a gestational age at birth >36 weeks. Data regarding atopic dermatitis ever, early life exposure to household smoking and family history of asthma and

atopy were obtained retrospectively by review of medical records from the hospitalisation for bronchiolitis and supplemented by information from questionnaires at follow-up.

Anthropometry at follow-up was measured by study nurses or collected from questionnaires for those not participating in the clinical examinations. Personal smoking was defined based on questionnaires, and missing responses from two subjects were interpreted as negative answers in the analyses.

2.5 | Statistics

Continuous data were presented as means with standard deviations (SD) and compared by Student's *t* test if normally distributed or as medians and interquartile ranges and compared by Mann–Whitney *U* test if not normally distributed. Categorical data were presented as counts and percentages and compared by Pearson chi-square test. Multiple imputation by iterative chained equations resulting in 100 completed data sets (random seed 123456) was performed to handle missing data on the virus (RSV vs. non-RSV), weight and the level of eosinophils during hospitalisation for bronchiolitis, birthweight, atopic dermatitis, household smoking, body mass index (BMI), asthma, atopy and lung function. Sex, age at hospitalisation for bronchiolitis, gestational age at birth <36 weeks, family history of atopy, age at follow-up and personal smoking were included as auxiliary variables. When analysing phenotypes of combinations of asthma and atopy, an otherwise equal separate multiple imputation was performed including the four-category phenotype variable instead of separate variables for asthma and atopy. Linear, logistic and multi-nominal logistic regressions were performed to study the associations between the level of eosinophils and different outcomes. The distribution of the levels of eosinophils was highly skewed and

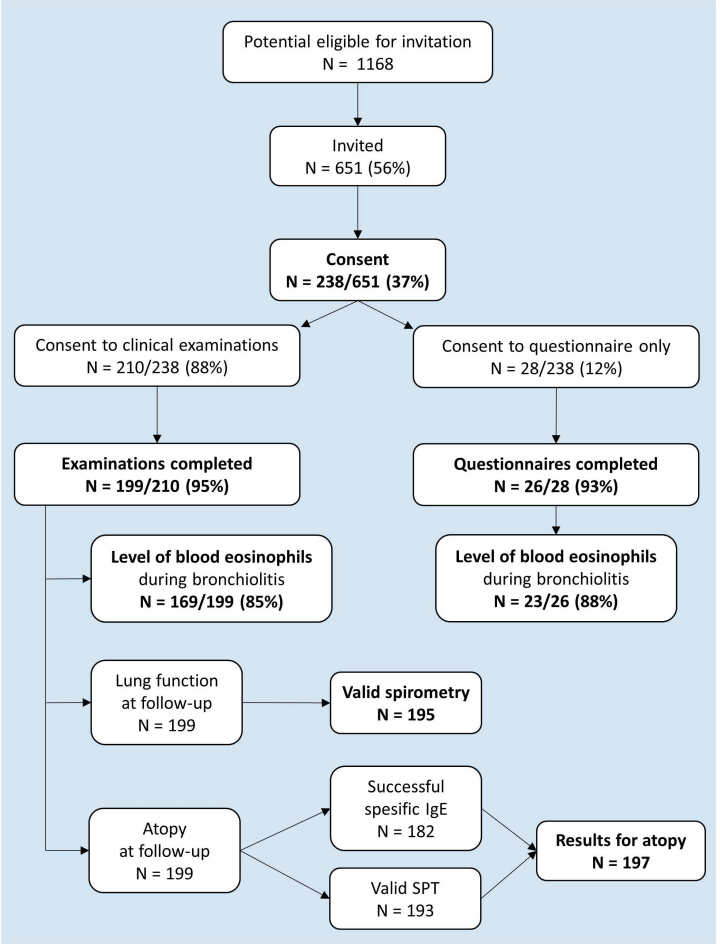

FIGURE 1 Overview of study subjects. SPT, skin prick test.

TABLE 1 Background and clinical characteristics at the hospitalisation for bronchiolitis

	All subjects		RSV		Non-RSV		
Background	***N***		***N***		***N***		***p* value***
Male, *n* (%)	225	117 (52.0)	128	62 (48.4)	64	34 (53.1)	0.540
Gestational age at birth <36 weeks, *n* (%)	225	4 (1.8)	128	3 (2.3)	64	1 (1.6)	0.721
Birth weight, grams, mean (SD)	197	3526 (619)	110	3515 (667)	58	3528 (530)	0.898
Early life exposure to household smoking, *n* (%)	182	39 (21.4)	105	22 (21.0)	48	9 (18.8)	0.753
Family history of atopy, *n* (%)	225	165 (73.3)	128	90 (70.3)	64	49 (76.6)	0.361
Atopic dermatitis ever, *n* (%)	217	55 (25.4)	125	24 (19.2)	62	19 (30.7)	0.080
At hospitalisation for bronchiolitis							
Age at hospitalisation, months, median (quartiles)	225	4.2 (2.3, 6.8)	128	3.8 (2.0, 5.8)	64	4.5 (2.4, 7.8)	**0.041**
Weight at hospitalisation, grams, mean (SD)	197	6911 (1905)	112	6539 (1823)	54	7107 (1818)	0.062
History of bronchopulmonary obstruction, *n* (%)	225	32 (14.2)	128	13 (10.2)	64	12 (18.8)	0.095
Length of hospital stay, days, median (quartiles)	225	3.0 (1.0, 4.0)	128	3.0 (2.0, 5.5)	64	2.0 (1.0, 3.0)	**0.001**
Corticosteroids (inhaled/systemically) given during admission, *n* (%)	225	15 (6.7)	128	6 (4.7)	64	6 (9.4)	0.206
Leukocytes count/μL, mean (SD)	212	12 303 (3851)	119	12 295 (3868)	62	12 976 (3943)	0.266
Eosinophils % of leukocytes, median (quartiles)	192	1.0 (0.3, 2.2)	108	0.7 (0.3, 1.5)	57	1.8 (0.7, 2.8)	**<0.001**
Eosinophils count/μL, median (quartiles)	192	110 (40, 234)	108	80 (32, 169)	57	190 (74, 407)	**<0.001**
Eosinophils count >300/μL, *n* (%)	192	41 (21.4)	108	14 (13.0)	57	19 (33.3)	**0.002**
Eosinophils count >100/μL, *n* (%)	192	103 (53.7)	108	47 (43.5)	57	40 (70.2)	**0.001**

Abbreviations: *N*, number of subjects with available data; *n*, number of subjects with the characteristic described; RSV, respiratory syncytial virus; SD, standard deviation.

**p* values comparing the RSV and the non-RSV group from Student's *t* test for normally distributed variables given as mean (SD), Mann–Whitney *U* test for continuous variables not normally distributed given as median (quartiles) and Pearson chi-squared test for dichotomous variables.

Bold values denote statistical significance at the $p < 0.05$ level.

therefore transformed using the natural logarithm after adding 0.1 to all values to include subjects with a level of eosinophils at zero. Unadjusted and adjusted odds ratios (OR), relative risk ratios (RRR) or regression coefficients (β) with 95% confidence intervals (CI) were calculated. We adjusted for the following pre-specified variables: age at follow-up, sex, family history of atopy, RSV status and age at hospitalisation for bronchiolitis. Other potential confounders were handled by sensitivity analyses (Figure S1). The impact of viral aetiology was assessed by including an interaction term between the level of eosinophils and RSV status.

Stata version 17.0 (*Stata Corp LLC*, *TX*, *USA*) was used for all analyses. *p* values of <0.05 were considered statistically significant.

2.6 | Ethics

The study was approved by the Norwegian Regional Committee on Medical Research Ethics (2014/1930/REK west). Signed statements of informed consent were obtained from all subjects and also from parents if the subjects were younger than 18 years of age.

3 | RESULTS

3.1 | Study subjects

An overview of the inclusion process is given in Figure 1 and described in more detail previously.[6] In total, 1168 eligible infants were admitted for bronchiolitis in the study region during the inclusion period, of whom 651 (56%) were invited to the follow-up at 17–20 years of age. Of the invited subjects, 238 (37%) consented to participate, 199 completed the clinical examinations and 26 returned the questionnaire only, without taking part in the examinations. The level of blood eosinophils was measured during the hospitalisation for bronchiolitis in 192 of the consenting subjects.

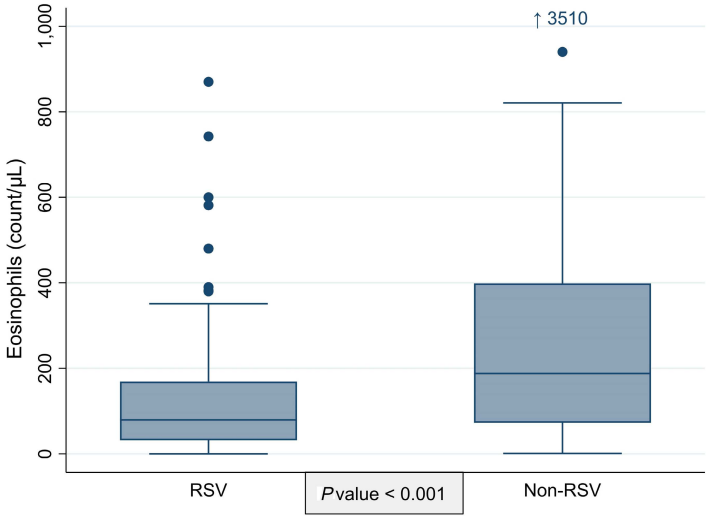

FIGURE 2 Box plot depicting levels of eosinophils during respiratory syncytial virus (RSV) bronchiolitis and non-RSV bronchiolitis. Within each box, the horizontal lines denote median values; boxes extend from the 25th to the 75th percentile; whiskers denote adjacent values (i.e. values within the 1.5 interquartile range of the 25th and 75th percentile of each group); dots denote observations outside the range of adjacent values. Differences are tested by Mann–Whitney *U* test.

TABLE 2 Clinical characteristics at follow-up of 225 young adults hospitalised for bronchiolitis in infancy

	All subjects		RSV		Non-RSV		
	N		*N*		*N*		*p* value*
Age, years, median (quartiles)	225	19.4 (18.6, 20.3)	128	19.0 (18.3, 19.8)	64	19.5 (18.7, 20.2)	**0.045**
BMI, kg/m^2, median (quartiles)	223	23.5 (21.0, 27.4)	127	23.4 (21.0, 26.9)	63	23.2 (21.0, 27.1)	0.900
Height, cm, median (quartiles)	224	172.5 (167.0, 181.8)	127	171.7 (165.1, 181.3)	64	172.7 (168.0, 181.0)	0.489
Weight, kg, median (quartiles)	224	70.0 (63.1, 83.4)	128	68.5 (62.1, 81.6)	63	70.0 (63.1, 83.7)	0.498
Personal smoking, *n* (%)	225	20 (8.9)	128	10 (7.8)	64	7 (10.9)	0.472
Atopy, *n* (%)	197	90 (45.7)	110	34 (30.9)	55	32 (58.2)	**0.001**
Asthma ever, *n* (%)	223	81 (36.3)	127	41 (32.3)	64	25 (39.1)	0.352
Current asthma, *n* (%)	224	58 (25.9)	128	31 (24.2)	64	17 (26.6)	0.724
Phenotypes	196		110		55		
Healthy, *n* (%)		82 (41.8)		62 (56.4)		15 (27.3)	**<0.001**
Atopic non-asthmatic, *n* (%)		64 (32.7)		22 (20.0)		25 (45.5)	**0.001**
Non-atopic asthma, *n* (%)		25 (12.8)		14 (12.7)		8 (14.6)	0.746
Atopic asthma, *n* (%)		25 (12.8)		12 (10.9)		7 (12.7)	0.730
Lung function							
FVC z-score, mean (SD)	195	0.02 (0.94)	110	−0.08 (0.96)	54	0.10 (0.93)	0.261
FEV_1 z-score, mean (SD)	195	−0.38 (1.03)	110	−0.44 (1.02)	54	−0.42 (1.10)	0.913
FEV_1/FVC z-score, mean (SD)	195	−0.67 (0.99)	110	−0.62 (1.01)	54	−0.88 (0.87)	0.107
FEF_{25-75} z-score, mean (SD)	195	−0.70 (1.00)	110	−0.68 (1.01)	54	−0.85 (1.00)	0.316

Abbreviations: BMI, body mass index; FEF_{25-75}, forced expiratory flow between 25% and 75% of the forced vital capacity; FEV_1, forced expiratory volume in first second; FVC, forced vital capacity; N, number of subjects with available data; n, number of subjects with the characteristic described; RSV, respiratory syncytial virus; SD, standard deviation.

*p values comparing the RSV and the non-RSV group from Student's t test for normally distributed variables given as means (SD), Mann–Whitney U test for continuous variables not normally distributed given as median (quartiles) and Pearson chi-squared test for dichotomous variables.

Bold values denote statistical significance at the $p < 0.05$ level.

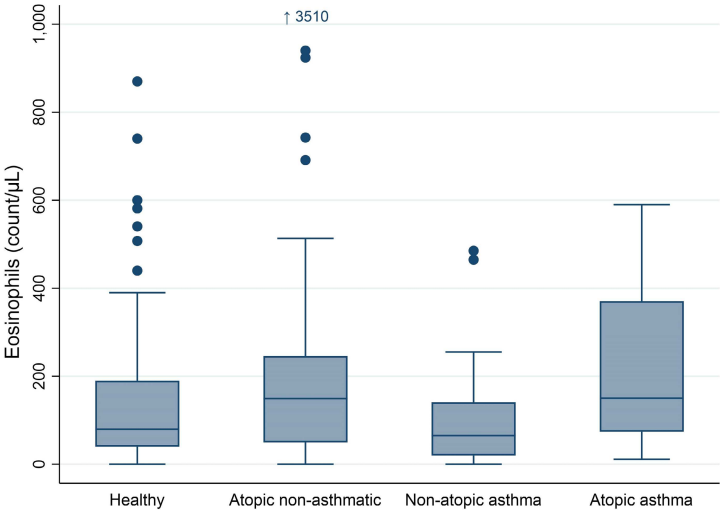

FIGURE 3 Box plot depicting levels of blood eosinophils during bronchiolitis for different phenotypes regarding asthma and atopy at follow-up. Within each box, the horizontal lines denote median values; boxes extend from the 25th to the 75th percentile; whiskers denote adjacent values (i.e. values within the 1.5 interquartile range of the 25th and 75th percentile of each group); dots denote observations outside the range of adjacent values.

3.2 | Background factors and clinical characteristics

The background characteristics did not differ between the RSV group and the non-RSV group (Table 1). As previously described,[6] the non-RSV group were older at hospitalisation and had a shorter length of hospital stay than the RSV group (Table 1). The non-RSV group had higher levels of eosinophils during the hospitalisation for bronchiolitis than the RSV group (Figure 2, Table 1). Also, more subjects in the non-RSV group had a level of eosinophils exceeding a cut-off set to 300 or 100/µL (Table 1).

Subjects in the non-RSV group had a higher prevalence of atopy and were older at follow-up compared to the RSV group, but anthropometry, personal smoking, the prevalence of asthma and lung function variables did not differ between the virus groups (Table 2).[6] The atopic non-asthmatic phenotype was more frequent in the non-RSV group and the healthy phenotype was more frequent in the RSV group, but the frequency of the other phenotypes did not differ between the two virus groups (Table 2). The level of eosinophils during bronchiolitis for each phenotype based on current asthma and atopy at follow-up is illustrated in Figure 3.

3.3 | Associations between the level of eosinophils and atopy and respiratory morbidity

Associations between the level of blood eosinophils during bronchiolitis and different outcomes in young adult age are shown in Table 3 for imputed data.

3.3.1 | Atopy, asthma and phenotypes

The level of eosinophils during bronchiolitis was positively associated with atopy at follow-up in the unadjusted analysis, but not after adjusting for potential confounders (Table 3). There were no associations between the level of eosinophils during bronchiolitis in infancy and asthma ever or current asthma in young adult age (Table 3). A negative association was found between the level of eosinophils during bronchiolitis and the non-atopic asthmatic phenotype, but we found no associations between the level of eosinophils during bronchiolitis and the other phenotypes (Table 3).

3.3.2 | Lung function

The level of eosinophils during bronchiolitis was negatively associated with forced vital capacity (FVC) and forced expiratory volume in first second (FEV_1) at follow-up, but there was no association between the level of eosinophils and FEV_1/FVC (Table 3 and Figure 4). There was a tendency for a negative association between the level of eosinophils and forced expiratory flow between 25% and 75% of the forced vital capacity (FEF_{25-75}) (Table 3 and Figure 4).

3.3.3 | Impact of viral aetiology

We found no interactions between the level of eosinophils and RSV status for atopy, asthma, different phenotypes or lung function parameters, meaning that the associations between the level of eosinophils and the various outcomes did not differ between the RSV group and the non-RSV group (data not shown).

3.3.4 | Sensitivity analyses

Analyses with the imputed outcomes left out of the data set did not change the results. The results from analyses on complete cases given in Table S1 did not differ notably from the imputed data set. Including atopic dermatitis, gestational age at birth, birthweight

TABLE 3 Associations between the level of eosinophils during bronchiolitis in infancy and atopy, asthma and lung function in 225 young adults

Atopy and asthma	**OR (95% CI)**	***p* value***
Atopy, unadjusted	1.27 (1.03, 1.56)	**0.026**
Atopy, adjusted[a]	1.18 (0.94, 1.47)	0.144
Asthma ever, unadjusted	1.01 (0.86, 1.20)	0.867
Asthma ever, adjusted[a]	0.99 (0.83, 1.18)	0.934
Current asthma, unadjusted	0.97 (0.81, 1.16)	0.734
Current asthma, adjusted[a]	0.96 (0.79, 1.16)	0.657
Phenotypes	**RRR (95% CI)**	***p* value***
Healthy	Reference	
Atopic non-asthmatic, unadjusted	1.13 (0.89, 1.42)	0.307
Atopic non-asthmatic, adjusted[a]	1.01 (0.77, 1.31)	0.964
Non-atopic asthma, unadjusted	0.81 (0.65, 1.02)	0.068
Non-atopic asthma, adjusted[a]	0.76 (0.60, 0.98)	**0.031**
Atopic asthma, unadjusted	1.44 (0.97, 2.15)	0.072
Atopic asthma, adjusted[a]	1.37 (0.88, 2.11)	0.162
Lung function	***β* (95% CI)**	***p* value***
FVC z- score, unadjusted	−0.09 (−0.17, −0.01)	**0.024**
FVC z- score, adjusted[b]	−0.11 (−0.19, −0.02)	**0.014**
FEV_1 z- score, unadjusted	−0.11 (−0.19, −0.03)	**0.011**
FEV_1 z- score, adjusted[b]	−0.12 (−0.21, −0.03)	**0.010**
FEV_1/FVC z- score, unadjusted	−0.02 (−0.11, 0.07)	0.633
FEV_1/FVC z- score, adjusted[b]	−0.01 (−0.10, 0.08)	0.808
FEF_{25-75} z- score, unadjusted	−0.08 (−0.16, 0.01)	0.074
FEF_{25-75} z- score, adjusted[b]	−0.07 (−0.16, 0.01)	0.098

Note: Results from unadjusted and adjusted logistic, multi-nominal logistic and linear regression analyses. Missing data were handled by multiple imputation.

The distribution of levels of eosinophils was highly skewed and therefore transformed using the natural logarithm after adding 0.1 to all values.

Abbreviations: CI, confidence interval; FEF_{25-75}, forced expiratory flow between 25% and 75% of the forced vital capacity; FEV_1, forced expiratory volume in first second; FVC, forced vital capacity; OR, odds ratio (from logistic regression); RRR, relative risk ratio (from multinominal regression); RSV, respiratory syncytial virus; β, regression coefficient (from linear regression).

[a]Adjusted for age, sex, family history of atopy, RSV status and age at hospitalisation for bronchiolitis.

[b]Adjusted for family history of atopy, RSV status and age at hospitalisation for bronchiolitis.

*p values from Wald tests. Bold values denote statistical significance at the $p < 0.05$ level.

and household smoking in the analyses did not change the results. Removal of the outlier with an eosinophil blood cell count at 3510/μL (Figure 2) also did not change the results notably.

4 | DISCUSSION

In this historical cohort study, we found associations between the level of blood eosinophils during bronchiolitis in infancy and respiratory outcomes measured in young adult age. The level of eosinophils was negatively associated with lung function. We found a positive association with atopy in the unadjusted analysis, but not after adjustment for potential confounders, and not with asthma. However, when studying phenotypes of asthma and atopy, the level of eosinophils was negatively associated with non-atopic asthma. None of these associations differed between the RSV group and the non-RSV group.

The level of eosinophils during bronchiolitis was associated with atopy in young adult age in the unadjusted analysis, but this finding did not remain significant after adjusting for potential confounders. In a prospective study of newborns of whom the majority had atopic heredity, eosinophilia during infancy was associated with atopy during the first 6 years of life.[22] However, if these associations also apply to a post-bronchiolitis population is less studied. In our follow-up at 11 years, the level of eosinophils during bronchiolitis was not associated with atopy.[16] The reason for the divergent results is not known, but could partly relate to different study populations and age at follow-up.

The level of eosinophils was higher in infants hospitalised for non-RSV bronchiolitis compared to RSV bronchiolitis. This is in line with other studies,[17,19] and may suggest different pathophysiology with a Th2-high eosinophilic inflammation being more pronounced in non-RSV bronchiolitis.[9] Eosinophils are central effectors of allergic inflammation and are linked to atopy.[23] Correspondingly, and consistent with others, the non-RSV group in this study had more atopy than the RSV group at follow-up.[24–27] On this basis, one could suspect RSV status to impact the association between the level of eosinophils and atopy, but there was no interaction between the level of eosinophils and virus group for neither atopy nor any of the other outcomes.

This study does not support that asthma in young adulthood can be predicted based on the level of eosinophils during bronchiolitis in infancy. The result is in line with the Finnish follow-up at the same age,[17] but contrasts another recently published Finnish study which found increased blood eosinophil count during viral wheezing before 2 years of age to be an independent predictive factor for asthma in early adulthood.[28] Our finding is also contrary to post-bronchiolitis studies reporting associations between blood eosinophils and an increased risk of asthma during childhood.[15,16,19] Asthma in adult age is more heterogeneous in that Th2-low neutrophilic inflammation is

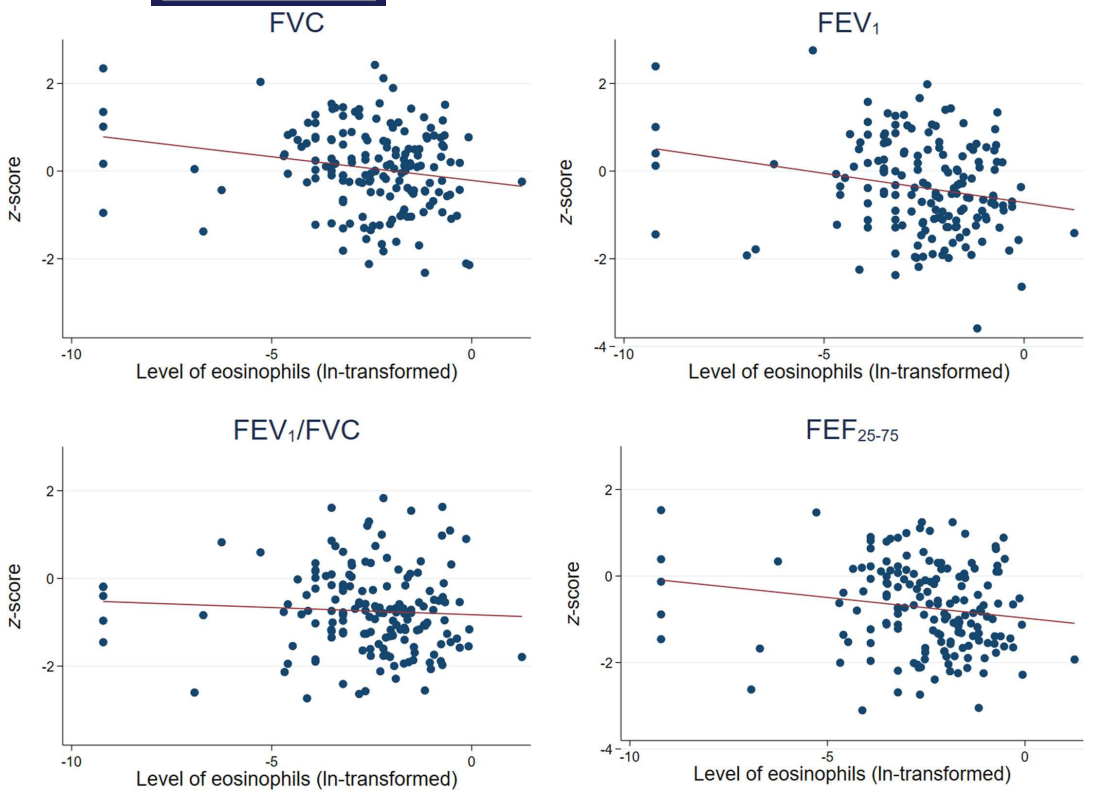

FIGURE 4 Scatter plots depicting lung function at follow-up versus ln-transformed levels of blood eosinophils during bronchiolitis. The y-axes depict lung function variables in z-scores, and the x-axes depict levels of blood eosinophils during bronchiolitis transformed using the natural logarithm after adding 0.1 to all values. Fitted values are represented by the red line. FEF_{25-75}, forced expiratory flow between 25% and 75% of the forced vital capacity; FEV_1, forced expiratory volume in first second; FVC, forced vital capacity.

more common than in childhood asthma.[12,13] This means that the proportion of subjects with late-onset Th2-low neutrophilic asthma will be higher in adults with a previous history of bronchiolitis compared to children with the same history, and may partly explain why eosinophils measured during bronchiolitis are not associated with subsequent asthma in young adult age.

There was a negative association between the level of eosinophils during bronchiolitis and non-atopic asthma. However, as bronchiolitis in general increases the risk of subsequent asthma, the interpretation should not be that eosinophilia during bronchiolitis protects against subsequent asthma. Rather, if an infant with a high level of eosinophils during bronchiolitis presents with asthma in early adult life, the asthma phenotype is more likely to be atopic than non-atopic.

The level of eosinophils during bronchiolitis was associated with lower lung function in young adult age both for FVC and FEV_1, but not the FEV_1/FVC ratio, suggesting a tendency towards a more restrictive than obstructive lung function pattern. A negative association with FEV_1 was found also in our previous follow-up at 11 years.[16] In the Finnish cohort, a high level of eosinophils during bronchiolitis was associated with irreversible airway obstruction at 28–31 years of age.[14] These differences may partly be explained by different ages both at the hospitalisation for bronchiolitis and at follow-up. The Finnish study included children with bronchiolitis up to 24 months of age, contrasting our cut-off at 12 months.[14] Inclusion of older subjects with bronchopulmonary obstruction during the second year of life may increase the heterogeneity of the post-bronchiolitis group, by potentially including more subjects in whom the bronchiolitis represents early onset asthma with a subsequent higher risk of long-term obstructive lung function.

Our study emphasises clinical and pathophysiological differences between RSV bronchiolitis and non-RSV bronchiolitis. Children with RSV bronchiolitis tend to be younger at hospitalisation. They have impaired lung function,[6] which might either be present prior to the respiratory insult of bronchiolitis, or a result of the acute infection, but with a normal inflammatory response with suppressed eosinophils during bronchiolitis. This may further result in persistently impaired lung function with increasing clinical relevance after the peak of lung function is passed in early adulthood,[29] but is neither associated with subsequent atopy nor eosinophilic inflammation. On

the other hand, children with non-RSV bronchiolitis tend to be older at hospitalisation, have higher levels of eosinophils and more atopy in young adult age.

The main strengths of this study were the high number of subjects with clinical data on lung function and atopy, and the inclusion of children hospitalised for both RSV bronchiolitis and non-RSV bronchiolitis, allowing us to study differences between virus groups. Only children hospitalised during their first year of life were included, facilitating a more homogeneous study population.[30] A main weakness was the modest participation rate potentially increasing the risk of selection bias. In addition, the lack of specific viral aetiologies in the non-RSV group disallowed further studies of subsets of this group. Missing data represent a limitation, but this was handled by multiple imputation to achieve higher statistical power and less skewness in the estimates than analyses limited to subjects with complete data would provide.

5 | CONCLUSIONS

The level of eosinophils during bronchiolitis in infancy was negatively associated with lung function in young adult age, but we found no associations with atopy or asthma. These associations were not different after RSV bronchiolitis compared to non-RSV bronchiolitis.

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We are grateful to all children, young adults, and parents who have taken part in this study. Our special thanks are also extended to the nurses at the Paediatric Clinical Trial Unit at Haukeland University Hospital and the study nurses in Stavanger for executing the clinical examinations.

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CONFLICT OF INTEREST

The authors declare that they have no potential conflict of interest related to the manuscript content.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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Figure S1. Simplified directed acyclic graph with confounders in purple solid line frames and mediators in orange dotted line frames. Regression analyses were adjusted for the potential confounders: sex, family history of atopy, RSV-status, and age at hospitalisation for bronchiolitis. Other potential confounders marked by * were handled by sensitivity analyses.

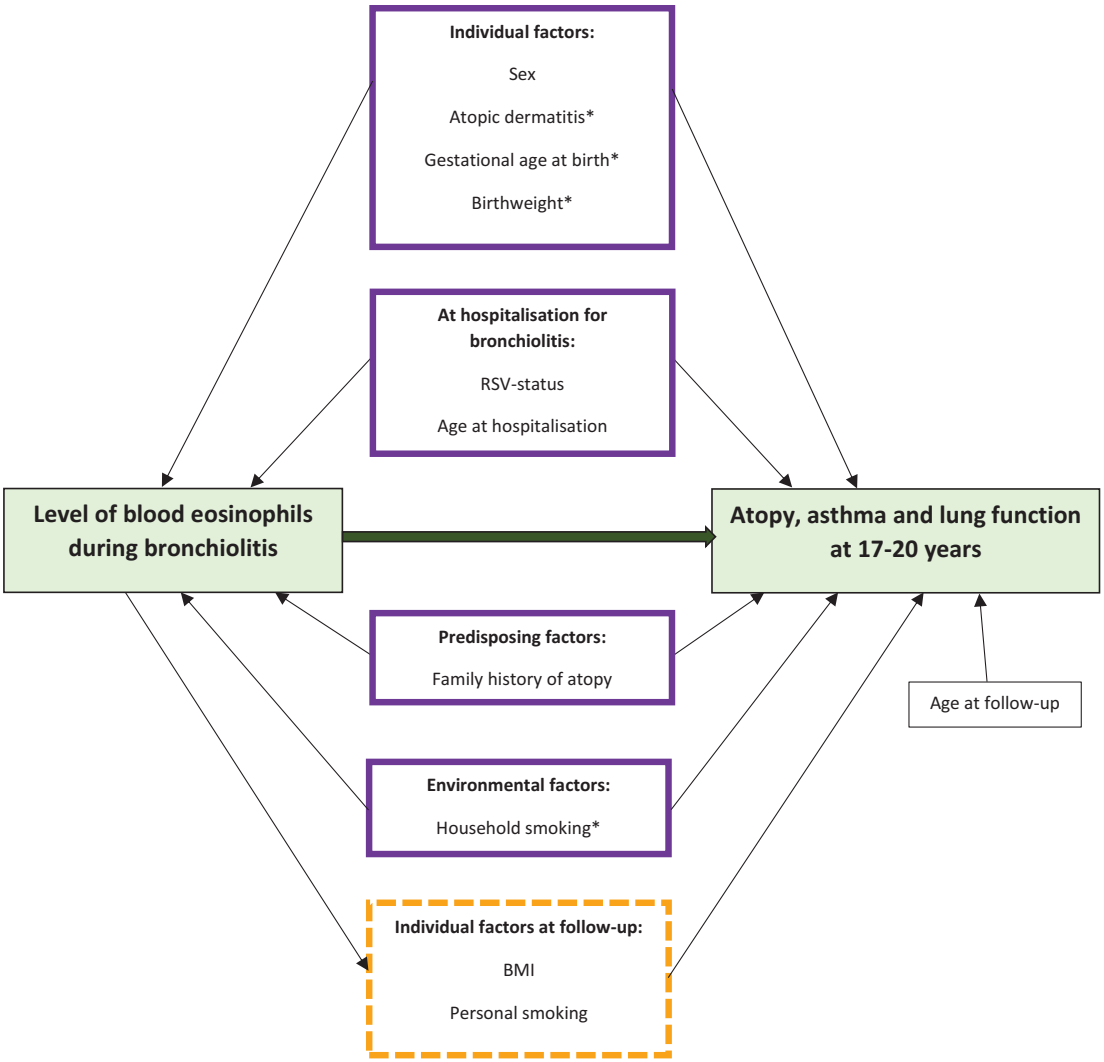

Table S1. Associations between the level of eosinophils during bronchiolitis in infancy and atopy, asthma and lung function in young adults

Atopy and asthma	**N**	**OR (95% CI)**	***p* value***
Atopy, unadjusted	167	1.26 (1.02, 1.55)	**0.029**
Atopy, adjusted [a]	141	1.26 (0.96, 1.65)	0.097
Asthma ever, unadjusted	191	1.00 (0.85, 1.18)	0.990
Asthma ever, adjusted [a]	164	0.97 (0.80, 1.18)	0.757
Current asthma, unadjusted	192	0.96 (0.80, 1.14)	0.633
Current asthma, adjusted [a]	165	0.95 (0.77, 1.17)	0.615
Phenotypes	**N**	**RRR (95% CI)**	***p* value***
Healthy		Reference	
Atopic non-asthmatic, unadjusted	167	1.12 (0.89, 1.42)	0.340
Atopic non-asthmatic, adjusted [a]	141	1.07 (0.78, 1.47)	0.653
Non-atopic asthma, unadjusted	167	0.81 (0.64, 1.02)	0.069
Non-atopic asthma, adjusted [a]	141	0.77 (0.59, 1.00)	0.054
Atopic asthma, unadjusted	167	1.44 (0.96, 2.15)	0.078
Atopic asthma, adjusted [a]	141	1.52 (0.90, 2.58)	0.120
Lung function	**N**	**β (95% CI)**	***p* value***
FVC z- score, unadjusted	165	-0.11 (-0.19, -0.03)	**0.011**
FVC z- score, adjusted [b]	140	-0.15 (-0.24, -0.05)	**0.003**
FEV_1 z- score, unadjusted	165	-0.13 (-0.22, -0.04)	**0.005**
FEV_1 z- score, adjusted [b]	140	-0.16 (-0.26, -0.05)	**0.005**
FEV_1/FVC z- score, unadjusted	165	-0.03 (-0.11, 0.05)	0.433
FEV_1/FVC z- score, adjusted [b]	140	-0.01 (-0.11, 0.09)	0.840
FEF_{25-75} z- score, unadjusted	165	-0.10 (-0.18, -0.01)	**0.031**
FEF_{25-75} z- score, adjusted [b]	140	-0.09 (-0.20, 0.01)	0.075

Note: Results from unadjusted and adjusted logistic, multi-nominal logistic and linear regression analyses on complete cases. The distribution of levels of eosinophils was highly skewed and therefore transformed using the natural logarithm after adding 0.1 to all values.

Abbreviations: CI, confidence interval; FEF_{25-75}, forced expiratory flow between 25% and 75% of the forced vital capacity; FEV_1, forced expiratory volume in first second; FVC, forced vital capacity; OR, odds ratio (from logistic regression); RRR, relative risk ratio (from multinominal regression); RSV, respiratory syncytial virus; β, regression coefficient (from linear regression).

[a] Adjusted for age, sex, family history of atopy, RSV status and age at hospitalisation for bronchiolitis.

[b] Adjusted for family history of atopy, RSV status and age at hospitalisation for bronchiolitis.

*p values from Wald tests. Bold values denote statistical significance at the $p < 0.05$ level.

IV

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Original Research

Are BMI and adipokines associated with asthma, atopy and lung function in young adults previously hospitalized for bronchiolitis?

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ABSTRACT

Background: Children hospitalized for bronchiolitis have increased risk of asthma and low lung function persisting into adulthood, but the underlying mechanisms are poorly understood. Body mass index (BMI) and adipokines are associated with respiratory morbidity. We aimed to investigate if associations between BMI and adipokines and the outcomes asthma, atopy, and lung function differed between young adults previously hospitalized for bronchiolitis and control subjects.

Methods: This sub study of a historical cohort enrolled 185 young adults previously hospitalized for bronchiolitis and 146 matched control subjects. Exposures (BMI and the adipokines: adiponectin, leptin, resistin, and ghrelin) and outcomes (asthma, atopy, and lung function) were measured cross-sectionally at 17–20 years of age. Associations were tested in regression models, and differences between the post-bronchiolitis- and control group were tested by including interaction terms.

Results: BMI was associated with asthma and lung function, but we did not find that the associations differed between the post-bronchiolitis- and control group. We also found some associations between adipokines and outcomes, but only associations between adiponectin and forced vital capacity (FVC) and between resistin and current asthma differed between the groups (p-value interaction term 0.027 and 0.040 respectively). Adiponectin tended to be positively associated with FVC in the post-bronchiolitis group, with an opposite tendency in the control group. Resistin was positively associated with current asthma only in the control group.

Conclusion: The increased prevalence of asthma and impaired lung function observed in young adults previously hospitalized for bronchiolitis do not seem to be related to growth and fat metabolism.

1. Introduction

Bronchiolitis is a common viral lower respiratory tract infection in early childhood [1,2] associated with subsequent asthma and impaired lung function persisting into young adulthood [3–6]. The underlying mechanisms of these associations are poorly understood, but factors related to viral etiology, genetics and different premorbid and life-style factors may be involved [7]. Asthma after bronchiolitis is heterogeneous, and not necessarily related to atopy or eosinophilic inflammation [3,8,9]. A post-bronchiolitis multicenter cohort study found that different growth trajectories gave differential risks for developing asthma by age 5 years, with the highest probability in children with persistent obesity [10]. This indicates that growth and body mass index (BMI) may impact on subsequent respiratory morbidity after bronchiolitis.

Obesity is an increasing problem worldwide [11,12], and elevated BMI is associated with asthma and impaired lung function [13–15]. Inflammatory markers of fat metabolism have been studied as factors potentially involved in these associations [16,17]. Adipokines are mainly produced by adipose tissue and associated with BMI [18,19]. The adipokines may impact on lung function and the development of asthma

Abbreviations: SPT, skin prick test.

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Abbreviations

β = regression coefficient
BMI = body mass index
CI = confidence interval
CV = coefficient of variation
FEF_{25-75} = forced expiratory flow between 25 and 75% of the forced vital capacity
FEV_1 = forced expiratory volume in first second
FVC = forced vital capacity
OR = odds ratio
RSV = respiratory syncytial virus
SD = standard deviation

in different ways. Leptin and resistin may act pro-inflammatory, whereas adiponectin and ghrelin have mainly anti-inflammatory effects [18–20]. Some studies have also found associations between different adipokines and atopy [21–23], but others show contradicting results [24–26].

As previous bronchiolitis, elevated BMI, markers of fat metabolism, and patterns of growth trajectories all have been associated with asthma and low lung function [3,10,13,18], it is relevant to study possible interaction effects between these parameters, in order to elucidate the long-lasting and perhaps life-long respiratory consequences of bronchiolitis. We therefore aimed to investigate if associations between BMI and adipokines and the outcomes asthma, atopy, and lung function were different in young adults previously hospitalized for bronchiolitis compared to control subjects with no such history.

2. Materials and methods

2.1. Study design

This is a sub study of a historical cohort enrolling young adults hospitalized for bronchiolitis in infancy and matched control subjects [3]. Both the exposures (BMI and adipokines) and the outcomes (asthma, atopy and lung function) were measured at 17–20 years of age.

2.2. Post-bronchiolitis group

The post-bronchiolitis group consisted of young adults hospitalized for bronchiolitis during the first year of life in Stavanger and Bergen, Norway between October 1996 and May 2001 as described in more details previously [3,27]. Based on European guidelines, bronchiolitis was defined as an acute viral respiratory tract infection during the first year of life with fever, tachypnea, dyspnea, prolonged expiration and wheeze on auscultation [1]. Exclusion criteria were use of inhaled or systemic corticosteroids prior to the hospitalization, previous hospitalization for bronchiolitis, severe neonatal or other pre-existing chronic lung disease, and prematurity <32 weeks of gestation.

2.3. Control group

A control group not hospitalized for bronchiolitis, but matched to each subject in the post-bronchiolitis group on date of birth, sex and gestational age at birth, was established by searching the hospital's birth protocols [3].

2.4. Exposures

Height and weight were measured by study nurses at follow-up, and BMI (kg/m^2) was calculated.

The serum levels of four different adipokines were analyzed; adiponectin (μg/ml), leptin (ng/ml), resistin (ng/ml), and ghrelin (pg/ml). Blood samples were drawn from the subjects at 17–20 years of age, centrifuged and aliquoted, and sera were stored at −80 °C. Serum adipokines were measured by electrochemiluminiscence on Meso® QuickPlex SQ 120 Imager (*Meso Scale Diagnostics, Rockville, MD, USA)* using the following assay-kits: R-PLEX® Human Adiponectin, R-PLEX® Human Resistin, and U-PLEX® Human Ghrelin and Leptin *(Meso Scale Diagnostics, Rockville, MD, USA)*. The samples were diluted using provided sample diluent to 1:8000 for adiponectin, 1:50 for resistin, and 1:4 or 1:10 for ghrelin and leptin. All analyses were performed from August 2021 to February 2022, and carried out in accordance with the procedures from the manufacturer. Two independent in-house control samples were analyzed to assess the different assay's coefficient of variation (CV). For adiponectin, the intra- and inter-assay CV were <9.2% and <10.1%, for leptin <5.4% and <12.7%, for resistin <13.1% and <11.6%, and for ghrelin <8.5% and <15.3% respectively.

2.5. Outcomes

Asthma ever was defined as a positive answer to the question *have you ever been diagnosed with asthma by a doctor?* Current asthma was defined as asthma ever combined with symptoms of asthma and/or use of asthma medication during the last 12 months. Atopy was defined as either a positive skin prick test [28] and/or a positive allergen panel or specific immunoglobulin E for at least one common allergen. Lung function was measured by spirometry according to established guidelines [29] and presented as z-scores [30]. Clinical examinations were performed from April 2015 to March 2020, and methods for recording of outcomes at follow-up are described in more details previously [3].

2.6. Covariates/confounders

Prior to the analyses, factors possibly influencing both the exposures and outcomes were identified as potential confounders.

As described previously [3], birthweight and gestational age at birth were collected from birth protocols, and clinical data from the hospital stay for bronchiolitis from medical records. Data regarding atopic dermatitis, family history of atopy, household smoking, and personal smoking were collected from questionnaires at follow-up. Ever exposed to smoking was defined as positive answer to either household smoking and/or personal smoking. Missing values regarding smoking were interpreted as negative answers.

2.7. Statistical analysis

Continuous data were presented as mean with standard deviation (SD) and compared by Student's *t*-test if normally distributed, or as median and interquartile range and compared by Mann–Whitney *U* test if not normally distributed. Categorical data were presented as count and percentage, and compared by Pearson chi-square test.

Associations between BMI and outcomes were analyzed by mixed effects logistic and linear regression analyses with p-values from Wald test. BMI had a non-linear relationship to outcomes, and was therefore included in the analyses as a 3-knot restricted cubic spline to allow for flexibility. The direction of the associations is described by predicted proportions and means of the outcomes with 95% confidence interval (CI) for given BMI levels and with all covariates at their respective mean values. Differences in the mentioned associations between the post-bronchiolitis group and the control group were assessed by including an interaction term between BMI and group (i.e. post-bronchiolitis vs. control), and illustrated in plots of predicted proportions and means.

Associations between the different adipokines and outcomes were analyzed by mixed effects logistic and linear regression analyses and presented as odds ratio (OR) or regression coefficient (β) with 95% CI, and corresponding p-values from Wald test. The distributions of all four adipokines were skewed, and therefore they were transformed using the

natural logarithm before entering the regression analyses. Differences in these associations between the post-bronchiolitis group and the control group were assessed by including an interaction term between the different adipokines and group. The interactions are presented as OR or β with 95% CI. In a scenario with no difference in associations between the post-bronchiolitis group and the control group, OR would be equal to 1, and β would be equal to 0. BMI was handled as a confounder, and adjusted for in the main analyses. As BMI also can be considered as a mediator, analyses without adjusting for BMI are in addition given in the supplemental Table A.1.

Potential correlations between matched individuals were allowed for by including a random intercept term in the models. All effect estimates were adjusted for potential confounders. P-values <0.05 were considered statistically significant. Differences between groups were considered significant if the p-values of the interaction term were <0.05, and stratified analyses were subsequently performed. Stata version 17.0 (*Stata Corp LLC, TX, USA*) was used for analyses.

2.8. Ethics

The study was approved by the Norwegian Regional Committee on Medical Research Ethics (2014/1930/REK west). Signed statements of informed consent were obtained from all subjects and from parents if the subjects were younger than 18 years of age.

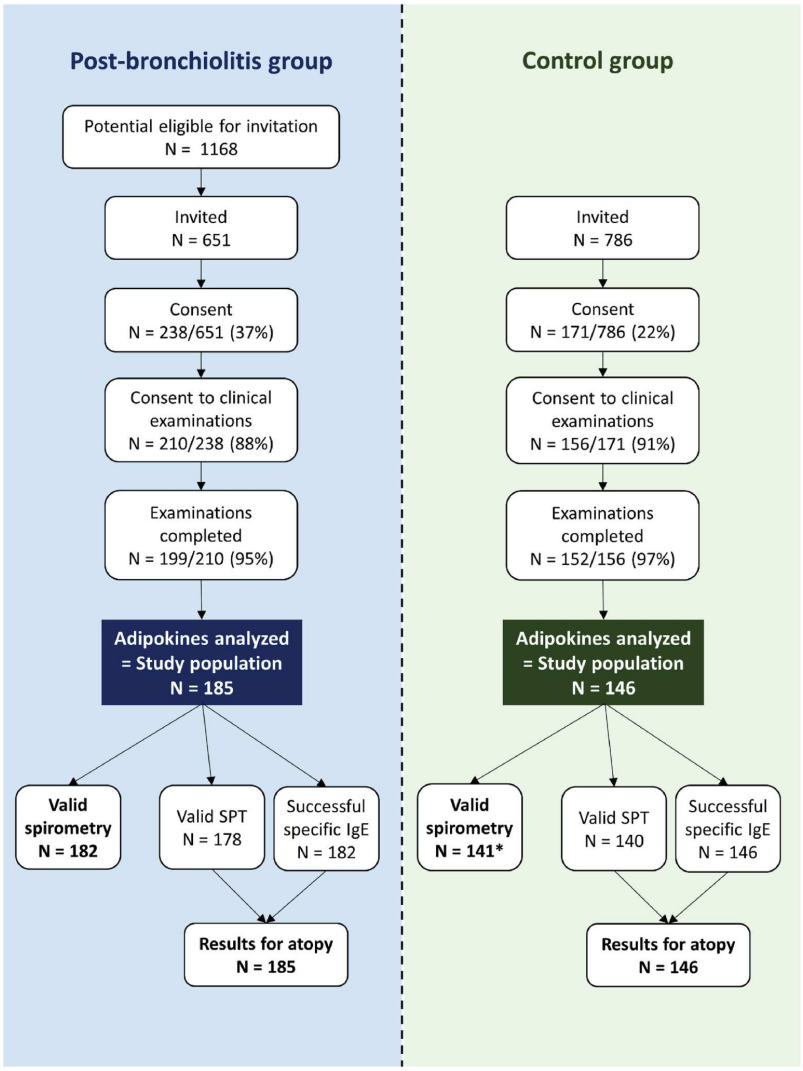

Fig. 1. Overview of study subjects in the post-bronchiolitis group and the control group. *Abbreviations:* SPT = skin prick test; FVC = forced vital capacity. *N = 142 for FVC.

3. Results

3.1. Study subjects

A detailed overview of the study subjects is given in Fig. 1, and described in more details previously [3]. In the post-bronchiolitis group, 185 subjects had results from analyses of serum adipokines and were included in this study. Of these, 103 (56%) subjects tested positive for respiratory syncytial virus (RSV) during the hospitalization for bronchiolitis, whereas 52 (28%) subjects tested negative for RSV, and 30 (16%) subjects did not undergo viral testing. One subject had a non-detectable level of leptin, and this result was excluded due to suspicion of a blood sample error.

In the control group, 146 subjects had results from analyses of serum adipokines and were included in this study.

3.2. Background and clinical characteristics (Table 1)

The post-bronchiolitis group had more often been exposed to smoking, and had a tendency for lower birthweight compared to the control group, but otherwise the groups did not differ regarding background or clinical characteristics.

BMI tended to be slightly higher in the post-bronchiolitis group, but the levels of the four different adipokines did not differ between the post-bronchiolitis group and the control group.

As previously described [3], the prevalence of both asthma ever and current asthma were higher in the post-bronchiolitis group, but the occurrence of atopy did not differ between the two groups. Forced expiratory volume in first second (FEV_1), the ratio FEV_1/forced vital capacity (FVC), and forced expiratory flow between 25% and 75% of FVC (FEF_{25-75}), were lower in the post-bronchiolitis group.

3.3. Associations between BMI and outcomes (Table 2)

In the regression analyses including all subjects, BMI was associated with both asthma ever and current asthma, but not with atopy. We found associations between BMI and FVC, FEV_1, and the ratio between the two, but not FEF_{25-75}. The directions of the associations between BMI and the various outcomes were non-linear, but with an overall tendency for a positive association for asthma, FVC, and FEV_1, and an overall tendency for a negative association for FEV_1/FVC. Fig. 2 illustrates the associations by plots of predicted proportions for asthma and atopy, and predicted mean z-scores for lung function variables for each of the two groups at increasing levels of BMI. We found no statistically significant interactions between BMI and group for any of the outcomes, but a tendency for a more pronounced inverted U-shaped curve for FVC, FEV_1, and FEF_{25-75} in the post-bronchiolitis group.

3.4. Associations between adipokines and outcomes (Table 3)

3.4.1. Adiponectin (Table 3a)

For all subjects, we found no associations between adiponectin and asthma, atopy, or lung function. We found an interaction between adiponectin and group for FVC. Stratified analyses showed a tendency towards a positive association in the post-bronchiolitis group with the opposite tendency in the control group, but none of these were statistically significant. Otherwise we found no interactions between adiponectin and group for any of the outcomes.

3.4.2. Leptin (Table 3b)

For all subjects, we found no associations between leptin and asthma or atopy, but leptin was negatively associated with FVC, FEV_1 and FEF_{25-75}. For the ratio FEV_1/FVC, we found a negative association in the unadjusted analyses, but only a tendency to the same in the adjusted analyses. We found no interactions between leptin and group for any of the outcomes.

Table 1
Background characteristics, clinical characteristics at 17–20 years of age, exposures, and outcomes in young adults hospitalized for bronchiolitis in infancy and control subjects.

Variable, category	Post-bronchiolitis group		Control group		P-value[a]
Background characteristics	N		N		
Males, *n (%)*	185	95 (51.4)	146	70 (48.0)	0.538
Gestational age at birth <36 weeks, *n (%)*	167	3 (1.8)	145	1 (0.7)	0.386
Birth weight, grams, *mean (SD)*	161	3540 (624)	145	3664 (519)	0.061
Atopic dermatitis, *n (%)*	175	39 (22.3)	143	29 (20.3)	0.664
Family history of atopy, *n (%)*	185	122 (66.0)	146	92 (63.0)	0.580
Household smoking ever, *n (%)*	185	63 (34.1)	146	38 (26.0)	0.115
Personal smoking, *n (%)*	185	20 (10.8)	146	8 (5.5)	0.084
Ever exposed to smoking, *n (%)*	185	71 (38.4)	146	40 (27.4)	**0.036**
Clinical characteristics at 17–20 years of age					
Age, years, *median (quartiles)*	185	19.5 (18.6, 20.3)	146	19.3 (18.6, 20.6)	0.577
Height, cm, *median (quartiles)*	185	172.5 (167.5, 181.5)	146	174.4 (167.5, 182.0)	0.484
Weight, kg, *median (quartiles)*	185	70.0 (63.8, 83.7)	146	71.0 (62.0, 80.1)	0.406
Exposures					
BMI, kg/m^2, *median (quartiles)*	185	23.6 (21.2, 27.7)	146	22.7 (21.2, 25.7)	0.071
Adiponectin, µg/ml, *median (quartiles)*	185	22.9 (17.8, 29.0)	146	23.5 (18.0, 28.7)	0.991
Leptin, ng/ml, *median (quartiles)*	184	5.1 (0.9, 12.5)	146	3.5 (0.9, 9.7)	0.234
Resistin, ng/ml, *median (quartiles)*	185	1.6 (1.3, 2.0)	146	1.6 (1.4, 1.9)	0.555
Ghrelin, pg/ml, *median (quartiles)*	185	190.5 (128.1, 306.8)	146	210.5 (134.0, 302.7)	0.442
Outcomes					
Asthma ever, *n (%)*	183	68 (37.2)	145	33 (22.8)	**0.005**
Current asthma, *n (%)*	184	48 (26.1)	145	19 (13.1)	**0.004**
Atopy, *n (%)*	185	86 (46.5)	146	70 (48.0)	0.792
FVC z-score, *mean (SD)*	182	0.03 (0.94)	142	0.10 (0.98)	0.509
FEV_1 z-score, *mean (SD)*	182	−0.36 (1.02)	141	−0.08 (0.95)	**0.010**
FEV_1/FVC z-score, *mean (SD)*	182	−0.65 (1.00)	141	−0.32 (0.99)	**0.003**
FEF_{25-75} z-score, *mean (SD)*	182	−0.68 (0.99)	141	−0.32 (0.94)	**0.001**

Abbreviations: N = number of subjects with available data; n = number of subjects with the characteristic described; SD = standard deviation; BMI = body mass index; FVC = forced vital capacity; FEV_1 = forced expiratory volume in first second; FEF_{25-75} = forced expiratory flow between 25 and 75% of the forced vital capacity.

[a] P-values comparing the post-bronchiolitis group and the control group from Student's t-test for normally distributed variables given as means (SD), Mann Whitney *U* test for continuous variables not normally distributed given as median (quartiles), and Pearson Chi Square test for dichotomous variables. Bold values denote statistical significance at the $P < 0.05$ level.

Table 2
Associations between BMI and asthma, atopy, and lung function in young adults hospitalized for bronchiolitis in infancy and control subjects. Results from unadjusted and adjusted mixed effects logistic and linear regression analyses with corresponding p-values for main analyses and interactions.

		Predicted proportions at specific levels of BMI, % (95% CI)			Association BMI-outcome	Interaction BMI*Group
	N	BMI = 17.5 kg/m^2	BMI = 22.5 kg/m^2	BMI = 36.0 kg/m^2	P-value[a]	P-value[b]
Asthma ever, unadjusted	328	16.2 (5.4, 27.0)	28.7 (23.1, 34.3)	44.8 (28.9, 60.8)	**0.014**	0.188
Asthma ever, adjusted[c]	303	13.2 (2.6, 23.8)	27.2 (21.2, 33.1)	42.9 (26.1, 59.7)	**0.028**	0.299
Current asthma, unadjusted	329	9.6 (1.1, 18.0)	17.7 (12.9, 22.4)	36.9 (21.5, 52.3)	**0.011**	0.091
Current asthma, adjusted[c]	304	6.6 (0, 14.1)	15.5 (10.6, 20.5)	34.2 (18.3, 50.2)	**0.020**	0.131
Atopy, unadjusted	331	42.9 (26.9, 58.9)	47.3 (41.1, 53.5)	47.5 (31.6, 63.4)	0.863	0.981
Atopy, adjusted[d]	294	53.5 (34.6, 72.5)	47.0 (39.9, 54.2)	44.6 (26.7, 62.6)	0.757	0.826
		Predicted means at specific levels of BMI, z-scores (95% CI)				
Lung function	N	BMI = 17.5 kg/m^2	BMI = 22.5 kg/m^2	BMI = 36.0 kg/m^2	P-value[a]	P-value[b]
FVC z-score, unadjusted	324	−0.70 (−1.00, −0.40)	0.02 (−0.10, 0.14)	0.48 (0.19, 0.78)	**<0.001**	0.303
FVC z-score, adjusted[e]	300	−0.73 (−1.05, −0.42)	0.01 (−0.11, 0.13)	0.49 (0.20, 0.79)	**<0.001**	0.336
FEV_1 z-score, unadjusted	323	−0.72 (−1.04, −0.40)	−0.23 (−0.35, −0.10)	−0.13 (−0.45, 0.18)	**0.007**	0.140
FEV_1 z-score, adjusted[e]	299	−0.76 (−1.10, −0.42)	−0.24 (−0.37, −0.11)	−0.11 (−0.43, 0.21)	**0.006**	0.205
FEV_1/FVC z-score, unadjusted	323	0.03 (−0.29, 0.35)	−0.44 (−0.56, −0.32)	−1.03 (−1.34, −0.71)	**<0.001**	0.065
FEV_1/FVC z-score, adjusted[e]	299	−0.00 (−0.33, 0.33)	−0.46 (−0.58, −0.33)	−0.98 (−1.29, −0.67)	**<0.001**	0.078
FEF_{25-75} z-score, unadjusted	323	−0.56 (−0.89, −0.24)	−0.50 (−0.62, −0.37)	−0.66 (−0.98, −0.35)	0.652	0.258
FEF_{25-75} z-score, adjusted[e]	299	−0.63 (−0.96, −0.30)	−0.52 (−0.65, −0.39)	−0.61 (−0.93, −0.30)	0.758	0.287

Abbreviations: BMI = body mass index; CI = confidence interval; FVC = forced vital capacity; FEV_1 = forced expiratory volume in first second; FEF_{25-75} = forced expiratory flow between 25 and 75% of the forced vital capacity.
BMI had a non-linear relationship to outcomes, and was therefore included in the analyses as a 3-knot restricted cubic spline to allow for flexibility. The predicted proportions and means are assessed at BMI at 17.5, 22.5, or 36 kg/m^2 respectively, and with all other covariates set to sample means.
[a] P-values from Wald tests from models not including an interaction term. Bold values denote statistical significance at the $P < 0.05$ level.
[b] P-values from Wald tests for the interaction term between BMI and group (i.e. post-bronchiolitis vs. control). The unadjusted models include main effects for both BMI and group, and the interaction term between them, but no other adjustment variables. Bold values as described under[a].
[c] Adjusted for sex, age of gestation at birth, birthweight, hospitalization for bronchiolitis, exposure for smoking, age at follow-up, and atopy at follow-up.
[d] Adjusted for sex, age of gestation at birth, birthweight, hospitalization for bronchiolitis, exposure to smoking, atopic dermatitis, family history of atopy, and age at follow-up.
[e] Adjusted for age of gestation at birth, birthweight, hospitalization for bronchiolitis, exposure for smoking, and atopy at follow-up.

3.4.3. Resistin (Table 3c)

For all subjects, we found no associations between resistin and asthma, atopy or lung function. An interaction was found between resistin and group for current asthma, and stratified analyses showed a positive association between resistin and current asthma only in the control group. Otherwise we found no interactions between adiponectin and group for any other outcomes.

3.4.4. Ghrelin (Table 3d)

For all subjects, we found ghrelin to be negatively associated with FVC in the unadjusted analyses, but not in the adjusted analyses, and not with asthma, atopy or any other lung function parameters. We found no interactions between ghrelin and group.

3.4.5. BMI as a potential mediator

Results from regression analyses with BMI handled as a mediator, and thereby excluded from the adjustment variables, are given in the supplemental Table A.1. In this scenario, we found adiponectin to be negatively associated with current asthma, leptin to be positively associated with asthma and negatively associated with FEV_1/FVC, and ghrelin to be negatively associated with FVC. Differences between the post-bronchiolitis group and the control group did not differ notably from analyses adjusted for BMI (Table A.1, Table 3a,3b,3c,3d).

4. Discussion

This is the first study to explore if the increased prevalence of asthma and impaired lung function seen in subjects with previous hospitalization for bronchiolitis are related to growth and fat metabolism. Our primary aim was to investigate if the associations between BMI and adipokines and respiratory outcomes differed between the post-bronchiolitis group and the control group. Asthma and low lung function were clearly more common in the post-bronchiolitis group, but the associations between BMI and adipokines and outcomes did not differ notably from what were observed in the control group. Thus, the high risk of asthma and low lung function observed after bronchiolitis in our [3] and other studies [4–6] do not seem to be related to growth or markers of fat metabolism.

4.1. BMI

In line with studies from the general population, BMI was associated with lung function and asthma, but not atopy [13–15]. However, the associations between BMI and the outcomes did not differ between the post-bronchiolitis group and the control group. In a recent multicenter study, different growth trajectory profiles after bronchiolitis did influence the risk for developing asthma during childhood [10]. Unfortunately, we do not know if the growth trajectories of our post-bronchiolitis group have developed differently throughout childhood compared to the control group. At young adult age, we found only a tendency for higher BMI in the post-bronchiolitis group, but more asthma and a lower lung function. Nevertheless, the association between BMI and asthma, atopy, and lung function did not differ between the post-bronchiolitis group and the control group. To our knowledge, no others have studied these associations in a young adult post-bronchiolitis population, but our results are in line with a study showing no association between weight status and asthma or allergy in adolescents with a previous history of bronchiolitis [31].

4.2. Adipokines

4.2.1. Adiponectin

Adiponectin is a predominantly anti-inflammatory adipokine which is decreased in obesity [18 32], and in some studies positively associated with lung function [33], and negatively associated with asthma [16 18 19]. We found no associations between adiponectin and asthma or atopy. Neither did we find any differences in associations between the post-bronchiolitis group and the control group. However, the estimates

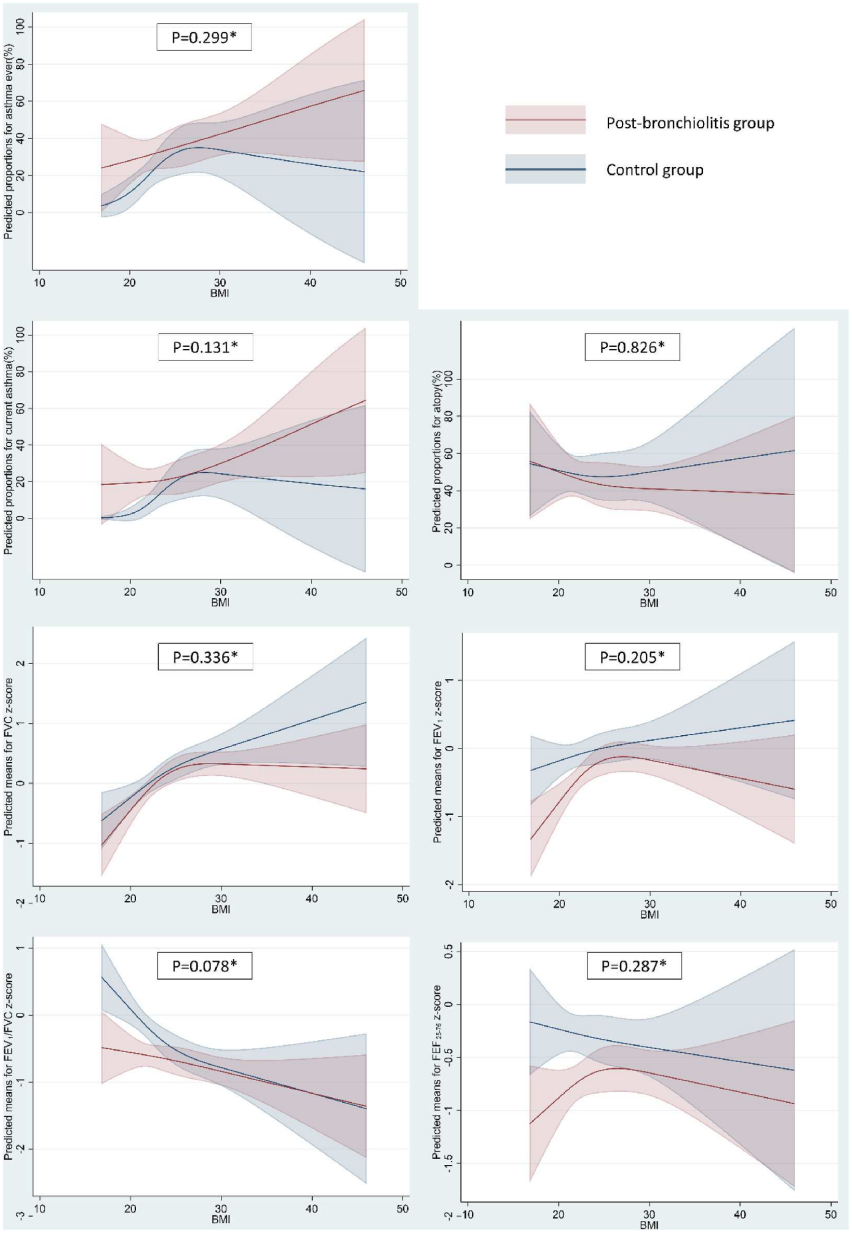

Fig. 2. Associations between BMI and asthma, atopy and lung function in the post-bronchiolitis group and the control group. Plots showing predicted proportions and means with 95% confidence intervals for each of the two groups, with all other covariates set to sample means. The x-axis depicts BMI, and the y-axis depicts the predicted proportions (%) or means (z-scores) for each outcome. *P-values for the interaction term between BMI and group (i.e. post-bronchiolitis vs. control) from Wald tests. *Abbreviations:* BMI = body mass index; FVC = forced vital capacity; FEV1 = forced expiratory volume in first second; FEF25-75 = forced expiratory flow between 25 and 75% of the forced vital capacity.

of the interaction between adiponectin and group for asthma and atopy showed large variance, and hence larger studies are necessary to confirm if there are indeed no differences between the two groups.

The association between adiponectin and FVC differed between the post-bronchiolitis group and the control group with a tendency towards a positive association in the post-bronchiolitis group in line with other studies in general populations [33]. This finding may indicate a possible anti-inflammatory protective effect of adiponectin on FVC only in the

Table 3a
Associations between adiponectin and asthma, atopy, and lung function in young adults hospitalized for bronchiolitis in infancy and control subjects. Results from unadjusted and adjusted mixed effects logistic and linear regression analyses with corresponding p-values for main analyses and interactions.

				Interaction LnAdiponectin*Group	
	N	OR (95% CI)	P-value[a]	OR (95% CI)	P-value[b]
Asthma ever, unadjusted	328	0.78 (0.41, 1.48)	0.445	1.00 (0.26, 3.85)	0.996
Asthma ever, adjusted [c]	303	1.12 (0.50, 2.51)	0.777	1.18 (0.27, 5.05)	0.826
Current asthma, unadjusted	329	0.50 (0.21, 1.19)	0.116	4.68 (0.67, 32.42)	0.118
Current asthma, adjusted [c]	304	0.48 (0.16, 1.45)	0.195	5.03 (0.62, 40.70)	0.130
Atopy, unadjusted	331	1.07 (0.59, 1.93)	0.821	0.70 (0.21, 2.31)	0.561
Atopy, adjusted [d]	294	1.79 (0.84, 3.81)	0.129	1.57 (0.40, 6.21)	0.520
Lung function	**N**	**β (95% CI)**	**P-value [a]**	**β (95% CI)**	**P-value [b]**
FVC z-score, unadjusted	324	−0.13 (−0.41, 0.16)	0.391	0.58 (0.02, 1.15)	**0.041**
FVC z-score, adjusted [e]	300	0.03 (−0.26, 0.31)	0.855	0.61 (0.07, 1.15)	**0.027**
FVC z-score, adjusted [e], only post-bronchiolitis	159	0.31 (−0.07, 0.69)	0.115		
FVC z-score, adjusted [e], only control subjects	141	−0.25 (−0.67, 0.16)	0.232		
FEV_1 z-score, unadjusted	323	−0.04 (−0.34, 0.26)	0.793	0.39 (−0.20, 0.98)	0.193
FEV_1 z-score, adjusted [e]	299	0.03 (−0.28, 0.33)	0.856	0.34 (−0.26, 0.94)	0.263
FEV_1/FVC z-score, unadjusted	323	0.09 (−0.21, 0.39)	0.563	−0.45 (−1.05, 0.14)	0.136
FEV_1/FVC z-score, adjusted [e]	299	−0.01 (−0.30, 0.29)	0.952	−0.58 (−1.15, 0.00)	0.051
FEF_{25-75} z-score, unadjusted	323	0.06 (−0.24, 0.35)	0.694	−0.15 (−0.73, 0.43)	0.606
FEF_{25-75} z-score, adjusted [e]	299	0.07 (−0.23, 0.37)	0.663	−0.26 (−0.84, 0.33)	0.395

Abbreviations: OR = odds ratio (from logistic regression); β = regression coefficient (from linear regression); CI = confidence interval; FVC = forced vital capacity; FEV_1 = forced expiratory volume in first second; FEF_{25-75} = forced expiratory flow between 25 and 75% of the forced vital capacity; BMI = body mass index.
The distribution of adiponectin was highly skewed and therefore transformed using the natural logarithm.

[a] P-values from Wald tests from models not including an interaction term. Bold values denote statistical significance at the $P < 0.05$ level.

[b] P-values from Wald tests for the interaction term between adiponectin and group (i.e. post-bronchiolitis vs control). The unadjusted models include main effects for both adiponectin and group, and the interaction term between them, but no other adjustment variables. Bold values as described under [a].

[c] Adjusted for BMI, sex, age of gestation at birth, birthweight, hospitalization for bronchiolitis, exposure for smoking, age at follow-up, and atopy at follow-up.

[d] Adjusted for BMI, sex, age of gestation at birth, birthweight, hospitalization for bronchiolitis, exposure to smoking, atopic dermatitis, family history of atopy, and age at follow-up.

[e] Adjusted for BMI, age of gestation at birth, birthweight, hospitalization for bronchiolitis, exposure for smoking, and atopy at follow-up.

post-bronchiolitis-group. However, the results are tainted with some uncertainty, with imprecise effect estimates in both groups, and previous research on this field is also not consistent. Neither two studies in children nor a Norwegian population-based study in adults found an association between adiponectin and FVC [24,26,34].

4.2.2. Leptin

Leptin is increased in obesity [32], acts pro-inflammatory, and is associated with impaired lung function [17,26,35] and increased prevalence of asthma in some studies [18]. We found leptin to be negatively associated with lung function consistent with studies from the general population [17,26,35], but not with asthma or atopy. However, the associations between leptin and the outcomes did not differ between the post-bronchiolitis group and the control group.

4.2.3. Resistin

Resistin is increased in obesity [19,32], and has been suggested to have a pro-inflammatory effect regarding asthma [36]. However, the literature is sparse on this field, and results are inconsistent both in children [25,37] and adults [38,39]. We found no association between resistin and asthma, atopy or lung function, but we found an interaction between resistin and group for current asthma. This indicates that the association differs between the post-bronchiolitis group and the control group. In our study, resistin was positively associated with asthma only in the control group. No others have studied this association in a post-bronchiolitis population.

4.2.4. Ghrelin

Ghrelin is reduced in obesity [32], acts anti-inflammatory, and has been shown to ameliorate asthma in mice [20]. We found no associations between ghrelin and asthma, atopy or lung function, and the associations did not differ between the post-bronchiolitis group and the control group.

4.2.5. The role of BMI in the association between adipokines and outcomes

In our main analyses, BMI was included as a confounder due to impact on both the exposures (adipokines are mainly produced in fatty tissue [32]) and the outcomes [13–15]. The true effect of adipokines on the outcomes is then obtained by adjusting the analyses for BMI.

On the other hand, adipokines might be involved in hunger-regulation and energy-balance, and hence have an impact on BMI. By this, BMI may act as a mediator in the association between adipokines and outcomes. In this scenario, the total effect of adipokines on the outcomes is obtained by *not* adjusting for BMI, as it is the sum of the direct effect between adipokines and outcomes and the indirect effect mediated through BMI. The indirect effect would be suppressed if BMI was adjusted for.

The results from the analyses not adjusted for BMI with all subjects included were similar to those adjusted for BMI, with a few exceptions. The negative association between leptin and lung function found in our main analyses, was not present when not adjusting for BMI. This is in line with other studies in children [26], and suggests an independent effect of leptin on lung function, not mediated by BMI, as also emphasized by others [17 35]. According to this, we can infer that the association between leptin on lung function is more likely to be caused by an inflammatory effect rather than a mechanical effect due to obesity.

Our main finding, that the associations between the different adipokines and outcomes did not differ notably between the post-

Table 3b
Associations between leptin and asthma, atopy, and lung function in young adults hospitalized for bronchiolitis in infancy and control subjects. Results from unadjusted and adjusted mixed effects logistic and linear regression analyses with corresponding p-values for main analyses and interactions.

				Interaction LnLeptin*Group	
	N	OR (95% CI)	P-value [a]	OR (95% CI)	P-value [b]
Asthma ever, unadjusted	327	1.07 (0.93, 1.23)	0.361	1.06 (0.80, 1.43)	0.673
Asthma ever, adjusted [c]	302	1.05 (0.79, 1.40)	0.726	1.11 (0.81, 1.51)	0.511
Current asthma, unadjusted	328	1.17 (0.96, 1.42)	0.126	1.07 (0.72, 1.58)	0.751
Current asthma, adjusted [c]	303	0.97 (0.66, 1.44)	0.897	1.10 (0.72, 1.67)	0.671
Atopy, unadjusted	330	0.92 (0.81, 1.04)	0.199	1.05 (0.82, 1.36)	0.685
Atopy, adjusted [d]	293	0.90 (0.69, 1.17)	0.427	1.08 (0.81, 1.45)	0.583
Lung function		β (95% CI)	P-value [a]	β (95% CI)	P-value [b]
FVC z-score, unadjusted	323	0.05 (−0.01, 0.11)	0.096	0.03 (−0.09, 0.15)	0.625
FVC z-score, adjusted [e]	299	−0.08 (−0.15, −0.00)	**0.037**	0.02 (−0.09, 0.14)	0.680
FEV_1 z-score, unadjusted	322	−0.02 (−0.08, 0.04)	0.559	−0.01 (−0.13, 0.12)	0.896
FEV_1 z-score, adjusted [e]	298	−0.12 (−0.20, −0.04)	**0.003**	−0.02 (−0.15, 0.10)	0.742
FEV_1/FVC z-score, unadjusted	322	−0.12 (−0.18, −0.06)	**<0.001**	−0.06 (−0.19, 0.06)	0.309
FEV_1/FVC z-score, adjusted [e]	298	−0.07 (−0.15, 0.01)	0.069	−0.07 (−0.19, 0.06)	0.293
FEF_{25-75} z-score, unadjusted	322	−0.05 (−0.11, 0.01)	0.125	−0.07 (−0.19, 0.05)	0.257
FEF_{25-75} z-score, adjusted [e]	298	−0.08 (−0.16, −0.01)	**0.035**	−0.08 (−0.20, 0.04)	0.208

Abbreviations: OR = odds ratio (from logistic regression); β = regression coefficient (from linear regression); CI = confidence interval; BMI = body mass index; FVC = forced vital capacity; FEV_1 = forced expiratory volume in first second; FEF_{25-75} = forced expiratory flow between 25 and 75% of the forced vital capacity.
The distribution of leptin was highly skewed and therefore transformed using the natural logarithm.
[a] P-values from Wald tests from models not including an interaction term. Bold values denote statistical significance at the $P < 0.05$ level.
[b] P-values from Wald tests for the interaction term between leptin and group (i.e. post-bronchiolitis vs. control). The unadjusted models include main effects for both leptin and group, and the interaction term between them, but no other adjustment variables. Bold values as described under [a].
[c] Adjusted for BMI, sex, age of gestation at birth, birthweight, hospitalization for bronchiolitis, exposure for smoking, age at follow-up, and atopy at follow-up.
[d] Adjusted for BMI, sex, age of gestation at birth, birthweight, hospitalization for bronchiolitis, exposure to smoking, atopic dermatitis, family history of atopy, and age at follow-up.
[e] Adjusted for BMI, age of gestation at birth, birthweight, hospitalization for bronchiolitis, exposure for smoking, and atopy at follow-up.

Table 3c
Associations between resistin and asthma, atopy, and lung function in young adults hospitalized for bronchiolitis in infancy and control subjects. Results from unadjusted and adjusted mixed effects logistic and linear regression analyses with corresponding p-values for main analyses and interactions.

				Interaction LnResistin*Group	
	N	OR (95% CI)	P-value [a]	OR (95% CI)	P-value [b]
Asthma ever, unadjusted	328	1.13 (0.57, 2.26)	0.725	0.29 (0.07, 1.27)	0.102
Asthma ever, adjusted [c]	303	0.93 (0.39, 2.20)	0.871	0.23 (0.05, 1.15)	0.073
Current asthma, unadjusted	329	2.08 (0.86, 5.01)	0.104	0.13 (0.02, 0.92)	**0.041**
Current asthma, adjusted [c]	304	1.76 (0.55, 5.62)	0.342	0.09 (0.01, 0.90)	**0.040**
Current asthma, adjusted [c], only post-bronchiolitis	160	0.71 (0.22, 2.33)	0.572		
Current asthma, adjusted [c], only control subjects	143	8.22 (1.16, 58.08)	**0.035**		
Atopy, unadjusted	331	1.54 (0.81, 2.93)	0.190	0.60 (0.16, 2.28)	0.450
Atopy, adjusted [d]	294	1.41 (0.64, 3.09)	0.393	0.46 (0.10, 2.12)	0.323
Lung function		β (95% CI)	P-value [a]	β (95% CI)	P-value [b]
FVC z-score, unadjusted	324	0.14 (−0.17, 0.45)	0.368	−0.05 (−0.68, 0.57)	0.865
FVC z-score, adjusted [e]	300	−0.11 (−0.43, 0.21)	0.517	−0.12 (−0.75, 0.50)	0.699
FEV_1 z-score, unadjusted	323	0.02 (−0.31, 0.34)	0.914	−0.07 (−0.72, 0.59)	0.845
FEV_1 z-score, adjusted [e]	299	−0.10 (−0.45, 0.25)	0.567	−0.13 (−0.82, 0.56)	0.710
FEV_1/FVC z-score, unadjusted	323	−0.26 (−0.58, 0.07)	0.118	0.19 (−0.46, 0.85)	0.560
FEV_1/FVC z-score, adjusted [e]	299	−0.07 (−0.41, 0.27)	0.697	0.25 (−0.41, 0.92)	0.460
FEF_{25-75} z-score, unadjusted	323	−0.09 (−0.41, 0.23)	0.570	0.05 (−0.59, 0.69)	0.881
FEF_{25-75} z-score, adjusted [e]	299	−0.06 (−0.41, 0.28)	0.711	0.04 (−0.63, 0.71)	0.908

Abbreviations: OR = odds ratio (from logistic regression); β = regression coefficient (from linear regression); CI = confidence interval; BMI = body mass index; FVC = forced vital capacity; FEV_1 = forced expiratory volume in first second; FEF_{25-75} = forced expiratory flow between 25 and 75% of the forced vital capacity.
The distribution of resistin was highly skewed and therefore transformed using the natural logarithm.
[a] P-values from Wald tests from models not including an interaction term. Bold values denote statistical significance at the $P < 0.05$ level.
[b] P-values from Wald tests for the interaction term between resistin and group (i.e. post-bronchiolitis vs control). The unadjusted models include main effects for both resistin and group, and the interaction term between them, but no other adjustment variables. Bold values as described under [a].

[c] Adjusted for BMI, sex, age of gestation at birth, birthweight, hospitalization for bronchiolitis, exposure for smoking, age at follow-up, and atopy at follow-up.
[d] Adjusted for BMI, sex, age of gestation at birth, birthweight, hospitalization for bronchiolitis, exposure to smoking, atopic dermatitis, family history of atopy, and age at follow-up.
[e] Adjusted for BMI, age of gestation at birth, birthweight, hospitalization for bronchiolitis, exposure for smoking, and atopy at follow-up.

bronchiolitis group and the control group, remains the same whether BMI is treated as a confounder or a mediator.

4.3. General considerations

We found no differences in the associations between BMI and outcomes, and very few differences in associations between adipokines and outcomes between the two groups. Hence, associations between growth and markers of fat metabolism and asthma, atopy and lung function in young adults previously hospitalized for bronchiolitis do not seem to differ notably from that of the general population. The prevalence of asthma is increased after viral bronchiolitis in adult age [3–6], but the underlying mechanisms are poorly described, and the clinical picture appears heterogeneous. Given the results of this study, neither BMI nor inflammation linked to fat metabolism seem to be major explanatory factors for the increased risk of respiratory morbidity observed after bronchiolitis. Thus, the underlying mechanisms leading to asthma and impaired lung function persisting into young adulthood after viral bronchiolitis in infancy still remain unclear, and in the need of further investigation.

4.4. Strengths and limitations

The main strength of this study was the high number of study subjects. The main weakness was the modest participation rate potentially increasing the risk of selection bias. Another weakness was the lack of data on growth trajectories throughout childhood and adolescence. The high number of statistical analyses performed entails a risk of obtaining false positive results due to random variation. This aspect calls for extra caution when interpreting positive results in particular and was also taken into account in our considerations, although our main conclusions were negative.

5. Conclusion

The associations between BMI and adipokines and the outcomes asthma, atopy and lung function in young adults hospitalized for bronchiolitis did not differ notably from that of control subjects. Our results speak against BMI and adipokines, and hence growth and fat metabolism, as explanatory factors for the persisting increased respiratory morbidity observed up till young adult age after bronchiolitis in infancy.

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CRediT authorship contribution statement

Karen Galta Sørensen: Conceptualization, Methodology, Formal analysis, Investigation, Writing – original draft, Writing – review & editing, Visualization, Funding acquisition. **Knut Øymar:** Conceptualization, Methodology, Investigation, Writing – review & editing, Visualization, Supervision, Project administration. **Grete Jonsson:**

Table 3d
Associations between ghrelin and asthma, atopy, and lung function in young adults hospitalized for bronchiolitis in infancy and control subjects. Results from unadjusted and adjusted mixed effects logistic and linear regression analyses with corresponding p-values for main analyses and interactions.

				Interaction LnGhrelin*Group	
	N	OR (95% CI)	P-value [a]	OR (95% CI)	P-value [b]
Asthma ever, unadjusted	328	1.18 (0.83, 1.68)	0.355	0.87 (0.41, 1.84)	0.708
Asthma ever, adjusted [c]	303	1.44 (0.93, 2.23)	0.102	0.87 (0.39, 1.91)	0.724
Current asthma, unadjusted	329	0.84 (0.53, 1.32)	0.445	0.72 (0.26, 1.98)	0.524
Current asthma, adjusted [c]	304	0.95 (0.52, 1.73)	0.861	0.64 (0.21, 1.95)	0.436
Atopy, unadjusted	331	1.23 (0.88, 1.70)	0.221	0.78 (0.40, 1.54)	0.479
Atopy, adjusted [d]	294	1.39 (0.92, 2.09)	0.120	1.02 (0.48, 2.17)	0.968
Lung function		β (95% CI)	P-value [a]	β (95% CI)	P-value [b]
FVC z-score, unadjusted	324	−0.20 (−0.35, −0.05)	**0.010**	0.13 (−0.19, 0.45)	0.417
FVC z-score, adjusted [e]	300	−0.11 (−0.28, 0.05)	0.184	0.21 (−0.10, 0.53)	0.177
FEV_1 z-score, unadjusted	323	−0.13 (−0.30, 0.03)	0.108	0.13 (−0.19, 0.46)	0.427
FEV_1 z-score, adjusted [e]	299	−0.10 (−0.28, 0.08)	0.294	0.22 (−0.11, 0.56)	0.194
FEV_1/FVC z-score, unadjusted	323	0.11 (−0.05, 0.28)	0.176	0.04 (−0.29, 0.36)	0.831
FEV_1/FVC z-score, adjusted [e]	299	0.02 (−0.16, 0.19)	0.850	0.03 (−0.30, 0.35)	0.874
FEF_{25-75} z-score, unadjusted	323	−0.04 (−0.20, 0.12)	0.655	0.09 (−0.23, 0.41)	0.598
FEF_{25-75} z-score, adjusted [e]	299	−0.03 (−0.20, 0.15)	0.780	0.16 (−0.17, 0.49)	0.337

Abbreviations: OR = odds ratio (from logistic regression); β = regression coefficient (from linear regression); CI = confidence interval; BMI = body mass index; FVC = forced vital capacity; FEV_1 = forced expiratory volume in first second; FEF_{25-75} = forced expiratory flow between 25 and 75% of the forced vital capacity.
The distribution of ghrelin was highly skewed and therefore transformed using the natural logarithm.
[a] P-values from Wald tests from models not including an interaction term. Bold values denote statistical significance at the $P < 0.05$ level.
[b] P-values from Wald tests for the interaction term between ghrelin and group (i.e. post-bronchiolitis vs control). The unadjusted models include main effects for both ghrelin and group, and the interaction term between them, but no other adjustment variables. Bold values as described under [a].
[c] Adjusted for BMI, sex, age of gestation at birth, birthweight, hospitalization for bronchiolitis, exposure for smoking, age at follow-up, and atopy at follow-up.
[d] Adjusted for BMI, sex, age of gestation at birth, birthweight, hospitalization for bronchiolitis, exposure to smoking, atopic dermatitis, family history of atopy, and age at follow-up.
[e] Adjusted for BMI, age of gestation at birth, birthweight, hospitalization for bronchiolitis, exposure for smoking, and atopy at follow-up.

Methodology, Investigation, Validation, Writing – review & editing. **Ingvild Dalen:** Methodology, Writing – review & editing, Visualization, Supervision. **Thomas Halvorsen:** Conceptualization, Investigation, Writing – review & editing, Visualization, Supervision. **Ingvild Bruun Mikalsen:** Conceptualization, Methodology, Investigation, Writing – review & editing, Visualization, Supervision.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.rmed.2023.107149.

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Table A.1. Associations between the different adipokines and asthma, atopy, and lung function in young adults hospitalized for bronchiolitis in infancy and control subjects. Results from adjusted mixed effects logistic and linear regression analyses with BMI handled as a mediator (not adjusted for) with corresponding p-values for main analyses and interactions.

						Interaction LnAdipokine*Group		
Adipokine	Outcome	N	Effect estimate (95% CI)		P-value [a]	Effect estimate (95% CI)		P-value [b]
Adiponectin	Asthma ever [c]	303	OR	0.84 (0.40, 1.75)	0.636	OR	1.15 (0.29, 4.67)	0.840
	Current asthma [c]	304	OR	0.34 (0.12, 0.98)	**0.045**	OR	4.23 (0.58, 31.00)	0.156
	Atopy [d]	294	OR	1.84 (0.88, 3.81)	0.103	OR	1.54 (0.39, 6.06)	0.534
	FVC z-score [e]	300	β	-0.11 (-0.41, 0.18)	0.450	β	0.58 (0.01, 1.15)	**0.047**
	FVC z-score, adjusted [e], only post-bronchiolitis	159	β	0.17 (-0.23, 0.57)	0.398			
	FVC z-score, adjusted [e], only control subjects	141	β	-0.37 (-0.81, 0.06)	0.091			
	FEV_1 z-score [e]	299	β	-0.05 (-0.36, 0.26)	0.748	β	0.35 (-0.26, 0.95)	0.260
	FEV_1/FVC z-score [e]	299	β	0.10 (-0.20, 0.40)	0.518	β	-0.52 (-1.12, 0.07)	0.084
	FEF_{25-75} z-score [e]	299	β	0.06 (-0.23, 0.36)	0.668	β	-0.24 (-0.82, 0.35)	0.428
Leptin	Asthma ever [c]	302	OR	1.26 (1.02, 1.56)	**0.029**	OR	1.11 (0.81, 1.52)	0.502
	Current asthma [c]	303	OR	1.36 (1.02, 1.83)	**0.038**	OR	1.14 (0.73, 1.77)	0.571
	Atopy [d]	293	OR	0.91 (0.75, 1.10)	0.323	OR	1.09 (0.82, 1.45)	0.558
	FVC z-score [e]	299	β	0.05 (-0.01, 0.11)	0.108	β	0.03 (-0.09, 0.15)	0.630
	FEV_1 z-score [e]	298	β	-0.03 (-0.09, 0.04)	0.406	β	-0.02 (-0.15, 0.11)	0.784
	FEV_1/FVC z-score [e]	298	β	-0.13 (-0.19, -0.07)	**<0.001**	β	-0.07 (-0.19, 0.05)	0.267
	FEF_{25-75} z-score [e]	298	β	-0.06 (-0.12, 0.01)	0.077	β	-0.08 (-0.20, 0.05)	0.213
Resistin	Asthma ever [c]	303	OR	1.16 (0.52, 2.63)	0.714	OR	0.24 (0.05, 1.18)	0.079
	Current asthma [c]	304	OR	2.41 (0.80, 7.23)	0.116	OR	0.11 (0.01, 0.91)	**0.040**
	Current asthma, adjusted [c], only post-bronchiolitis	160	OR	0.97 (0.31, 3.01)	0.955			
	Current asthma, adjusted [c], only control subjects	143	OR	9.73 (1.53, 62.03)	**0.016**			
	Atopy [d]	294	OR	1.34 (0.62, 2.90)	0.456	OR	0.47 (0.10, 2.12)	0.322
	FVC z-score [e]	300	β	0.04 (-0.29, 0.37)	0.827	β	-0.15 (-0.82, 0.51)	0.646
	FEV_1 z-score [e]	299	β	-0.04 (-0.39, 0.30)	0.802	β	-0.16 (-0.85, 0.54)	0.661
	FEV_1/FVC z-score [e]	299	β	-0.20 (-0.54, 0.14)	0.258	β	0.26 (-0.43, 0.94)	0.464
	FEF_{25-75} z-score [e]	299	β	-0.07 (-0.41, 0.26)	0.661	β	0.03 (-0.64, 0.70)	0.933
Ghrelin	Asthma ever [c]	303	OR	1.08 (0.74, 1.58)	0.697	OR	0.78 (0.36, 1.70)	0.527
	Current asthma [c]	304	OR	0.67 (0.39, 1.16)	0.156	OR	0.57 (0.19, 1.69)	0.311
	Atopy [d]	294	OR	1.36 (0.93, 1.97)	0.111	OR	1.01 (0.48, 2.14)	0.979

FVC z-score [e]	300	β	-0.23 (-0.39, -0.07)	**0.004**	β	0.11 (-0.22, 0.43)	0.511
FEV_1 z-score [e]	299	β	-0.14 (-0.31, 0.03)	0.100	β	0.17 (-0.17, 0.51)	0.316
FEV_1/FVC z-score [e]	299	β	0.14 (-0.02, 0.30)	0.096	β	0.11 (-0.22, 0.44)	0.524
FEF_{25-75} z-score [e]	299	β	-0.01 (-0.17, 0.15)	0.902	β	0.16 (-0.17, 0.49)	0.336

Abbreviations: BMI = body mass index; OR = odds ratio (from logistic regression); β = regression coefficient (from linear regression); CI = confidence interval; FVC = forced vital capacity; FEV_1 = forced expiratory volume in first second; FEF_{25-75} = forced expiratory flow between 25 and 75% of the forced vital capacity.

The distribution of all four adipokines were highly skewed and therefore transformed using the natural logarithm.

[a] P-values from Wald tests from models not including an interaction term. Bold values denote statistical significance at the $P < 0.05$ level.

[b] P-values from Wald tests for the interaction term between BMI and group (i.e. post-bronchiolitis vs. control). Bold values as described under [a].

[c] Adjusted for sex, age of gestation at birth, birthweight, hospitalization for bronchiolitis, exposure for smoking, age at follow-up, and atopy at follow-up.

[d] Adjusted for sex, age of gestation at birth, birthweight, hospitalization for bronchiolitis, exposure to smoking, atopic dermatitis, family history of atopy, and age at follow-up.

[e] Adjusted for age of gestation at birth, birthweight, hospitalization for bronchiolitis, exposure for smoking, and atopy at follow-up.

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