UNIWERSYTET MEDYCZNY im. PIASTÓW ŚLĄSKICH we WROCŁAWIU

Katedra i Zakład Immunologii Klinicznej

DRIES VAN ELST

MECHANISMS OF ASTHMA ENDOTYPE ASSOCIATED WITH THE METABOLIC SYNDROME – THE EFFECTS OF HORMONE STIMULATION ON THE BRONCHIAL SMOOTH MUSCLE CELLS

or

BADANIA MECHANIZMÓW ENDOTYPU ASTMY ZWIĄZNEGO Z ZESPOŁEM METABOLICZNYM – WPŁYW HORMONÓW NA FUNKCJE KOMÓREK MIĘŚNI GŁADKICH OSKRZELI

Rozprawa doktorska

Promotor pracy:

Prof. dr hab. Marek Jutel

Promotor pomocniczy

Dr Magdalena Zemelka-Wiącek

Wrocław 2022

Acknowledgement

I would like to acknowledge and express my sincere gratitude to my PhD supervisor prof. dr. hab. **Marek Jutel** and co-promotor Dr **Magdalena Zemelka-Wiącek**, for stimulating me to perform this scientific research, for the opportunities they provided to broaden my horizons, for their valuable advice in the laboratory and at the coffee table, and for their understanding and patience throughout the PhD process.

I would also like to thank the reviewers, prof. dr. hab. **Magdalena Czarnecka-Operacz**, prof. dr. hab. **Radosław Gawlik** and prof. dr. hab. **Maciej Chałubiński**, for taking the time to read and review this thesis.

I would like to thank all **the members of the department of Clinical Immunology**, but especially **Angieszka**, **Anna** and **Paweł**, for aiding me when I had difficulties with Polish bureaucracy, for assisting me in the lab and their endless patience with my broken Polish.

My PhD experience was greatly enhanced by two foreign stages I was able to undertake, one at the Swiss Institute of Allergy and Asthma research and one at the otorhinolaryngology department of the Ghent University hospital. I would like to express my sincere thanks to SIAF's prof. **Cezmi Akdis** and all the other members of SIAF that guided and aided me in my scientific endeavours, with special thanks to prof. **Milena Sokołowska** and **Andzej Eljaszewicz** who were always available, in- and outside the lab. My gratitude also goes out to Prof. Dr. **Philippe Gevaert** and his team, for welcoming me with open arms and allowing me to work on exciting projects.

This work could not have been realised without the support of my family, **Mama, Papa, Sofie** en **Brecht**, and the many friends I made along the way. I would also like to offer them my deepest gratitude.

Lastly, I would like to, from the bottom of my heart, thank **Zuzanna Łukasik**, for always believing in me, for the endless aid and support you give me and for being an unbelievable source of power and inspiration for me. Thank you.

Contents

Abbreviations

Abbreviation	Explanation
AMP	adenosine monophosphate
APC	antigen-presenting cells
BAL	bronchoalveolar lavage
BCR	B cell receptor
BMI	body mass index
BSMC	bronchial smooth muscle cell
CBA	cytometric bead array
CDC	Centre for Disease Control and Prevention
CRP	C-reactive protein
DAMP	damage-associated molecular patterns
Der p 1	endopeptidase 1, a house dust mite allergen
DMSO	dimethyl sulfoxide
EAACI	European Academy of Allergy and Clinical Immunology
FBS	fetal bovine serum
Fel d 1	secretoglobin, a cat allergen
FEV1	forced expiratory volume in the first second
FGFa	fibroblast growth factor 1 (gene)
GATA3	GATA3 binding protein
GC	glucocorticosteroid
GINA	Global Initiative for Asthma
GR	glucocorticoid receptor
HBSMC	human bronchial smooth muscle cell
HDC	histidine decarboxylase
HR1	histamine receptor 1
HR2	histamine receptor 2

HR3	histamine receptor 3
HR4	histamine receptor 4
ICAM-1	inflammatory intracellular adhesion molecule 1
ICD-11	International Classification of Diseases
IFN-γ	Interferon-gamma
IgE	Immunoglobulin E
IgG4	Immunoglobulin G4
IL-10	interleukin 10
IL-12	interleukin 12
IL-13	interleukin 13
IL13R	IL-13 receptor
IL-17	interleukin 17
IL-1β	interleukin 1 beta
IL-2	interleukin 2
IL-25	interleukin 25
IL-33	interleukin 33
IL-4	interleukin 4
IL4R	interleukin 4 receptor
IL-5	interleukin 5
IL-6	interleukin 6
IL-8	interleukin 8
IL-9	interleukin 9
ILC	innate lymphoid cell
INSR	insulin receptor
IR	insulin resistance
ISAAC	International Study of Asthma and Allergies in Childhood
JAK1	Janus kinase 1
JAK3	Janus kinase 3

LEPR	leptin receptor
LOD	limit of detection
MAPK	mitogen-activated protein kinase
MCP	monocyte chemoattractant protein
MCP-1	monocyte chemoattractant protein 1
NR3C1	glucocorticoid receptor (gene)
NSAIDs	non-steroidal anti-inflammatory drugs
OR	odds ratio
PAMP	pathogen-associated molecular patterns
PBS	phosphate-buffered saline
PEF	peak expiratory flow
PRR	pattern recognition receptors
RBP4	retinol-binding protein 4
ROS	reactive oxygen species
RR	relative risk
RT-PCR	real time-polymerase chain reaction
SDS	standard deviation scores
STAT6	signal transducer and activator of transcription 6
TCR	T cell receptor
TLR4	Toll-like receptor 4
TNF	tumor necrosis factor
TNS	trypsin neutralizing solution
Treg	T regulatory cell
TSLP	thymic stromal lymphopoietin
TSLPR	thymic stromal lymphopoietin receptor
US	United States
veh	vehicle, medium-treated (control)
WHO	World Health Organization

Introduction

1. Allergic inflammation

The immune system is a finely balanced, complex network of cells and molecular mediators ensuring protection of the host from a broad range of pathogens and tissue injury, while maintaining tolerance to self- and external innocuous antigens. Dysregulation of immune responses lies at the root of various chronic conditions, including cancer, autoimmune disorders and allergic diseases.

1.1. Allergic diseases – definition, classification and epidemiology

Hypersensitivity is defined as conditions causing reproducible symptoms or signs and initiated by exposure to a defined stimulus at a dose tolerated by normal subjects. The mechanism of hypersensitivity reactions is either driven by the immune system and referred to as *allergy*, or non-immunologic (also referred to as nonallergic hypersensitivity). *Allergy* is therefore a mechanism of immune-driven hypersensitivity to innocuous foreign antigens, leading to the development of a range of signs and symptoms, recognized as different disease entities such as allergic rhinitis, asthma or atopic dermatitis[1]. Allergic reactions can be triggered by almost any foreign protein and arise via several immune pathways.

The term *allergic diseases* comprises a large group of chronic conditions driven by an inappropriate or exaggerated immune reaction to innocuous foreign antigens[2]. They are the most common chronic conditions in the 21st century, affecting over 25% of the world's population. Since allergic immune responses can occur against a broad range of external antigens in all tissues, disease symptoms are incredibly diverse. Moreover, clinical presentation of allergic diseases changes throughout life, with food allergies and eczema most commonly affecting infants, and respiratory symptoms developing at a later age[3]. This commonly observed progression of allergy symptoms is known as the atopic march[4]. Versatile clinical manifestation of allergic diseases poses a risk to the correct diagnosis and calculation of the true disease burden. The latest revision of the International Classification of Diseases (ICD-11) attempts to reflect the complex etiopathogenesis and broad range of symptoms of allergic and hypersensitivity conditions[5], [6].

Allergic diseases have been distinguished as a subcategory of the diseases of the immune system and classified in accordance with their predominant symptomatology. However, specific disease entities which share pathogenetic background with allergic diseases, such as asthma, chronic rhinosinusitis, nasal polyps and atopic eczema, have been described separately. Table 1 presents the current classification of hypersensitivity and allergic conditions.

Table 1. Current classification of hypersensitivity and allergic conditions according to the International Classification of Diseases, 11th revision (ICD-11)

ICD-11 code			Coded elsewhere
4A80	Allergic or hypersensitivity disorders involving the respiratory tract		Vasomotor or allergic rhinitis (CA08), Aspergillus-induced allergic or hypersensitivity conditions (CA82.4), Chronic rhinosinusitis (CA0A), Asthma (CA23), Nasal polyp (CA0J), Hypersensitivity pneumonitis due to organic dust (CA70)
4A81	Allergic or hypersensitivity disorders involving the eye		Allergic conjunctivitis (9A60.02), Acute atopic conjunctivitis (9A60.01), Atopic keratoconjunctivitis (9A60.0Y)
4A82	Allergic or hypersensitivity disorders involving skin or mucous membranes		Allergic contact dermatitis (EK00), Photo-allergic contact dermatitis (EK01), Allergic contact utricaria (EK10), Allergic contact sensitization (EK12), Utricaria, angioedenma and other urticarial disorders (EB00-EB0Y), Atopic eczema (EA80), Allergy to substances in contact with the skin (EK5Y)
4A83	Allergic or hypersensitivity disorders involving the gastrointestinal tract		Allergic gastritis (DA42.4), Allergic duodenitis (DA51.3), Allergic or dietetic colitis (DB33.2), Allergic or dietetic enteritis of small intestine (DA94.2)
4A84	Anaphylaxis		

4A85	Complex allergic or hypersensitivity conditions		
4B03	Eosinophilia		
4A8Y	Allergic or hypersensitivity conditions of other specified type		
4A8Z	Allergic or hypersensitivity conditions of unspecified type		

The current disease taxonomy thus does not cover the information of disease endotypes describing asthma, rhinitis and atopic dermatitis, which are defined by distinct pathophysiological mechanisms. An EAACI Task Force is currently re-assigning allergic disease to categories linked to intrinsic disease biology together with traditional signs and symptoms, to improve understanding of disease mechanisms, prevention and treatment.

Large-scale epidemiological studies corroborate that the prevalence of allergic diseases has drastically increased over the last century. It is estimated that over 25% of the world's population is affected by hypersensitivity or allergic disorders. The prevalence of allergic diseases differs between countries, and peaks in highly-developed, industrialized regions[7]. The International Study of Asthma and Allergies in Childhood, conducted in three phases between 1995 and 2001, has concluded that as many as 1 in 5 children in industrialized countries experiences symptoms of atopic dermatitis, allergic rhinitis or asthma[8]. In 2014 the World Health Organization (WHO) estimated that over 300 million patients worldwide were suffering from asthma, and if the rising trend continues, this number is expected to grow to 400 million by 2025[9]. Despite important progress in the management of asthma, the disease still accounts for as many as 180.000 deaths annually[10]. The incidence of the most severe form of allergic disorders, anaphylactic reactions, has also increased as shown by the rates of hospitalisations due to anaphylactic shock, with food and drugs as the main triggers[11]–[13].

Most epidemiological studies focus on the prevalence of asthma, while only a minority assess the burden of food allergies and atopic eczema[14]. Different research methods have been

employed in these studies, including public health databases and non-standardized patient-reported questionnaires, rendering direct comparison of the results impossible. However, despite methodological incompatibility, it is clear that variability in the prevalence and severity of allergic diseases is linked to the characteristics of the patients' local environment.

The highest prevalence of allergic diseases has been reported in highly urbanized areas and non-affluent neighbourhoods[8], [15]–[17]. Lower income and socio-economic family status has also been associated with a lesser disease control and higher number of emergency consultations. This is also reflected in the data collected by the Centre for Disease Control and Prevention (CDC)[18]. High exposure to pollution and unhealthy lifestyle in low-income industrialized areas has been suggested as the main factor driving the inequality in the prevalence of allergic diseases between communities within the same region[19]. Despite a significant progress in the understanding of the pathogenesis of allergic sensitization, current knowledge does not explain the patterns of prevalence of allergic diseases.

The socio-economic cost of allergic diseases includes decrease in the quality of patient's life and premature deaths, direct costs of healthcare, chronic complications and comorbidities associated with allergies, and indirect costs measured by work absence, loss of productivity and presentism[20]. Atopic dermatitis, affecting over 10% of children in the United States, is estimated to generate a yearly cost of over $5 billion[21]. The estimated annual direct cost of only airway allergies in the United States is about $20 billion[5]. This sum is more than doubled when considering the total incremental cost of asthma to society, calculated to be around $56 billion yearly[22]. The disease impairs normal activity in children, already causing a significant decrease in the quality of life, which aggravates with the patient's age, as long-term complications of the disease arise[23], [24]. Importantly, implementation of accurate preventive measures and appropriate disease control could alleviate the burden of asthma, especially in high-risk patient groups[25]. The variability in the severity of the impact of allergic diseases follows the same patterns as disease prevalence[26], [27]. While further proving severe socio-environmental health inequalities in regards to allergic diseases, this represents an important opportunity for researchers and healthcare professionals. In many countries worldwide asthma has been recognised as a priority disorder in government health strategies. Identifying links between the environment, lifestyle and disease susceptibility

could lead to the development of better preventive measures and further improvement in the management of allergic diseases.

1.2. Overview of immune mechanisms in allergic diseases

1.2.1. Immune tolerance and allergic sensitization

The pivotal role of the immune system is to sense and respond to harmful triggers from the outside world, while remaining actively unresponsive to self antigens and innocuous foreign antigens. This state of *immune tolerance* is maintained by a diverse range of processes that prevent responses detrimental to the host.

Two main mechanisms prevent the maturation and activation of potentially self-reactive lymphocytes: central and peripheral tolerance. Central tolerance refers to the regulatory mechanisms at the early stages of T and B cell development in the generative lymphoid organs. These include clonal deletion, clonal diversion, receptor editing and anergy of thymocytes and immature B cells expressing autoreactive T or B cell receptors (TCR or BCR, respectively)[28], [29]. In this way, potentially self-reactive lymphocytes are eliminated.

Peripheral tolerance is exerted in the peripheral lymphoid tissues, where unresponsiveness to self antigens is maintained. Naïve lymphocytes require costimulatory signals for eliciting the adaptive immune response, survival and expansion[30]. Such signals are provided in a complex spatiotemporal manner by antigen-presenting cells (APCs). Lack of the costimulatory signal can result in failure to recognize antigens, termed clonal ignorance, or lead to apoptosis or anergy (functional inactivation without cell death)[30].

These forms of immune tolerance maintenance, however, do not explain how the immune system differentiates between potentially harmful and inoffensive challenges from the outside world. Host's barrier tissues, such as the skin and mucosal membranes of the respiratory and the gastrointestinal tract, are constantly exposed to countless antigens of microbial and environmental origin. Cellular components of the barrier tissues are equipped with a range of pattern recognition receptors (PRR) sensing pathogen- and damage-associated molecular patterns (PAMP and DAMP, respectively)[31]. Molecular patterns recognized by PRRs are highly conserved products of microbial metabolism, such as bacterial lipopolysaccharide or fungal β-glucan. In physiological conditions, these molecular triggers remain in the lumen of the gastrointestinal and respiratory tract, both colonized by an

abundance of commensal bacteria and fungi. Interestingly, the same PRRs which contribute chiefly to defence against pathogens also recognize lumen-derived ligands from the commensal microbiome[32]. This steady-state constitutive activation of PRRs ensures the production of immune mediators protective to the epithelial barrier integrity, controlling the composition of commensal microbiota and inducing tolerance to foreign antigens[33]. However, binding of specific microbial molecules to PRRs on the immune cells populating the lamina propria of mucosal barrier tissues induces production of proinflammatory cytokines, which rapidly recruit innate immune cells to the infection site. These early responses are followed by activation of the adaptive immunity – a cascade of immune events aiming to protect the host from pathogen invasion[34].

The mucosal barriers come in contact with not only microbial components, but also ingested food-derived molecules, inhaled plant- and animal-derived molecules etc. While most of these harmless molecules remain invisible to the immune system, in allergy the immune tolerance to specific proteins (allergens) breaks, resulting in a reproducible, exaggerated reaction. Some of the most common allergens have been found to interact with the PRRs and elicit proinflammatory responses. For example, two frequent allergens: Der p 1 (house dust mite protein) and Fel d 1 (secretoglobin, protein produced by cat's secretory glands) bind bacterial lipopolysaccharide and therefore interact with bacteria-sensing PRRs. Moreover, bacterial contaminants have frequently been identified on pollen. Other allergens have been shown to bind PRRs directly, such as peanut or cockroach proteins[35], [36]. Finally, certain identified allergens function as proteases and may degrade epithelial tight junctions, causing a breach in the barrier integrity[37]. Damaged epithelial cells produce proinflammatory mediators and induce a pronounced immune response. Adaptive immune responses to allergens include antigen presentation by dendritic cells, activation and clonal proliferation of antigen-specific T and B lymphocytes, and the production of IgE antibodies specific for the allergen. These responses will be described in further detail in section 1.2.3.

With the steady increase in the prevalence of allergic diseases over the last century, several hypotheses on allergic sensitization have been proposed. It has been postulated that frequent use of antibiotic and excessive hygiene negatively affects commensal microbiome – host interactions, resulting in an increased susceptibility to allergic disorders. Latest research has shown that timely exposure to a diverse range of microbial triggers has a protective effect on

immune tolerance[38], [39]. Another popular concept relates to the aggravating pollution of the industrialized world. According to this hypothesis, omnipresent pollutants cause chronic micro damage to the mucosal barrier tissues and allow penetration of harmless allergens through the epithelial layers[40]. Strikingly, it has been demonstrated that household detergents even in very low concentrations can also cause epithelial cell damage[41]. As a result, allergens come into contact with tissue-resident immune cells and provoke immune responses. Lastly, the surge in allergic disorders has also been linked to a change in eating habits, including diet rich in fat and sugars (Western diet). This type of diet contains high amounts of monounsaturated fatty acids and advanced glycation end-products, of which the molecular structure resembles a DAMP family of proteins, named the alarmins. Intake of food rich in monosaturated fatty acids and advanced glycation end-products may thus result in allergic sensitization to bystander proteins[42].

To summarize, immune tolerance is an active state of unresponsiveness to self- and innocuous foreign antigens, maintained by all components of the immune system. A breach in immune tolerance is therefore a systemic disorder in the functioning of the immune system, with implications for host-microbiome interactions, susceptibility to infections and sterile inflammation. Global changes in lifestyle have been linked to the increase in allergic sensitization and malfunction of the immune system.

1.2.2. Hypersensitivity reactions

Although non-allergic hypersensitivity and allergic hypersensitivity reactions have been classified under the same group of immune system disorders, they are driven by different immune mechanisms. Four main immune mechanisms of hypersensitivity have been identified and associated with different disease entities, as proposed by Gell and Coombs in 1968[43] (Table 2).

Table 2. The Gell and Coombs classification of hypersensitivity reactions, based on different pathophysiological mechanisms.

Reaction type		Timeframe of immune response	Disease examples
Type I	IgE -mediated hypersensitivity	Immediate	Allergic rhinitis, ocular allergic conjunctivitis, atopic dermatitis, soft tissue angioedema, allergic asthma, anaphylaxis
Type II	IgG or IgM antibody-mediated hypersensitivity	Immediate	Autoimmune hemolytic anemia, immune thrombocytopenia, pemphigus vulgaris, Goodpasture syndrome, Graves disease, myasthenia gravis
Type III	Immune complex-mediated hypersensitivity	Immediate	Membranous nephropathy, reactive arthritis, systemic lupus erythematosus; extrinsic allergic alveolitis (hypersensitivity pneumonitis)
Type IV	Cytotoxic, cell-mediated hypersensitivity	Delayed	Contact dermatitis, coeliac disease, Hashimoto thyroiditis, host's reactions against graft

It should be noted that Type IV hypersensitivity can be further divided into subdivisions, namely Type IVa, Type IVb, Type IVc and Type IVd, corresponding to types of immune responses and pathological characteristics [44]. Type IVa is typically related to an IFN-γ mediated Th1 immune response, whereas Type IVb largely relies on IL-5 and IL-4 signalling for Th2 immunity encompassing eosinophilic inflammation. Type IVc relies on cytotoxic T cell activation with $CD4^+$ or $CD8^+$ mediated cell killing. Lastly, the hallmark of Type IVd is neutrophil recruitment and activation[44].

Type I hypersensitivity reactions underlie the immune mechanisms of allergic diseases. After allergic sensitization, IgE antibodies are produced by allergen-specific clones of mature B cells (plasma cells)[45]. In physiological conditions, this antibody class is chiefly involved in host defence against parasitic infection, which is reflected by the distribution of IgE at anatomical

sites prone to parasite entry: mucosal-associated lymphoid tissues in the airways and in the gastrointestinal tract[46]. Several innate immune cell types, namely mast cells, basophils and activated eosinophils, express high affinity receptors for IgEs antibodies. Upon repeated exposure to the allergen, cross-linking of the IgE-receptor complexes occurs, leading to activation of the sensitized cells and massive release of anaphylactogenic mediators that are responsible for the immediate hypersensitivity reaction[43].

1.2.3. Type 2 immune response

The type 2 immune response is a subset of so-called cell-mediated immunity, mediated by $CD4^+$ T lymphocytes. This immune response is occupied with phagocyte-independent defence against extracellular parasites such as helminths and extracellular bacteria (Figure 1). However, inappropriate activation of type 2 immune responses is a hallmark of allergic reactions, for example in allergic rhinitis, allergic asthma etc. Cytokines essential in the initiation of the Th2 immune response are IL-4 (interleukin 4), the cytokine most commonly associated with Th2 cells, IL-25, IL-33 and thymic stromal lymphopoietin (TSLP), produced by epithelial cells, basophils, eosinophils and mast cells[47]. Exposure of $CD4^+$ T cells to IL-4 will induce Th2 cell differentiation via binding to the IL4R. This will initiate phosphorylation and activation of Janus kinase 1 (JAK1) and JAK3, which in turn leads to the recruitment and activation of signal transducer and activator of transcription 6 (STAT6). STAT6, together with TCR signals will induce expression of the transcription factor GATA3[48]. Aside from stimulating Th2 cytokine expression, GATA3 blocks Th1 differentiation by suppressing expression of the IL-12 signalling pathway, sustaining the Th2 immune response. TSLP activates transcription pathways via binding with the TSLP receptor (TSLPR). This induces phosphorylation and activation of JAKs which in turn activate STATs including STAT1, STAT3, STAT4, STAT5a, STAT5b and STAT6[49].

Classical effector type 2 cytokines are IL-4, IL-5, IL-9 and IL-13. Alongside its role as Th2 inducer, IL-4 executes different effector function in relation to several cell types. It induces class switching in B cells with an antibody switch to the IgE and IgG4 isotype. Together with IL-13, IL-4 supresses Interferon-gamma-mediated (IFN-γ) macrophage activation and stimulates leukocyte recruitment via the promoting of adhesion molecule expression on endothelial cell surfaces and the secretion of chemokines[43]. IL-13 further stimulates goblet cell hyperplasia and mucus secretion in airway epithelial cells, aiding in the removal of

bacteria on epithelial surfaces during infections, while simultaneously serving as an large factor in airflow obstruction during allergic reactions[50] (Figure 1). IL-5 and IL-9 are the major drivers of, respectively, eosinophil and mast cell maturation, proliferation and activation[51].

Th2 cells thus maintain defence against helminthic and other extracellular infections via specialised mechanisms. B cells produce IgE antibodies stimulated by IL-4 bound to helminths or allergens which facilitates detection and attachment by IL-5-activated eosinophils, mast cells and basophils. These cells degranulate and release inflammatory mediators like histamine, leukotrienes and proteases, capable of breaking down helminth- and bacterial cell walls and perpetuating the Th2 immune response. Additionally, increased mucus production promotes the removal of bacteria and blocks further entry of invading organisms[43]. Lastly, IL-4 and IL-13 stimulate macrophages to produce anti-inflammatory cytokines and growth factors to initiate tissue repair. This results in scarring and fibrosis, another major component in allergic reactions.

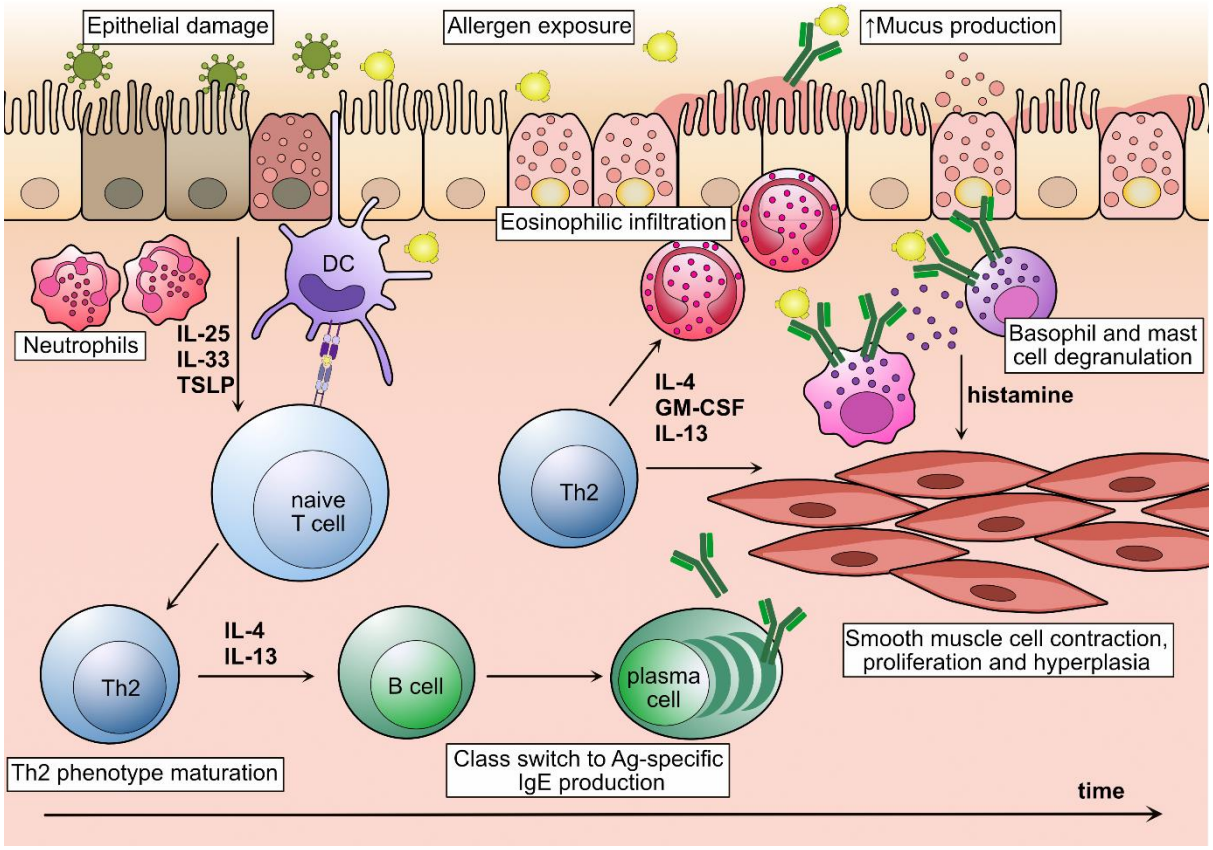

Figure 1. Initiation and propagation of Type 2 immune responses. Type 2 immune responses are provoked by helminths or allergens damaging the epithelial membrane. This leads to the secretion of IL-25, IL-33 and TSLP. These cytokines will activate dendritic cells which in turn will propagate the Th2 immune response via the stimulation of T cell differentiation towards a Th2 phenotype. Th2 cells in turn will produce IL-4 and IL-13, which in lymphoid organs will induce class switching of B cells to IgE production and in the affected tissue will activate eosinophils, mast cells and basophils. Basophil and mast cell degranulation will induce smooth muscle cell contraction, proliferation and hyperplasia. More immune cells are guided towards the site of inflammation and together they will orchestrate an immune response against helminths or induce an allergic reaction.

1.3. Asthma – clinical landscape, immunology and treatment

Asthma is a highly heterogenous, chronic, inflammatory disease of the respiratory system. The word *asthma* originates from the Greek *ásthma*, which means *panting* and was used to describe shortness of breath irrespective of the underlying cause[52]. In the 19th century a number of specific disease features has been ascribed to asthma, including paroxysmal character, the narrowing of airway lumen caused by both spasm of the bronchial muscles and swelling of the mucosal membrane, a link to allergic rhinitis, hypersensitivity to certain food types and familial occurrence[53]. Shortness of breath is paired with an increased production of thick mucus containing Charcot–Leyden crystals, which may plug smaller bronchi and worsen dyspnoea[54]. The symptoms vary over time and intensity, and can be triggered by infections, exercise, allergen or irritants exposure. While the clinical landscape of asthma remains unchanged, our understanding of the pathomechanisms underlying the disease has

improved greatly. Already in early scholarly descriptions, it had been acknowledged that rather than a symptomatic term, asthma should be perceived as a spectrum of respiratory tract pathologies, comprising different disease phenotypes and endotypes[55].

The following main complex phenotypes of asthma have been identified with relevance to clinical presentation and immune mechanisms[55]:

1. Early-onset allergic asthma
2. Early-onset allergic moderate-to-severe remodelled asthma
3. Late-onset non-allergic eosinophilic asthma
4. Late-onset non-allergic non-eosinophilic asthma

The proposed classification has been based on the age of disease onset and the presence or absence of allergic immune response underlying the disease pathogenesis. Of note, different classification systems for asthma phenotypes exist, focusing on the severity of symptoms and response to treatment, composition of the immune cell infiltrate in the inflamed airway, or exacerbating factor (aspirin-induced, exercise-induced, obesity-related, occupational asthma and other) [56]–[59]. Figure 12 (reprinted from [60]) illustrates the continuum of asthma heterogeneity. Further, the asthma subtypes as defined by the ICD-11 are visible in Table 3.

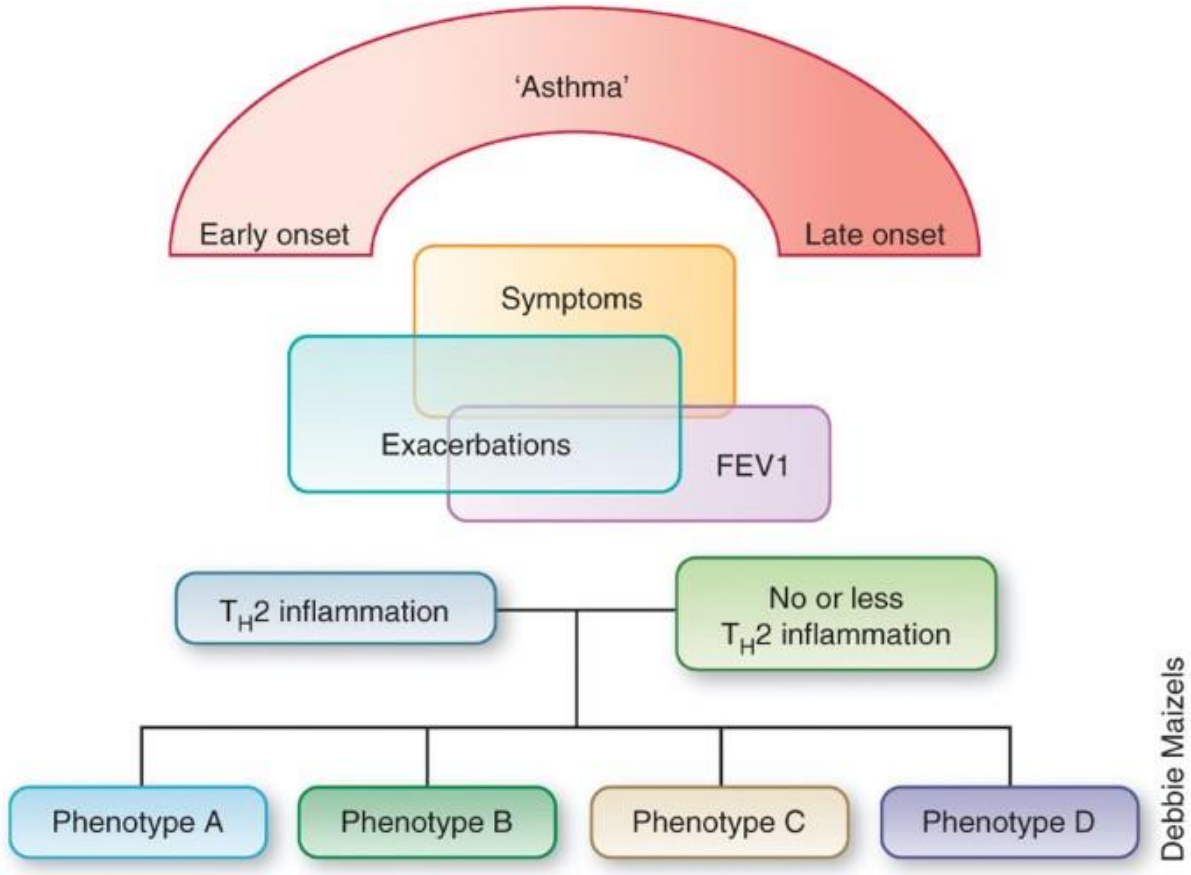

Figure 2. Schematic representation of the umbrella term 'asthma'. The key clinical features of severity (lung function, symptoms and exacerbations), inflammatory characteristics (particularly Th2 immunity) and their division into associated phenotypes are shown. However, these phenotypes have not yet been fully characterized. Reprinted from [60]

Table 3. Classification of asthma according to the 11th Revision of the International Classification of Diseases.

ICD-11 code	Asthma subtype	
CA23.0	Allergic asthma	
CA23.1	Non-allergic asthma	
CA.23.2	Other specified forms of asthma or bronchospasm	
	CA.23.20	Aspirin-induced asthma
	CA.23.21	Exercise-induced bronchospasm
	CA.23.22	Cough variant asthma
	CB02.0	Allergic pulmonary eosinophilia
	CA0A.0	Samter syndrome
CA.23.3.	Unspecified asthma	

Early-onset allergic asthma often begins at a pre-school age. Many children diagnosed with asthma have previous medical history of atopic eczema and food allergy[3]. A symptom pattern characteristic for this asthma phenotype is wheezing (high-pitched whistling sound made while breathing due to narrowing of the airways), often aggravating at night and reaching its peak in the morning. Wheezing is frequently provoked by upper respiratory tract infections or allergic exposure. It is, however, not exclusively specific for asthma, and up to 30% of children under three years of age may present with wheezing during common cold, and not develop asthma[61]. Therefore, only recurrent episodes of wheezing in children older than three years of age suggest the diagnosis of asthma. Children with asthma are more susceptible to respiratory infections, and these tend to be more sever and persist longer than in age-matched healthy children. Frequent viral infections at a very young age have been suggested to play a role in the development of early-onset asthma[62]. In this model, repetitive damage to the bronchial epithelium and activation of the resident immune cells cause a switch towards type 2 immunity and facilitate allergic sensitization [63]. Viral infections are also responsible for the majority of asthma exacerbations in children[64]–[66]. Asthmatic patients have been shown to have defective antiviral responses [67], [68]. Furthermore, it has been shown that use of antibiotics is much higher in asthmatic children compared to their non-asthmatic peers[69], [70]. A possible causal relationship has been suggested, pointing towards the importance of physiological host-microbiome interactions in the development of allergic diseases[71].

An important hindrance in the diagnosis of asthma in pre-school children is the difficulty in objective measurement of pulmonary function[72]. Functional respiratory tests remain the cornerstone of asthma diagnostics in adults and older children. Limitation in expiratory airflow and/or abnormally high variability in lung function belong to the diagnostic criteria formulated by Global Initiative for Asthma (GINA)[73]. These are assessed during spirometry on the basis of forced expiratory volume in the first second (FEV1) and peak expiratory flow (PEF). In clinical practice, airflow obstruction in asthma is characterized by variability and reversibility; these features refer to fluctuating improvement and deterioration in symptoms and lung function, and responsiveness to bronchodilator agents, respectively.

Allergic asthma is driven by type 2 immune responses and classified as type 1 hypersensitivity reaction. Following allergic immunization, allergen-specific IgE immunoglobulins are

produced by plasma cells. IgE antibodies bind to high-affinity Fcε receptors on the surface of basophils and mast cells present in the bronchial subepithelial layer. Allergen binding results in cross-linking of IgE-receptor complexes and activation of granulocytes, followed by immediate release of proinflammatory mediators. Immune mediators specific to basophils and mast cells include histamine, neural proteases and proteoglycans, IL-4, IL-5, IL-6 and IL-13 and arachidonic acid derivatives such as leukotrienes and prostaglandin D2[74]. Histamine is a central immune mediator in the pathogenesis of asthma attacks, by not only directly causing bronchoconstriction, but also regulating the maturation and activation of leukocytes[75], [76]. Histamine exerts its functions via four transmembrane receptors, belonging to the family of G protein-coupled receptors[77]. Downstream signalling differs between the receptor subtypes, with H_1 receptor (HR1) coupling to Gq protein and regulating Ca++ mobilization, H_2 receptor (HR2) coupling to Gs protein and stimulating cyclic adenosine monophosphate (AMP) production, while the H_3 (HR3) and H_4 receptor (HR4) inhibit cyclic AMP accumulation[78]. Thus, the effect of histamine signalling depends on the activated histamine receptor and cell type expressing it. With relevance to allergic inflammation, histamine signalling via HR1 and HR2 has been shown most important. H1R is expressed by a range of immune cells, including mast cells and basophils, eosinophils, macrophages and T and B lymphocytes. It promotes cellular migration by direct activation of the immune cells and vasodilatation. It is further underscored by the fact that antihistaminic drugs targeting HR1 reduce eosinophil tissue infiltration[79]. Activation of HR1 on smooth muscle cells results in rapid bronchoconstriction. Moreover, histamine induces proliferation of bronchial smooth muscle cells *in vitro*[80]. HR2 signalling modulates the production of gastric acid and airway mucus. HR1 and HR2 exert opposite effects on adaptive immunity responses, with HR1 promoting antigen presentation and cellular immunity, and HR2 acting as a repressor of type 2 immunity[78].

In the first phase of the allergic immune response, allergen binding to the specific IgE antibodies leads to basophil and mast cell activation. Basophil and mast cell degranulation promotes eosinophil recruitment and activation. These act as effector cells and secrete highly charged basic proteins (major basic proteins and eosinophil cationic protein), peroxidase and several cytokines. The content of eosinophilic granules is toxic to bronchial epithelial cells,

causing further damage and perpetuation of inflammation. Eosinophil activation is further reinforced by Th2 cell function.

Eosinophils are the predominant infiltrating immune cell population in allergic asthma. Accordingly, patients with allergic asthma respond well to inhaled corticosteroids, which are the mainstay of therapy in allergic diseases[81]. In case of persistent asthma symptoms despite correct administration of medication, additional therapy with bronchodilators or other mode of action anti-inflammatory drugs should be considered. Short course treatment with oral corticosteroid can be considered in patients with severe, uncontrolled asthma exacerbations. Long-term intake of oral corticosteroid is to be avoided. Novel therapeutics have recently been developed for the treatment of severe allergic asthma. Biological medications interfering with IL4R and IL5R signalling, as well as agents directly blocking IgE antibodies, have been approved for patients with the most severe form of allergic asthma[82]. Figure 3 presents the current therapeutic strategy in adults and adolescents diagnosed with asthma (reprinted from the 2022 update of the Global Strategy for Asthma Management and Prevention [83]).

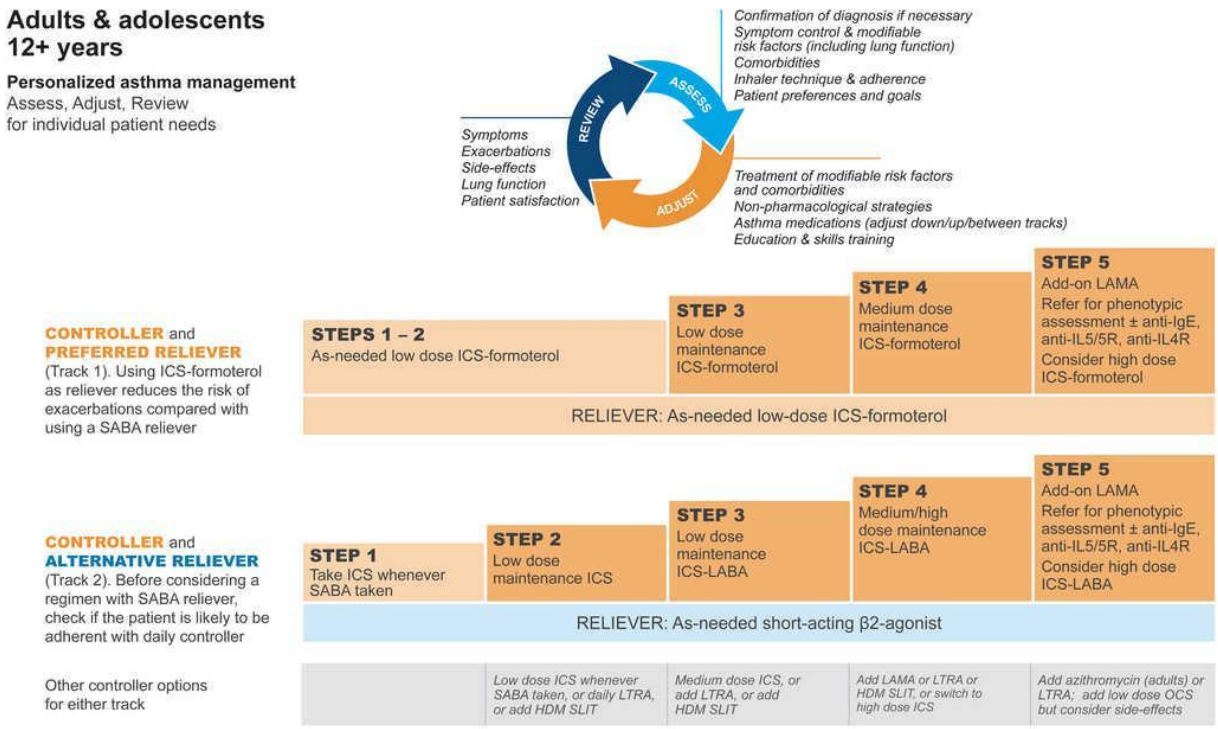

Figure 3. Therapeutic strategy in adults and adolescents diagnosed with asthma [83]

Regular and correct intake of medication is key to successful management of asthma and prevention of long-term complications. Due to the intermittent occurrence of disease symptoms, adherence to treatment among asthmatic patients is poor[84]. Many patients with a mild form of the disease who do not experience symptoms at rest, neglect controller medication and only rely on reliever drugs. Despite vast evidence of safety and minimal side-effects of inhaled corticosteroids, steroid-phobia is often reported[85]. Contrary to frequently expressed fears, inhaled corticosteroids decrease infection rates in asthmatic patients. Poor compliance has been linked to more frequent exacerbations and worse disease control followed by unfounded step-up in therapy[84]. Controlled asthma is defined as occurrence of disease symptoms requiring reliever medication less often than twice per week[86]. The level of disease control encompasses not only the patient's symptoms and limitations, but also the future risk of exacerbations and long-term disease complications[87].

Airway remodelling is the chief long-term complication of uncontrolled asthma. Chronic low-grade inflammation and recurrent flares result in permanent narrowing of the airways due to structural changes in the bronchial walls[88]. These include alterations of the bronchial epithelium, sub-epithelial membrane thickening, deposition of extracellular matrix and severe dysfunction of the smooth muscle tissue surrounding the airways[89]. Impaired relaxation-contraction function, accompanied by hypertrophy and hyperplasia of bronchial smooth muscle cells (BSMCs) is observed in asthmatic patients[90]. Increased BSMCs mass has been suggested to be the main denominator of airway remodelling, chiefly contributing to persistent narrowing of the airways[89]. It has been linked to more severe disease and persistent airflow obstruction[91], [92].

1.4 Bronchial smooth muscle cells in asthma

Bronchial smooth muscle cells (BSMCs) are active players in the pathophysiology of asthma, as they directly regulate the airway lumen, respond to a plethora of immune mediators and secrete cytokines and chemokines perpetuating the inflammatory cycle[89]. BSMCs react to inflammatory stimuli via a variety of receptors expressed on their membranes. IL-4 and IL-13, two key cytokines central in the pathogenesis of asthma produced by CD4+ T cells, basophils, mast cells and eosinophils, promote inflammatory processes and airway remodelling via binding to type I (IL4aR/common γ chain) or type II (IL4Ra/IL13Ra1) receptor systems[93]. Binding of the cytokines activates STAT6-dependent signalling pathways, inducing airway

hyperresponsiveness, smooth muscle cell proliferation, airway contractility and impaired responsiveness to muscle relaxants such as B-agonists[94]. As stated previously, histamine is another potent mediator of airway inflammation and interacts with BSMCs via histamine receptors HR1 and HR2 with the former promoting and the latter repressing Th2 type immune responses. HBSMs further express glucocorticoid receptors (GRs) on their membrane capable of interacting with glucocorticosteroids (GCs), anti-inflammatory drugs able to curve hypersensitivity responses and achieve asthma control[95]. Activated GRs bind to GC-responsive genes, stimulating expression of B_2-adrenergic receptors and anti-inflammatory proteins and preventing airway smooth muscle cell remodelling. In activated Th2 cells, GRs inhibit the transcription factor GATA3 in charge of regulating Th2 cytokine [96], [97].

Chemokines produced in large quantities by BSMCs amplify signals derived from inflammatory cells and produce a powerful chemotactic stimulus driving further recruitment[98] (Figure 4). BSMCs are capable of producing eotaxin, monocyte chemoattractant proteins (MCPs), IL-8 and IL-6, among others[99]. Briefly, eotaxin production is induced in response to the Th1 cytokines TNF and IL-1β, and Th2 cytokines IL-4 and IL-13 and drives eosinophil recruitment. MCPs are found in three isoforms, MCP-1 to 3, which are chemotactic mainly to monocytes (MCP-1), lymphocytes (MCP-2) and eosinophils, NK-cells, basophils and dendritic cells (MCP-3)[100]. IL-8 potently chemoattracts and activates neutrophils while evidence also points towards this chemokine exerting an effect on cell proliferation, contraction and survival in human BSMC (HBSMC)[99], [101]. Lastly, IL-6 is a pleiotropic cytokine mediating T cell infiltration into the lungs with immunoregulatory roles such as regulating effector $CD4^+$ T cell fate, promoting IL-4 production during Th2 differentiation, preventing Th1 differentiation and stimulating Th17 differentiation[99], [102].

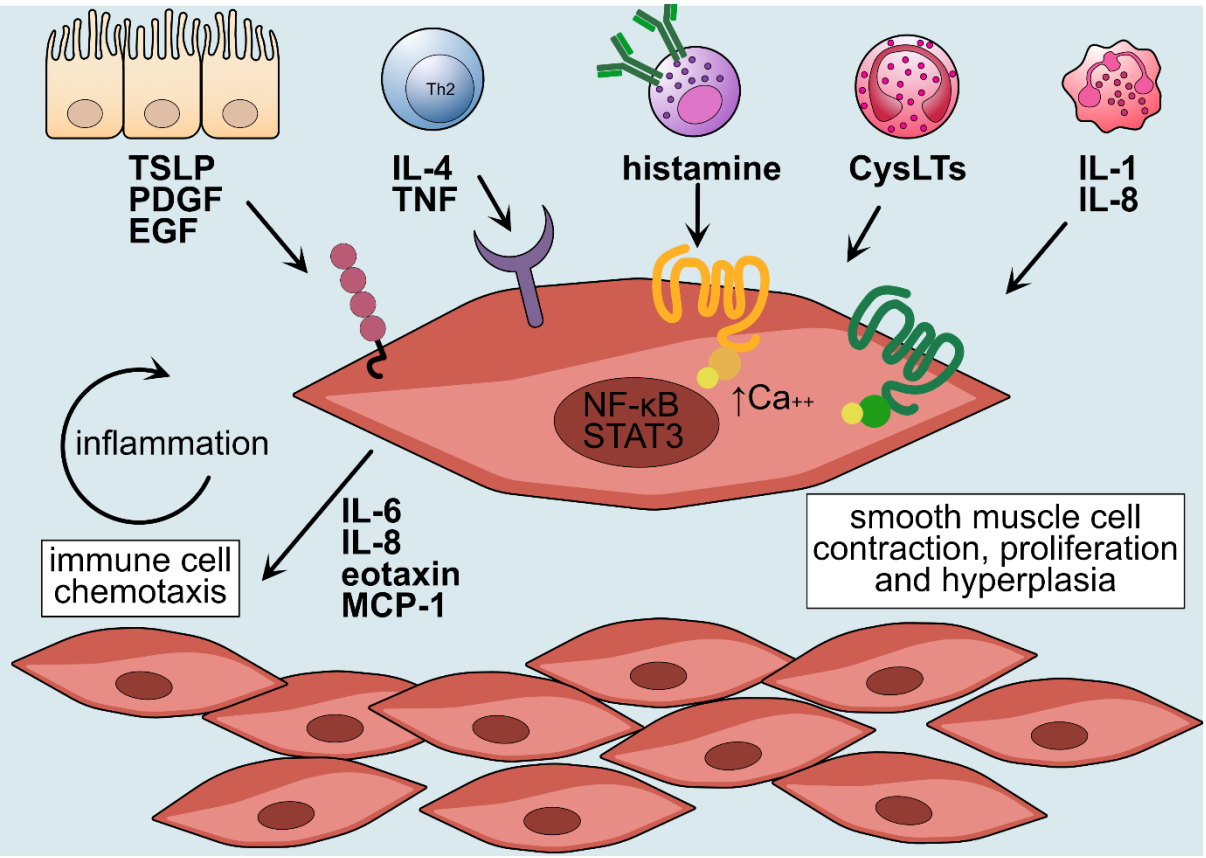

Figure 4. BSMCs respond to various inflammatory stimuli such as epithelium derived TSLP, Th2 cell produced IL-4 and TNF, histamine from mast cells and IL-1 and IL-8 from neutrophils, with different receptors on their membrane. BSMCs then amplify these signals via production of chemokines like eotaxin and MCP-1, further driving immune cell recruitment to the site of inflammation. Aside from promoting inflammatory processes, the HBSC also undergo airway remodelling via increased proliferation and hyperplasia.

Immune mediators secreted at each stage of allergic inflammatory response have been shown to induce BSMC hyperplasia. Allergen exposure-driven mast cell and basophil activation result in a secretory burst of histamine and lipid mediators such as thromboxane and leukotriene, found to promote BSMC proliferation[103], [104]. Infiltrating neutrophils release elastase and reactive oxygen species (ROS), which activate BSMC mitogenesis[105]. In later phases of the allergic immune response, inflammation- and hypoxia-induced mediators and growth factors are produced, further inducing BSMC hyperplasia[106], [107]. Not only the tissue-resident and infiltrating immune cells influence BSMC function. It has been shown that upon allergen exposure and viral infections, bronchial epithelial cells produce IL-6, IL-8 and metalloproteinase-9, which induce BSMC proliferation[108], [109]. Therefore, in uncontrolled asthma, repetitive activation of the immune system results in constant production of mediators promoting BSMC proliferation and hypertrophy, the hallmark of airway remodelling. Moreover, inflammatory mediators increase BSMC responsiveness to

contractile agonists and down-regulate BSMC expression of β_2 receptors, leading to lower response to bronchodilators[110].

Despite clear correlation between asthma control and the incidence and degree of airway remodelling, certain patients are more prone to developing this complication. The susceptibility is believed to have genetic background[111]. For that reason allergic asthma with airway remodelling has been recognized as a separate disease phenotype. Both allergic phenotypes are nevertheless driven by type 2 immune responses and share multiple pathogenetic traits.

A distinct immune background characterizes non-allergic asthma. The reported prevalence of this disease phenotype varies greatly between publications (from 10 % to over 40% of all asthma patients), making it difficult to estimate the real frequency of the pathology[112]–[115]. Non-allergic asthma is typically diagnosed at a later age in patients without atopic comorbidities[116]. Immune cell infiltrate in non-allergic asthma is varied, dividing it further into eosinophilic, neutrophilic, mixed and paucigranulocytic phenotypes[117].

Non-allergic eosinophilic asthma is often diagnosed in adulthood and is associated with chronic rhinosinusitis and nasal polyposis resistant to inhaled and nasal corticosteroids[118]. Hypersensitivity to non-steroidal anti-inflammatory drugs (NSAIDs), also referred to as aspirin-exacerbated respiratory disease, is also commonly seen in these patients[119]. While the origin of eosinophilia in allergic asthma has been extensively studied, the triggers for eosinophilia in non-allergic eosinophilic asthma remain to be elucidated. Type 2 innate lymphoid cells (ILC) have been shown to perpetuate eosinophilic infiltration in this disease phenotype[120]. This discrepancy in immune pathways leading to the emergence of seemingly similar disease phenotypes urges towards more in-depth investigation into the early events in asthma pathogenesis, and the need to define disease endotypes.

Non-allergic non-eosinophilic asthma phenotypes are associated with smoking, exposure to inhaled pollutants and obesity[121]–[125]. Smoking has been shown to induce neutrophilia in the airways of both asthmatic and non-asthmatic individuals[122]. In non-allergic asthma type 1 and type 17 immune responses predominate over type 2 reactions. Main immune mediators include IL-1β, IL-17, IL-33 and type I interferons[126], [127]. IL-17 is mechanistically linked to airway hyperresponsiveness and has been correlated with asthma severity and

resistance to inhaled corticosteroids[128]. Moreover, IL-17 signalling has been shown to contribute to BSMC hyperplasia[129].

Of importance, the prevalence of both allergic and non-allergic asthma has been increasing steadily. Latest clinical studies point towards the importance of patient phenotyping and stratification prior to initiating therapy in order to achieve best therapeutic response. While several lines of treatment, including targeted biological therapies for allergic asthma exist, the range of therapeutic possibilities is much smaller for patients with non-allergic asthma phenotypes. The link between comorbidities and immune events leading to asthma development require further investigation.

2. Metabolic syndrome and obesity

In parallel to the increase in the prevalence of allergic diseases, the rates of obesity have been surging worldwide. Obesity is defined as excessive body weight and accumulation of adipose tissue accompanied by chronic, low-grade, systemic inflammation[130]. It is an important component of the metabolic syndrome, defined by the Joint Interim Statement of the International Diabetes Federation Task Force on Epidemiology and Prevention; National Heart, Lung, and Blood Institute; American Heart Association; World Heart Federation; International Atherosclerosis Society; and International Association for the Study of Obesity, as the cooccurrence of at least three of the following five criteria[131]:

- Visceral obesity: a waist circumference of ≥ 94 cm in men and ≥ 80 cm in women in the West (values depend on country-specific definitions)
- Hypertriglyceridaemia: ≥ 150 mg per dL or on triglyceride-lowering medication
- Low levels of high-density lipoprotein cholesterol: < 40 mg per dL for men and < 50 mg per dL for women
- Elevated blood pressure: systolic blood pressure of ≥ 130 mmHg, diastolic blood pressure of ≥ 85 mmHg or antihypertensive drug treatment in a patient with a history of hypertension
- Increased glucose levels: fasting glucose levels of ≥ 100 mg per dL or drug treatment to lower increased levels of glucose

Of note, different definitions of metabolic syndrome exist, and the 2009 Joint Interim Statement applies the most stringent criteria to diagnose the disorder. Metabolic syndrome

is a predictor of insulin resistance, type 2 diabetes and cardiovascular disease[132]. It is associated with increased cardiovascular and overall mortality[133]. Importantly, an epidemiological link between obesity, metabolism dysregulation and asthma has been established[134]. The incidence of asthma has been shown to be higher in individuals with metabolic syndrome[134]. In overweight children asthma incidence increases by as much as 20%, the risk being highest for children with obesity [135]. Since 2015, obesity is listed as a major risk factor for asthma in children by the US CDC[136]. The exact pathophysiological links between asthma and metabolic syndrome remain to be elucidated.

2.1. Epidemiology of obesity and metabolic syndrome

According to the WHO, over 2 billion people worldwide are overweight or obese[137]. Obesity is often referred to as the pandemic of the 21st century, as the prevalence has risen dramatically and globally in the last 20 years. For statistical purposes, overweight is defined as a body mass index (BMI), which is a calculated proportion of adult's weight to height, equal to or more than 25. Obesity then compromises a BMI of more than 30.

Since 1999 the prevalence of obesity among adults in the United States has increased from 30.5% to 41.9% in 2020 [138]. In Europe, as much as 53% of adults are overweight[137].

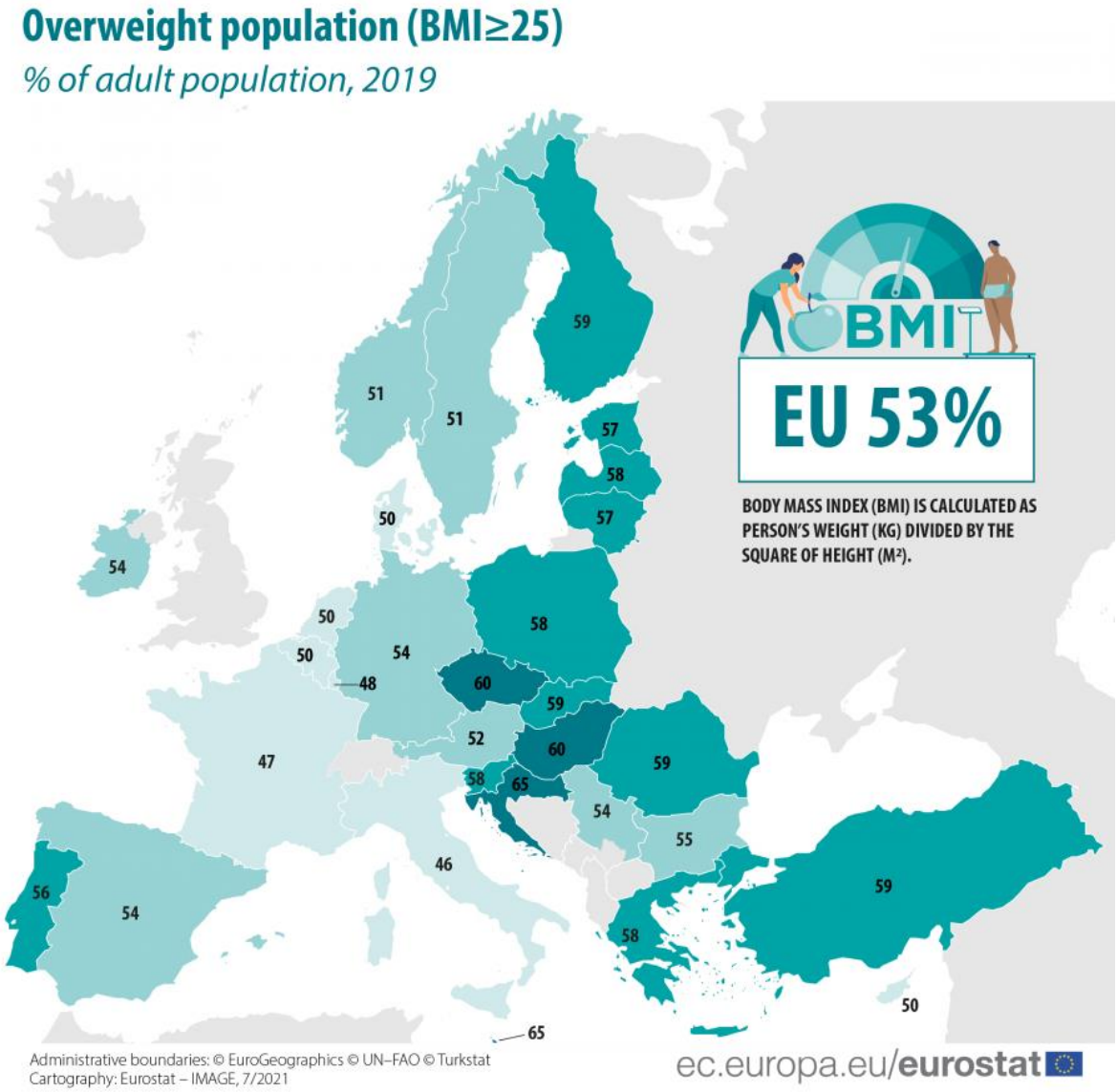

Figure 5. Overview of the overweigh population of Europe based on an average BMI ≥ 25, taken from Eurostat [137]

Shockingly, the pandemic of obesity is not confined to the adult population. The prevalence of obesity among children has been rising alarmingly, from just 4% in 1975 to over 18% in 2016[139]. It is estimated that over 125 million children worldwide are obese[139]. In children, overweight and obesity are defined upon scores based on the BMI standard deviation, adjusted for ethnicity[130].

Overweight and obesity are a global health challenge, affecting both high-income and developing countries. The burden is, however, disproportionately higher in certain ethical and social groups. In the United States, non-Hispanic black adults had the highest age-adjusted prevalence of obesity, reaching almost 50%, while non-Hispanic Asian adults were on the opposite end of the scale, with obesity prevalence of 16%. Obesity in adults and children has

been linked to lower socioeconomic status[140]. An updated meta-analysis of studies on the relation of socioeconomic status and obesity showed that in developed countries, obesity is inversely correlated with income[141]. Higher income translates to purchase capacity of higher quality, healthier products. This correlation was particularly strengthened by education and occupation determinants of socioeconomic status. In developing countries, a shift in obesity patterns in relevance to socioeconomic status has been observed, as a country's annual gross national product increased, leading to the same trends as seen in high-income countries[142]. These findings are strikingly similar to the global patterns of allergic diseases burden, further underscoring the epidemiological link between the entities and the burning issue of health inequality.

Metabolic syndrome is strictly related to visceral obesity and reflects consequent metabolic dysfunctions. The prevalence of the disorder has been rising in parallel to obesity rates. In 2012, the prevalence of metabolic syndrome among US adults reached 34.2% [143]. By 2016, the proportion of affected adults rose to 37%, and is estimated to increase further[144]. In the European population, the prevalence of metabolic syndrome (defined upon more stringent criterium of waist circumference than the one proposed by the Joint Interim Statement) reached 24.3% in 2014[145]. Based on the chief contribution of certain components to the diagnosis of the syndrome, country-specific clusters of metabolic syndrome have been found. The prevalence of the disorder differs between European countries and is significantly higher in the Polish adult population: almost 30% of Polish women and 40% of Polish men are affected[146]. Two large-scale, population-based studies conducted in 2003-2005 and in 2013-2014, namely the WOBASZ and WOBASZ II studies, further showed a significant increase in the prevalence of metabolic syndrome in Poland over a 10 year period of time[146]. The main components of the syndrome were abdominal obesity and elevated blood pressure for Polish women and men, respectively[147].

As for obesity, the prevalence of metabolic syndrome differs among ethnic and social groups within a country. In Poland, the incidence of metabolic syndrome is higher among people living in rural areas[148]. Lower education level has been identified as a risk factor for metabolic syndrome worldwide. Interestingly, in the US the ethnic group-specific prevalence of metabolic syndrome does not match the ethnical differences in obesity, as non-Hispanic whites are more likely than non-Hispanic blacks to suffer from the disorder[144]. In Europe,

the prevalence is generally higher among men, while in Middle Eastern countries a much higher percentage of women, compared to men, is affected[149], [150].

A recent meta-analysis of 169 studies on the prevalence and risk factors of metabolic syndrome in children and adolescents showed that up to 5% of children globally suffer from the disorder[151]. The analysis included data from 44 countries, with almost equally high prevalence across them, pointing towards the global burden of metabolic disorder among the youngest.

2.2. Pathophysiology of obesity and metabolic syndrome

The main factors leading to obesity are undeniably overeating and sedentary lifestyle. To put it in a simplistic perspective, excessive caloric intake to energy expenditure leads to fat tissue deposition[130]. Even a small daily positive energy balance contributes to cumulative weight gain over time[152]. The current obesity pandemic is mostly attributed to the general decrease in physical activity paired with significant change in eating patterns, related to global increase of food availability and the quality and nutritional value of affordable alimentary products.

The consumption of highly processed food (for example soft drinks, packaged snacks, confectionary, shelf-stable dishes or reconstituted meat products) has been unequivocally linked with of developing obesity and steady increase in BMI values[153], [154]. This type of food has only been introduced to the market about a century ago, and its availability and selection range has been steadily increasing ever since. Highly processed food is characterized by palatableness, high caloric value and relatively low nutritional value. Consumption of highly and ultra-processed food is associated with overeating behavioural patterns[155].

Food intake is regulated by an intricate, multidirectional network of hormones and neurotransmitters. Among them, two hormones have been identified to have a major influence of energy balance: leptin and ghrelin[156]. Leptin is predominantly synthetised in the adipocytes and small intestine enterocytes. It activates leptin receptors (LEPR) in the hypothalamus, and negatively regulates food intake by providing satiety signals. Ghrelin, produced by the enteroendocrine cells of the gastrointestinal system, has a counter role to leptin and promotes food intake. Its production peaks at the beginning of the meal and steadily decreases after.

It has been found that peripheral blood concentration of leptin is higher in obese individuals and the negative feedback loop between white adipose tissue and hypothalamus-regulated food intake is impaired[157]. Resistance to leptin has been proposed as one of the mechanisms of obesity development[158]. Specific dietary sugars and lipids have been shown to induce insensitivity to leptin, linking not only caloric intake but also modern diet composition to the development of obesity[159].

The function of leptin, however, is not limited to providing satiety signals, as its receptors are widely expressed across various tissue and cell types. Experiments in animal models of obesity proved that leptin regulates immune cell function[160]. Leptin was demonstrated to modulate T cell function and increase IL-2 and IFN-γ production *in vitro*, and show proliferative and anti-apoptotic effects[161], [162]. It is plausible that the increase in levels of circulating leptin affects immune cell function in obese individuals.

Hormonal disbalance and inflammation in obesity are affecting systemic metabolism, as evidenced by the increased prevalence of insulin resistance and type 2 diabetes in obese individuals. Insulin plays a key role in the regulation of metabolic processes such as stimulation of glucose uptake, synthesis of lipids and cell growth, proliferation and differentiation[163]. Insulin resistance (IR) is a hallmark of obesity-related metabolic dysfunction. It leads to sustained hyperglycaemia, not compensated by the upregulated pancreatic production of insulin. Furthermore, IR was strongly associated with allergic asthma in a large Danish cohort study[164].

The link between obesity and metabolic syndrome is not linear and the role of genetic susceptibility and environmental factors has to be underscored. One popular hypothesis places chronic inflammation at the centre of a vicious circle propelling metabolic dysfunction and obesity[165].

Chronic, low-grade, systemic inflammation is a recognized phenomenon in obesity. It is mostly attributed to proinflammatory mediators' production by the adipose tissue, which constitutes of not only adipocytes, but also several types of immune cells. Among these mediators, leptin, IL-6, TNF and adiponectin have been suggested to influence systemic immune responses. Obesity-associated inflammation is thought to be an important driver of

the increased risk of other non-communicable diseases in obese patients, including allergic diseases and non-allergic asthma.

3. Links between allergic inflammation, asthma and obesity

The striking comorbidity between asthma and obesity, their dramatic rise in prevalence worldwide and association of poor asthma control with metabolic dysfunction urged researchers to further investigate the link between these entities [166] A clear link in the rise in prevalence of asthma and the prevalence of obesity is clearly visible from large nation-wide studies (Figure 6).

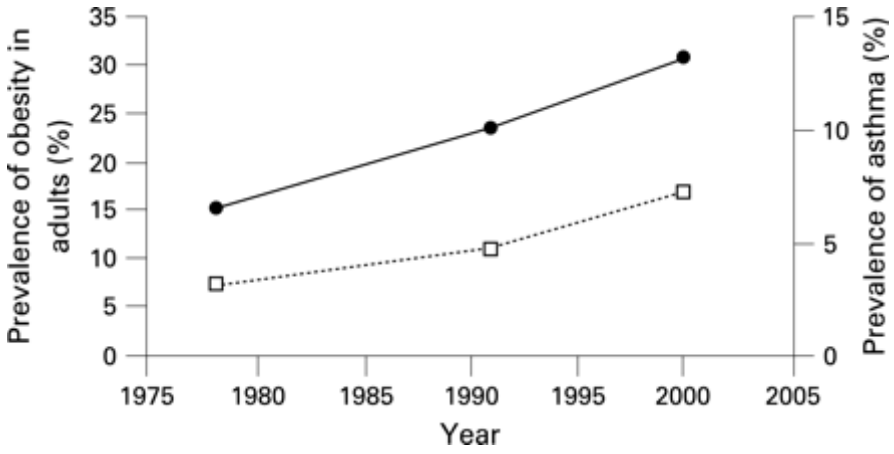

Figure 6. Trends in the prevalence of obesity and asthma over time in the US. Asthma, dotted line; Obesity, solid line. Figure reprinted from [166]

3.1. Epidemiological observations

Large-scale epidemiological studies show that overweight and obese individuals are more likely to have asthma, in comparison to the population characterized by normal range BMI. This association holds true for both children and adult patients across different countries. Furthermore, the prevalence of asthma is even higher in obese patients than in overweight individuals, suggesting a dose-dependent effect. Table 4 presents the results of major epidemiological studies on the prevalence of asthma with regards to overweight and obesity.

Table 4. The associations between overweight and obesity and the prevalence of asthma.

Year	Geographic area	Age group	Number of participants	Findings	Reference
1991-1993	Australia	Children	5,993	Higher BMI was associated with higher prevalence of atopy, wheeze and cough in girls only.	[167]
2010	Netherlands	Children	9,272	BMI-standard deviation scores (SDS) was related to current asthma in girls, but not in boys (adjusted odds ratio (OR) = 1.31 and = 1.01, respectively).	[168]
2014	Saudi Arabia	Children	1,264	BMI was associated with the prevalence of asthma in both pre-pubertal boys and girls (adjusted OR = 1.11 and = 1.38, respectively).	[169]
2011-2018	Germany	Children	157 with asthma and 471 without asthma	In atopic children, all overweight proxies were consistently positively associated with asthma prevalence.	[170]
1999 to 2004	USA	Adolescents	4,828	Overweight and obesity was associated with increased prevalence of asthma in girls but not in boys.	[171]
1970	Great Britain	Adults	8,960	The prevalence of asthma increased with increasing adult BMI. This association was not present for atopic eczema or hayfever.	[172]
1981	Netherlands	Adults	19,126	Severe overweight was associated with increased prevalence of asthma among women (OR = 1.80).	[173]
1995-1999	Sweden	Adults	309 with asthma and 352 without asthma	Increased BMI was a significant risk factor for incidence of asthma among both men and women (OR = 33 and 30, respectively).	[174]
1997-1998	USA	Adults	386 with asthma and	Increasing BMI and female sex were significant independent predictors of asthma prevalence.	[175]

			744 without asthma		
1995-2005	USA	Adults (women)	88,304	Abdominal obesity (defined as waist circumference > 88 cm) was associated with increased asthma prevalence, even among women with a normal BMI (adjusted OR = 1.37).	[176]
2001-2014	USA	Adults	Precise number unknown, data from the US National Health and Nutrition Examination Survey	The prevalence of asthma was higher in obese (14.6%) and overweight (9.1%) woman comparing to normal BMI range woman (7.9%). There were no significant differences in the prevalence of asthma among men stratified by weight status.	[177]
2012-2013	USA	Adults	2,860,305 overweight or obese adults	The relative risk of developing adult-onset asthma progressively increased with increase in BMI value categories, (12% increase for individuals with a BMI of 25.0–29.9 to almost 250% increase among individuals with a BMI ≥50 (HR = 2.49). The impact of obesity was greater for women than men.	[178]

Moreover, prospective observational cohort studies suggest that obesity has a causal effect on asthma development, as the incidence of asthma disease was higher among obese individuals than lean, age- and sex- matched persons.

Table 5 presents findings on the impact of overweight and obesity on asthma incidence.

Table 5. The impact of overweight and obesity on the incidence of asthma.

Time of observation	Geographic area	Age group	Number of participants	Findings	Reference

1974-1979	USA	Children	9,828	Obese girls had 2.2 times greater risk of incident asthma during 5-year follow-up.	[179]
1993-1998	USA	Children	3,792	The risk of new-onset asthma was higher among children with overweight or obese children, relative risk (RR) = 1.52, and = 1.60, respectively. The effect of obesity was higher in boys than in girls, and higher in nonallergic children than in children with atopic diseases.	[180]
1981-1998	Australia	Children, adolescents	2911	Increase in BMI z-score between 5 and 15 years of age was associated with an increased risk of asthma symptoms in adolescence.	[181]
1979-2000	USA	Children	4,393	Boys, but not girls, with BMIs $\geqslant$ 85th percentile were at increased risk of developing asthma (HR = 2.4)	[182]
1998-2008	USA	Children	285	Among children of asthmatic or atopic parents, overweight at 1 year of age was associated with a decreased risk of asthma and better lung function at the age of 6 and 8 years old. Overweight beyond infancy could confer a higher risk for asthma.	[183]
1995-1996	Taiwan	Adolescents	4,052	Girls with higher BMI had an increased risk of developing asthma during the 12-month follow-up period.	[184]
1971-1984	USA	Adults	9,456	The incidence of asthma was higher among obese individuals.	[185]
1975-1990	Finland	Adults	10,597	In a cohort of adult twins, obesity was a risk factor for new onset of asthma (multivariable adjusted OR = 3.00)	[186]

1990-1993	France	Adults (women)	67,229	Women with BMI ≥ 27 had a two-time higher risk of incident asthma than women with normal range BMI (multivariate RR = 2.02).	[187]
1990-2001	Northern Europe	Adults	16,191	Higher prevalence of obesity in study participants who reported new onset of asthma during the follow-up.	[188]
1991-1995	USA	Adults (women only)	85,911	The risk of developing adult-onset asthma increases steadily with the increase of BMI among adult women.	[189]
1994-1997	Canada	Adults	9,149	Obesity increases the risk of developing adult-onset asthma in women, but not in men (adjusted OR = 1.9 and = 1.1, respectively).	[190]
1994 to 2002	Norway	Adults	135,405	The relative risk of developing asthma was higher in obese and overweight men and women. The effect of obesity on asthma prevalence was higher in women.	[191]

The overall convergence of the results of these studies is high. Different trends on the impact of overweight and obesity on the prevalence and incidence of asthma incidence have been observed in relevance to age and sex[192], [193]. Among children, overweight and obesity has been shown to confer a higher risk of asthma in boys. It has also been associated with other atopic conditions, such as eczema and allergic rhinitis. A meta-analysis of 6 studies focusing on children obesity and asthma has shown that a positive correlation between BMI above the normal range and the incidence of asthma exists[135]. In adults, overweight and obesity has been associated with increased asthma prevalence and incidence chiefly among women, and often independent of atopy. This is in line with the epidemiological variation in new-diagnosis asthma phenotypes between children (predominantly allergic asthma) and adults (non-allergic asthma). Two main phenotypes of asthma comorbid with obesity have been described, with markedly different inflammatory profiles[194]. In early-onset allergic asthma, obesity increases disease severity and worsens control; in adult-onset non-allergic asthma, obesity seems to drive airway hypersensitivity. This phenotype, more prevalent in

women, is not associated with Th2 immune responses and is refractory to therapy with inhaled corticosteroids[195], [196]. Moreover, it is characterized by persistent decrease in lung function[195]. These associations have been repeated in independent study groups. The decrease in lung capacity with resistance to bronchodilators seen among obese patients could be the result of impaired ventilatory function, related to excessive adipose tissue deposition[197], [198]. Also in children populations, higher BMI has been associated with airway obstruction, but not allergic diseases[199].

The epidemiological links between asthma and metabolic syndrome are less evident. Several groups reported significant comorbidity of metabolic disorders (defined as the diagnosis of metabolic syndrome, insulin resistance or dyslipidaemia) and asthma[200]–[202]. Among the conditions defining metabolic syndrome, visceral obesity and impaired glucose tolerance have been associated with an increased incidence of asthma in adults and adolescents[134], [203]. In a 25-year follow-up US cohort, BMI has been found to be a stronger predictor of asthma development than the diagnosis of metabolic syndrome[204]. In this study, adjusting for BMI in prediction models resulted in loss of statistical significance of the association between metabolic syndrome and asthma, proving that visceral obesity is driving the epidemiological association[204]. Abdominal obesity was also the strongest predictor of impaired lung function[197]. In a clinical trial setting, obese patients who underwent bariatric surgery and successfully reduced weight showed striking improvement of lung function and asthma control[205]. These results have been replicated by a different[206].

It is important to note that in the cited literature, different methods have been used to estimate the prevalence of asthma. While some studies are based on official medical records and study-specific clinical examination, others refer to patient-reported symptoms and physiological characterisation. Moreover, a prospective study of Canadian adults suggested that adult asthma might often be overreported or overdiagnosed, however with no difference in the frequency of overdiagnosis between the obese and non-obese group[207]. In this regard it should be remembered that the intensity of asthma symptoms fluctuates over time and normal lung function at a single timepoint does not exclude the diagnosis of asthma.

Another important parameter in the assessment of disease burden is the level of asthma control. It is estimated that almost as many as 50% of asthmatic patients have insufficient disease control and experience regular exacerbations[84]. Suboptimal disease management

is associated with negative long-term outcomes and disease complications such as airway remodelling, disability and need of hospitalization. Several studies have reported inferior asthma control in patients with obesity in comparison to normal weight asthmatics[208]–[212]. Of note, the obese asthma phenotype has been associated with predominantly neutrophilic infiltration of the airways and worse response to inhaled corticosteroid treatment, which are the cornerstone of asthma[195], [196]. This has been confirmed by a randomized, double-blinded clinical trial which evaluated the response to asthma-controller medication and placebo in a heterogenic group of patients with moderate asthma. The response to inhaled corticosteroids was lower with increasing BMI, but interestingly, the same trend has been observed for placebo, suggesting that excessive fat deposition could influence the natural history of asthma control[213]. The symptoms intensity experienced by obese asthmatic patients has been evaluated as disproportionate for the level of airway inflammation as assessed by sputum omics-analysis[214]. This could be due to the impaired mechanical ventilation of the lungs in obese patients. It is further in line with the findings of an interventional study, in which weight loss was associated with significant improvement of asthma control and increase in forced vital capacity of the lungs, but not in changes of inflammatory parameters nor bronchial reactivity[215]. However, multiple studies focusing on systemic inflammation in obesity have shown that weight reduction results in lowering of C-reactive protein (CRP) levels[216]. It is plausible that this chronic proinflammatory status contributes to the disease pathology in asthma.

3.2. Immune crossroads of asthmatic inflammation and obesity

Summarizing the results of epidemiological studies, two major groups of obese asthmatic patients can be distinguished: early-onset allergic asthma patients, in whom obesity aggravates airway hyperresponsiveness; and late-onset non-allergic asthma patients, in whom obesity usually precedes the incidence of asthma[217]. These two clinical phenotypes also have distinct profiles of immune infiltration of the airways, with eosinophils predominating in allergic patients and neutrophils in patients without IgE-driven hypersensitivity. Obesity seems to contribute to disease burden in both phenotypes. This section will summarize the current knowledge of shared immune pathways in asthma and obesity, and highlight the directions of further research in this epidemiologically pressing field.

Obesity induces the state of low-grade, chronic, systemic inflammation[130], associated with increased serum levels of proinflammatory immune mediators, especially leptin, IL-6 and TNF[218], [219]. These proinflammatory cytokines, produced by both adipocytes and adipose tissue resident immune cells, influence systemic immune responses skewing them towards a more proinflammatory type. Strikingly, adipose tissue has lately been shown to accumulate within the lung tissue itself[220]. Adipose tissue area within the outer wall of larger airways positively correlated with BMI and bronchi wall thickness in both asthmatic and non-asthmatic patients. Accumulation of fat tissue positively correlated with neutrophilic infiltration in both groups, unequivocally pointing towards the proinflammatory environment of adipose tissue. Furthermore, in patient with fatal asthma, adipose tissue area also correlated with eosinophilic counts[220].

3.2.1. Leptin

Studies in animal models and *in vitro* setting have shown that leptin has diverse immunoregulatory functions[160]. Interestingly, leptin levels are not only higher in obese individuals, but have also been found to be higher in asthmatic patients in comparison to age-, sex- and BMI-matched non-asthmatic group[200], [201]. Leptin levels were associated with more severe asthma and higher rate of disease exacerbations[221]. In a study of allergic rhinitis paediatric patients, serum leptin concentration was significantly correlated with the levels of Th2 type cytokines[222]. These links have further been confirmed by mechanistic studies which have shown that leptin enhances allergic responses by modulating Th2 cell function. In a mouse model of allergic asthma, leptin deficiency was associated with attenuation of asthma symptoms and decreased eosinophilia[223]. Consistently, more severe asthmatic inflammation has been observed in obese mice with higher levels of leptin[224]. Furthermore, exogenous administration of leptin aggravated airway hyperresponsiveness in this model, as well as led to an increase in serum IgE levels[225]. Leptin has been demonstrated to promote the expression of Th2 type cytokines by naïve $CD4^+$ T cells and was shown to enhance proliferation and survival of Th2 cells and group 2 ILCs. Furthermore, leptin has been found to increase the responsiveness of Th2 cells[224]. However, the activating effect of leptin is not limited to Th2 cells, as leptin has also been shown to promote Th1 responses[161]. Of note, these findings refer to physiological levels of leptin, and not higher concentrations as found in obese individuals.

Latest studies showed that leptin and allergic sensitization might have an additive effect on airway hyperresponsiveness and long-term complications of asthmatic inflammation[226]. In an allergic model of asthmatic inflammation, leptin stimulation combined with allergic sensitization led to proliferation of lung fibroblasts and increase in tissue resistance. Interestingly, this study has shown a difference between the effect of leptin in female and in male mice, reflecting the epidemiological trends seen in human asthmatic patients[226]. This novel study touches on the topic of airway remodelling in obese asthma. Similar experiments focusing on bronchial smooth muscle cells are needed for a more complete picture of airway remodelling.

Sputum and bronchoalveolar lavage (BAL) fluid levels of leptin mirror the serum levels and are positively correlated with BMI[227], [228]. Leptin receptors are expressed by different cell types in the human lung tissue, which suggests a possible local mechanism of action. With importance to allergic airway inflammation, leptin was found to act as a survival factor for eosinophils, but also bronchial epithelial cells[229], [230]. This antiapoptotic effect has been accompanied by an increase in production of growth factors[230]. It would be interesting to further examine the effect of this enhanced secretory activity in the context of complex cell-cell interactions in the bronchial tissue.

Leptin has also been shown to affect cytokine and chemokine production by human lung fibroblasts, with a plausible effect on immune cell recruitment[231].

3.2.2. IL-6

Among the proinflammatory adipokines produced by the white fat tissue, IL-6 has been acknowledged as the marker of obesity-associated systemic inflammation. IL-6 is abundantly produced by the adipose tissue and its serum levels are significantly elevated in obese individuals. IL-6 induces release of leptin and insulin, counteracting glucose metabolism dysregulation[232]. In obesity, however, this protective mechanism is insufficient and higher IL-6 levels have been linked to the development of metabolic comorbidities[233]. Local effects of IL-6 signalling include polarization of adipose tissue resident macrophages into M2 phenotype, associated with anti-inflammatory functions, such as tissue repair and remodelling[234]. Interestingly, this macrophage phenotype is also promoted by Th2 cytokines[235]. The relationship is reciprocal as M2 macrophages modulate T cells towards a

Th2 immune response type. Therefore, systemically elevated IL-6 levels, as seen in obese patients, are though to promote hypersensitivity.

Higher levels of IL-6 have also been reported in the blood and in the airways of asthmatic patients, as evaluated upon the examination of induced sputum and BAL fluid[236]–[238]. IL-6 has been reported as a specific marker of asthmatic inflammation[236]. Furthermore, it was associated with significantly worse disease control and a higher number of exacerbations[239]–[241]. Strikingly, a recent study of over 400 patients with severe asthma has identified a distinctive disease phenotype characterized by high plasma IL-6 level, higher prevalence of obesity and diabetes and decreased lung function (FEV1). This phenotype was named exacerbation-prone asthma[240]. However, the level of circulating IL-6 did not correlate with any measures of type 2 inflammation, suggesting that the increase in systemic IL-6 is not a result of upstream hypersensitivity reactions[239]. This is in line with findings from the animal models of asthma, which demonstrated that IL-6 protected from allergic airway inflammation[102].

The relationship between upregulation of IL-6 signalling in obesity and a more severe asthma phenotype requires further mechanistic research. To date, it can be concluded that adipose tissue production of IL-6, rather than allergic immune responses, accounts for the elevation of systemic levels of this cytokine in obese asthmatic patients. While IL-6 can promote Th2 immunity, it is plausible that it contributes to aggravating asthma severity via different pathways, for example by acting on bronchial epithelial cells and inducing mucus hypersecretion[242].

3.2.3. TNF and IL-17

TNF and IL-17 are proinflammatory mediators classically associated with type 17 immune responses and autoimmunity. However, in asthma patients with predominantly neutrophilic infiltration of the airways, as seen in obese asthma phenotype, TNF and IL-17 have been found as the drivers of chronic inflammation[243], [244]. Importantly, this phenotype is characterized by worse response to inhaled corticosteroid treatment. Interestingly, in a mouse model of asthma neutralization of TNF led to increased response to therapy with corticoids[245]. Clinical trials of TNF blockade in asthma, however, yielded mixed results[246].

TNF is produced by adipocytes, and obese individuals have a significantly higher expression of TNF in adipose tissue[247], [248]. Serum levels of TNF correlate with serum IL-6 and are increased in obese patients, further associated with diabetes mellitus[249]. TNF acts as a chemoattractant for neutrophils and eosinophils, as well as increase their cytotoxic effect on bronchial epithelial cells. TNF also induces histamine release from mast cells, and therefore initiates the hypersensitivity response cascade[250]. Elevated levels of TNF in obese asthmatic patients could contribute to immune cell airway infiltration and sustain proinflammatory state of the tissue.

A positive feedback loop between neutrophils and Th17 cells exists: IL-17 secreted by Th17 cells promote neutrophil recruitment and activation while neutrophilic inflammation, on the other side, has recently been demonstrated to enhance T cell differentiation into Th17 in the pathogenesis of asthma[251], [252]. IL-17 however, is not exclusively produced by Th17 cells. Key insights into the pathogenesis of obese asthma came from animal studies. Diet-induced obesity in mice was accompanied by spontaneous development of airway hyperreactivity driven by IL-17 and inflammasome signalling. This airway hyperreactivity was entirely dependent on the innate arm of the immune system[253]. Importantly, IL-17 has been shown to act directly on smooth muscle cells and cause bronchoconstriction[254]. These results further suggest that endogenous cytokines and adipokines could initiate and contribute to airway hyperreactivity, either commencing the vicious circle of chronic airway inflammation (in late-onset non allergic obese asthma) or aggravating underlying hypersensitivity immune responses (in early-onset allergic asthma with obesity).

Rationale for undertaking of research

Prevalence of obesity has rapidly been increasing since the start of the 20th century, and with it the various associated comorbidities like cardiovascular diseases, diabetes mellitus, chronic kidney disease, many types of cancers and asthma[255]. Obesity increases the risk of asthma incidence by 92%. Patients with obesity and asthma are more likely to experience worse quality of life and increased asthma-related hospitalizations. Asthma is often more difficult to manage in obese patients, with more severe symptoms and worse lung function and a reduced response to asthma therapeutics[256]. Several mechanisms may be able to explain this association between obesity and severe asthma, such as decreased total respiratory system compliance, increased airway resistance and reduced lung capacity[257]. Another likely cause is a state of low-grade inflammation in obese patients, brought about by obesity-associated adipokines produced by adipose tissue and pro-inflammatory immune cells residing in the fat tissue[258]. Insulin and leptin, hormones produced in excess by adipose tissue in obesity, are two of these adipokines. While their effect at lower concentrations is regarded as anti-inflammatory, in excess their influence appears to propagate the inflammatory environment present in obesity. This effect has been studied on different cell types before such as adipocytes, fibroblasts and various immune cells, but research in smooth muscle tissue of the lungs is scarce. BSMCs are a cell type central to the pathophysiology of asthma, as chief mediators of airway remodelling and producers of chemokines and cytokines in response to pro- and anti-inflammatory stimuli[89]. Factors influencing these cells can have long-lasting effects on disease severity. We believe that exploring the influence of obesity hormones on HBSCs could yield valuable information regarding the immune mechanisms at play in obese asthma, which may lead to novel therapeutic targets in the future.

Hypotheses

Metabolic disease is epidemiologically and mechanically linked to severe asthma. Our molecular understanding of this link is still limited, but research has hinted at a role for hyperleptinemia and hyperinsulinemia caused by metabolic disease as key factors. With this study, we aim to elucidate the effect obesity hormones have on the production of various immune receptors as well as adipokines and cytokines. Further, given the association between the inflammatory environment generated by asthma and obesity, we investigate the effect of obesity hormones in tandem with exposure to inflammatory cytokines. Keeping these goals in mind, we propose the following hypotheses;

- Exposure of human bronchial smooth muscle cells (HBSMC) to increased concentrations of leptin or insulin will alter expression of immunomodulating receptors, with implications for downstream lung tissue remodelling as a consequence.
- Exposure of HBSMC to increased concentrations of leptin or insulin will augment production of adipokines and cytokines, resulting in the establishment of a pro-inflammatory environment associated with metabolic disease.
- A pro-inflammatory environment brought about by exposure to IL-4 or IFN-γ will provide an additive effect together with exposure to leptin or insulin in the expression of immunomodulating receptors in HBSMC.
- This pro-inflammatory environment will also provide an additive effect with exposure to leptin or insulin in the production of adipokines and cytokines by HBSMC.

Testing these hypotheses will allow us to further unfold the immunological mechanisms behind the link between asthma and metabolic disease.

Materials and methods

Cell culture

Primary human bronchial epithelial cells from non-cancerous, non-diseased human donors were purchased from Lonza Group Ltd (Basel, CH) and grown with the SmGM-2 Smooth Muscle Cell Growth Medium-2 BulletKit (Lonza Group Ltd, Basel, CH). This kit contains 500 mL SmBM Basal Medium and SmGM-2 SingleQuots supplements, namely 0.50 mL insulin, 1 mL hFGF-β, 0.50 mL GA-1000, 0.50 mL hEGF and 25.00 mL fetal bovine serum (FBS). All supplements were added to the medium before initiating cell culture. Before seeding HBSMC were rinsed with phosphate-buffered saline (PBS Solution without Ca^{2+}, Mg^{2+}, Genoplast, Rokocin, PL). Cells were seeded at a density of 5000 cells/cm^2 in plastic Corning T-75 and T-150 rectangular canted neck flasks with phenolitic-style cap without coating (Corning, New York, USA) and were only subjected to passaging 2-3 times before usage in experiments. All cells were kept at 37°C at a 5% CO_2 humidified atmosphere following protocols present at the Department of Clinical Immunology laboratory. Culture medium was changed every second day and cells were kept in 1 mL of medium per 5 cm^2 of culture vessel growth surface (T-75 flask 15 mL; T-150 flask 30 mL). Cells were passaged when flasks reached 75-90% confluency using 0.025% trypsin (5 mL, Lonza Group Ltd, Basel, CH) and trypsin neutralising solution (TNS) (6 mL, Lonza Group Ltd, Basel, CH). Cells were counted in Tryphan blue (0.4%, Thermo Fisher Scientific, Massachusetts, USA) using Neubauer cell counting chambers (Camlab, Cambridge, UK). Before start of experiments cells were transferred to Corning 24 well plates (Corning, New York, USA) at a density of 30000 cells per well (10 000 cells/cm^2 of growth surface). Excess cells were stored in liquid nitrogen in freezing medium containing cell culture grade dimethyl sulfoxide (DMSO) (PanReac Applichem, Darmstadt, DE) (10%), FBS (40%) and HBSMC growth medium (50%).

In vitro exposure

After HBSMC have grown for two to three days and reached 75% confluency in 24 well plates medium was changed and exposure started. HBSMC were stimulated with recombinant human IL-4 (BD Biosciences Pharmingen, San Diego, USA) at a concentration of 50 ng/mL, diluted with growth medium from a stock of 0.1 g/L, with recombinant human IFN-γ (BD Biosciences Pharmingen, San Diego, USA) at a concentration of 30 ng/mL from a stock of 0.25 g/L, or were grown in normal HBSMC medium. After 24 hours medium is changed and

different concentrations of leptin (Sigma-Aldrich, Massachusetts, USA) and insulin (Lonza Group Ltd, Basel, CH) are added. These compounds were dissolved in alcohol (ethyl alcohol for molecular biology, Chempur, Piekary Śląskie, PL) at a stock concentration of 1 mg/mL and 5 mg/mL, respectively. RNA isolation and harvesting of medium were performed at the second medium change, 8 hours and 24 hours after starting the second exposure with the obesity hormones. Before samples were taken, cells were rinsed with PBS.

Cell viability

Cell viability was assessed using a ReadyProbes™ Cell Viability Imaging Kit (Thermo Fisher Scientific, Massachusetts, USA). Images were made before samples were taken after 24 hours of exposure to obesity hormones or control medium using an EVOS M5000 Imaging System (Thermo Fisher Scientific, Massachusetts, USA).

Real-Time quantitative PCR

Total RNA was isolated using the RNeasy Mini Kit (Qiagen, Venlo, NL) in accordance with the manufacturer's instructions. Samples were stored at -80 °C until reverse transcription. Total isolated RNA was quantified using a nanodrop spectrometer (Thermo Fisher Scientific, Massachusetts, USA) and minimum 250 µg per sample was reverse transcribed to cDNA using the QuantiTect Reverse Transcription Kit (Qiagen, Venlo, NL) according to the manufacturer's instructions. The reaction mixtures for PCR amplification (14.7 µL) contained 5 ng cDNA at a concentration of 1 ng/µL, 7.35 µL SYBR™ Green PCR Master Mix (Applied Biosystems, Massachusetts, USA) and 8 µM forward and reverse primers in a 2.35 µL. Reactions were run on a LightCycler 480 instrument (Roche, Basel, CH) under the following conditions; an 8-minute pre-incubation at 95 °C followed by 45 cycles of 15 s at 95 °C (denaturation step), 30 s at 60 °C (annealing step) and 20s at 72°C (elongation). Clear LightCycler 480 Multiwell 96 Plates (Roche, Basel, CH) were used to run the reactions on, with each plate containing a negative control for each gene investigated. Gene expression was normalized using the housekeeping gene GADPH. Relative gene expression levels were calculated using the delta-delta Ct method. All assays were performed with technical replicates in duplo.

Table 6. Primer sequences of selected target genes

Target		Sequences	Product length (bp)
GADPH	F	5'-CAT GAG AAG TAT GAC AAC AGC CT-3'	113
	R	5'-AGT CCT TCC ACG ATA CCA AAG T-3'	
HR1	F	5'-TTT CTC TCT TTT CTG TGG GTT ATT C-3'	387
	R	5'-CTT GGG GGT TTG GGA TGG TGA CTT C-3'	
LEPR	F	5'-TGG GAA GAT GTT CCG AAC CC-3'	132
	R	5'-AGG CTC CAA AAG AAG AGG ACC-3'	
INSR	F	5'-TGA CAG ACT ATT TAG ACG TCC CG-3'	256
	R	5'-AGC TCT CGA AGG AGG GTG AT-3'	
IL13Ra1	F	5'-CGC GCC TAC GGA AAC TCA-3'	220
	R	5'-CCC CAC TTG CAG ACA AAT CC-3'	
IL4R	F	5'-ACC TGA CTT GCA CAG AGA CG-3'	153
	R	5'-AGG GCA TCT CGG GTT CTA CT-3'	
FGFa	F	5'-GGA AAG GCT GGA GGA GAA CC-3'	125
	R	5'-TCT GGC CAT AGT GAG TCC GA-3'	
NR3C1	F	5'-CAGT GTG CTT GCT CAG GAG A-3'	95
	R	5'-GTG AGG GTG AAG ACG CAG AA-3'	

Cytometric bead array

Medium was stored at -80 °C until experiments commenced. We used LEGENDplex Multi-Analyte Flow Assay Kits (BioLegend, San Diego, USA) to perform cytometric bead array (CBA). The Human Adipokine Panel with V-bottom Plate and Human Th Cytokine Panel with V-bottom Plate were used to quantify adiponectin, adipsin, retinol binding protein 4 (RBP4),

MCP-1, IL-1β , IP-10, IL-10, IL-8, leptin, IL-6, IFN-γ, resistin and TNF, and IL-2, IL-4, IL-5, IL-6, IL-9, IL-10, IL-13, IL-17A, IL-17F, IL-21, IL-22, IFN-γ and TNF, respectively. Assays were run according to the manufacturer's instructions on a BD FACS Canto2 (Becton Dickinson, New Jersey, USA). All assays were performed in duplo. The generated FCS files were analysed using BioLegend's LEGENDplex Data Analysis Software.

Statistical analysis

Statistical analysis was performed using the R programming language in the integrated development environment RStudio. Samples within each time point were checked for normality using the Shapiro-Wilk test, and differences between treatment groups were then assessed via one-way ANOVA and paired students' *t*-tests. In case of deviations in homogeneity of variance, the more rigorous paired Welch's *t*-test was used for the pairwise comparison of samples. Results depicted in the graphs are represented as a mean value with standard error of means (Mean ± SE). Statistical significance was assessed at $p < 0.05$.

Results

1. Assessment of cell death and proliferation

As a first parameter, we checked the effect of insulin and leptin on cell death and proliferation of HBSMC. For this purpose, images were taken with an EVOS M5000 Imaging System using ReadyProbes™ Cell Viability Imaging Kit. We did not observe any change in overall cell death in response to any of the leptin (Figure 7) or insulin (data not shown) exposures.

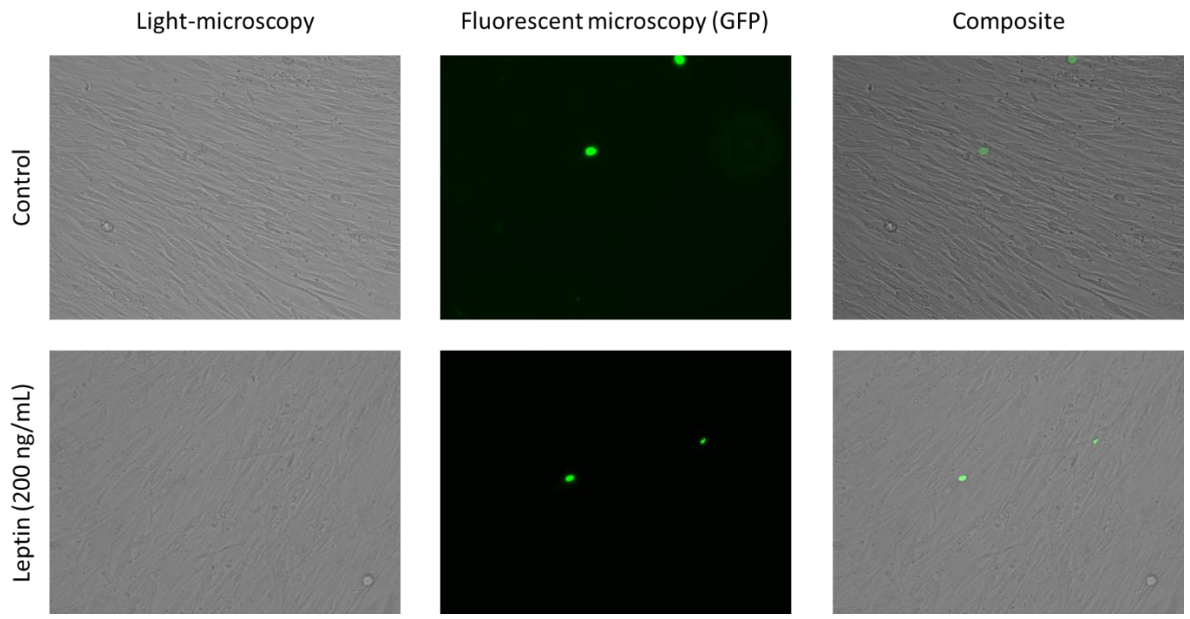

Figure 7. Superpositioned translight and green fluorescent images of control and 200 ng/mL leptin exposed HBSMCs in 24 well plates at the 24-hour time point. Typical images for confluent cell HBSMC cultures at passage 3 are shown.

To assess proliferation rate of HBSMC upon exposure to insulin or leptin concentrations, wells of every treatment were treated with trypsin to achieve detachment from plate bottoms and counted to check for differences in cell number. Comparisons of cell numbers using a one-way ANOVA and pairwise Welch's *t*-tests showed no significant differences between treatments (data not shown).

2. Assessment of immune cascade potency in HBSMCs

2.1. Response of HBSMCs after exposure to obesity hormones

The RNA levels of receptors HR1, IL4R, IL13R, LEPR, INSR, and NR3C1 in HBSMCs, together with fibroblast growth factor 1 (FGFa), were assessed after exposure to different leptin concentrations, namely 100, 200 and 1000 ng/mL, and after exposure to insulin, at 10 000, 50 000 and 250 000 pmol/mL. Expression of these inflammation-related receptors in HBSMCs was measured at 8 and 24 hours after exposure for HR1, IL4R, 13R, LEPR and INSR, and 8 hours after exposure for FGFa and NR3C1. An additional concentration of 2 000 pmol/mL was added as a treatment and samples of this exposure were only assessed at the 24-hour time point. The expression levels are compared to the vehicle (control medium) exposed HBSMC at the relevant time point resulting in the displayed relative expression levels.

2.1.1. Expression of immune receptors in response to leptin concentrations

After 8 hours of exposure, HR1 expression was nearly doubled at the lowest concentration compared to vehicle (Figure 8a). A significant decrease however was shown at the higher concentrations of 200 and 1000 ng/mL ($p < 0.05$). When expression levels were checked after 24 hours, the increase in HR1 at the 100 ng/mL concentration had subdued and was on par with the vehicle. At 200 and 1000 ng/mL a substantial decrease was still visible though no longer significant (Figure 8b).

a. b.

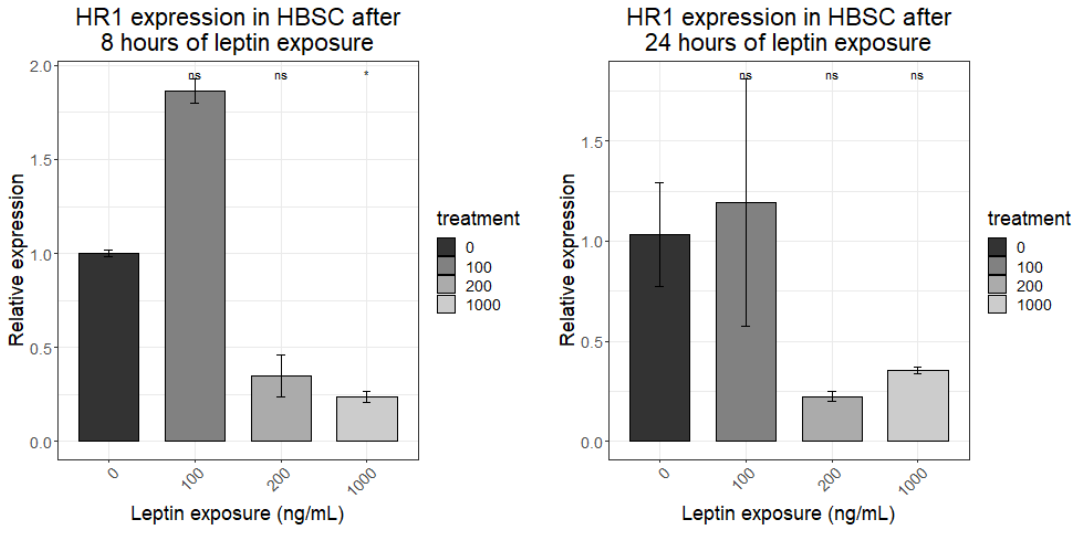

*Figure 8. Expression of HR1 after (a) 8 hours and (b) 24 hours of exposure to leptin. Significance based on paired Welch t-tests (p-value > 0.05, ns; <0.05, *).*

We observed the downregulation of IL13R expression after 8 hours of exposure, with a decrease after exposure to 200 ng/mL and a further, significant downregulation after exposure to the 1000 ng/mL leptin concentration (Figure 9a). This effect was also visible after 24 hours at 200 ng/mL and at 1000 ng/mL leptin, howbeit non-significant (Figure 9b).

a. b.

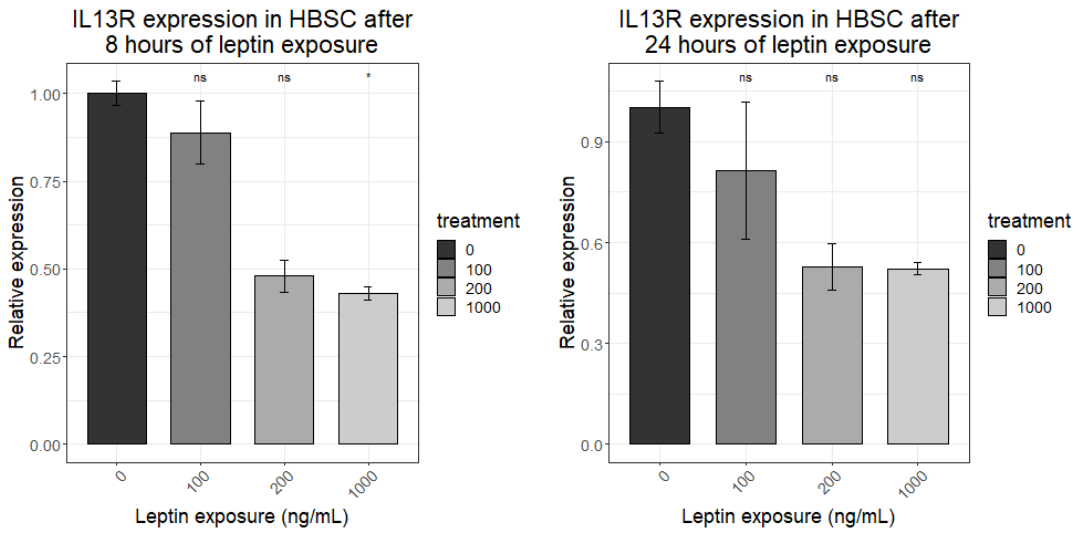

*Figure 9. Expression of IL13R after (a) 8 hours and (b) 24 hours of exposure to leptin. Significance based on paired Welch t-tests (p-value > 0.05, ns; <0.05, *).*

There was an apparent increase in LEPR expression at 100 ng/mL after 8 hours of leptin exposure, followed by a gradual dose-dependent decline as the leptin concentration increases at the first time point (non-significant) (Figure 10a). This decline appears to perpetuate after 24 hours, though overall levels of LEPR at this time point have decreased compared to the vehicle, with the most prominent reduction of expression visible at 200 and 1000 ng/mL (Figure 10b).

a. b.

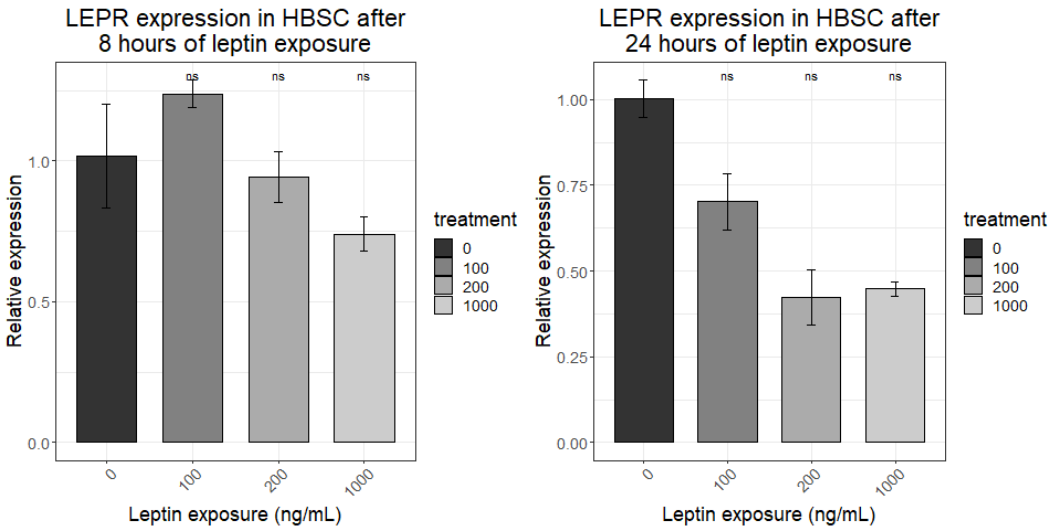

Figure 10. Expression of LEPR after (a) 8 hours and (b) 24 hours of exposure to leptin. Significance based on paired Welch t-tests (p-value > 0.05, ns).

NR3C1 expression levels remained unchanged after exposure to 100 ng/mL leptin after 8 hours, and were significantly decreased at the higher dosages (Figure 11). Expression of this receptor was not assessed at the 24-hour time point and could thus not be evaluated.

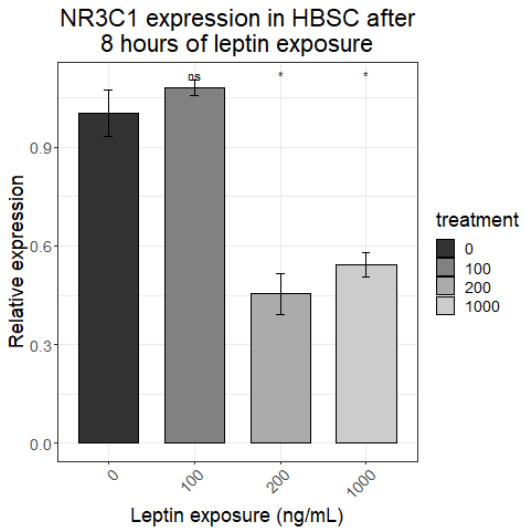

*Figure 11. Expression of NR3C1 after 8 hours of exposure to leptin. Significance based on paired Welch t- tests (p-value > 0.05, ns; <0.05, *).*

No changes were observed in the expression levels of IL4R, INSR or FGFA at any leptin dosage.

2.1.2. Expression of immune receptors in response to insulin concentrations

After 8 hours of exposure to insulin, HR1 expression was reduced in all samples, with a significant reduction visible at the two upper concentrations of 50 000 pmol/mL and 250 000 pmol/mL (Figure 12a). This reduction was only visible at the lowest concentration after 24 hours, with an observable gradual increase in expression at higher concentrations (Figure 12b).

a. b.

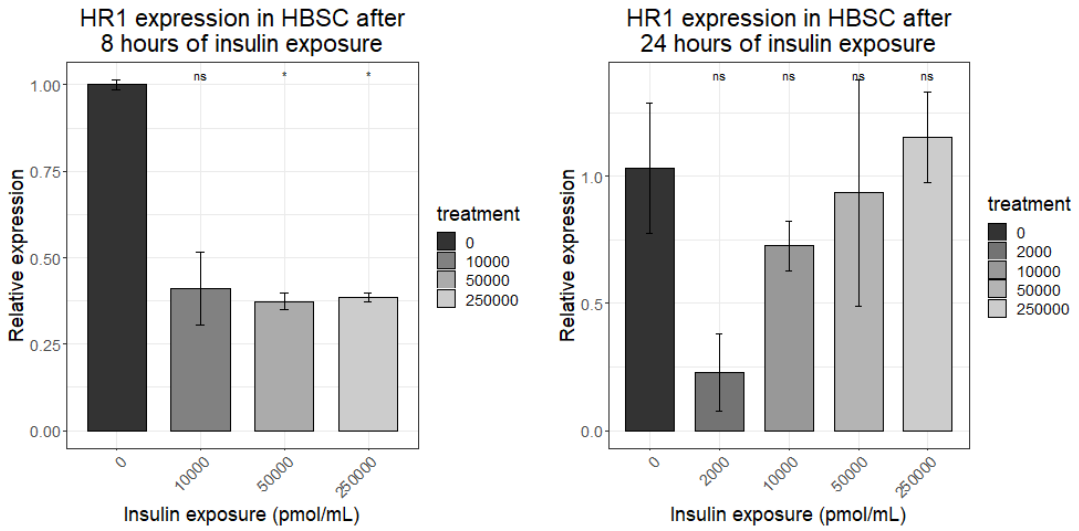

*Figure 12. Expression of HR1 after (a) 8 hours and (b) 24 hours of exposure to insulin. Significance based on paired Welch t-tests (p-value > 0.05, ns; <0.05, *).*

We observed a similar reduction at 8 hours at the higher insulin concentrations of 50 000 pmol/mL and 250 000 pmol/mL in IL4R expression, though non-significant (Figure 13a). Expression patterns at the lower concentrations were not checked for IL4R. After 24 hours of exposure to insulin, no differences were detected between the different concentrations and vehicle (Figure 13b).

a. b.

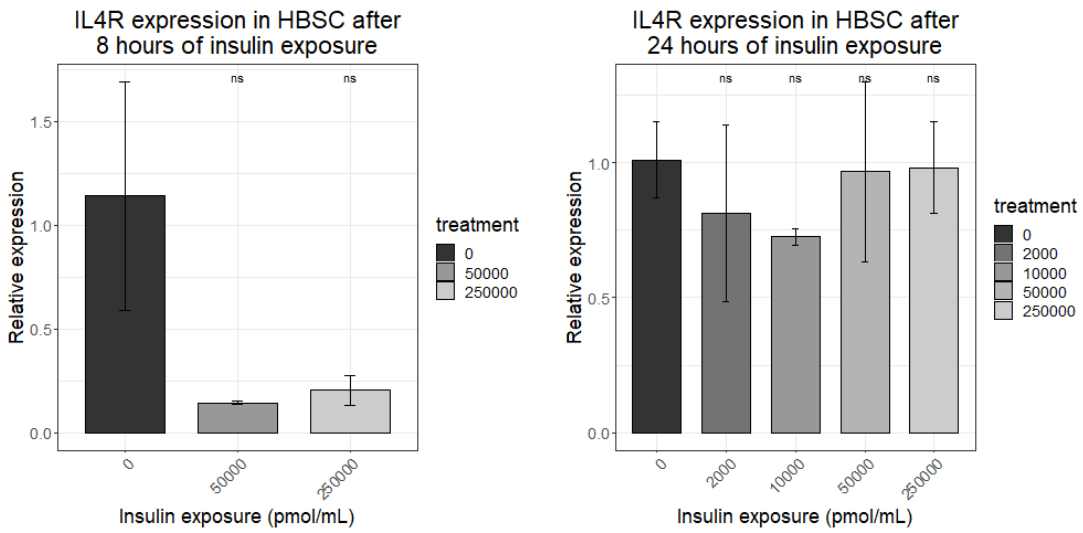

Figure 13. Expression of IL4R after (a) 8 hours and (b) 24 hours of exposure to insulin. Expression of IL4R in response to 2000 and 10 000 pmol/mL was not assessed at the 8-hour time point. Significance based on paired Welch t- tests (p-value > 0.05, ns).

IL13R expression was significantly downregulated at the 8-hour time point after insulin exposure at a concentration of 50 000 and 250 000 pmol/mL (Figure 14a). However, this appears no longer the case after 24 hours, with expression levels being similar to vehicle (Figure 14b).

a. b.

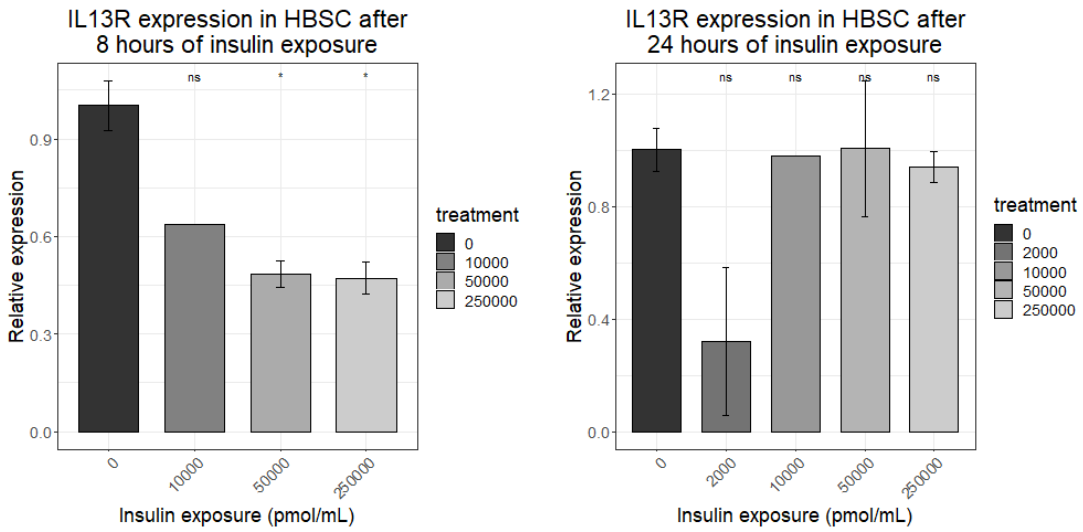

*Figure 14. Expression of IL13R after (a) 8 hours and (b) 24 hours of exposure to insulin. Significance based on paired Welch t-tests (p-value > 0.05, ns; <0.05, *).*

Similar to what we have observed in HR1, IL4R and IL13R, we have an apparent down-regulation of INSR in response to insulin after 8 hours (Figure 15a). This effect is not visible after 24 hours (Figure 15b).

a. b.

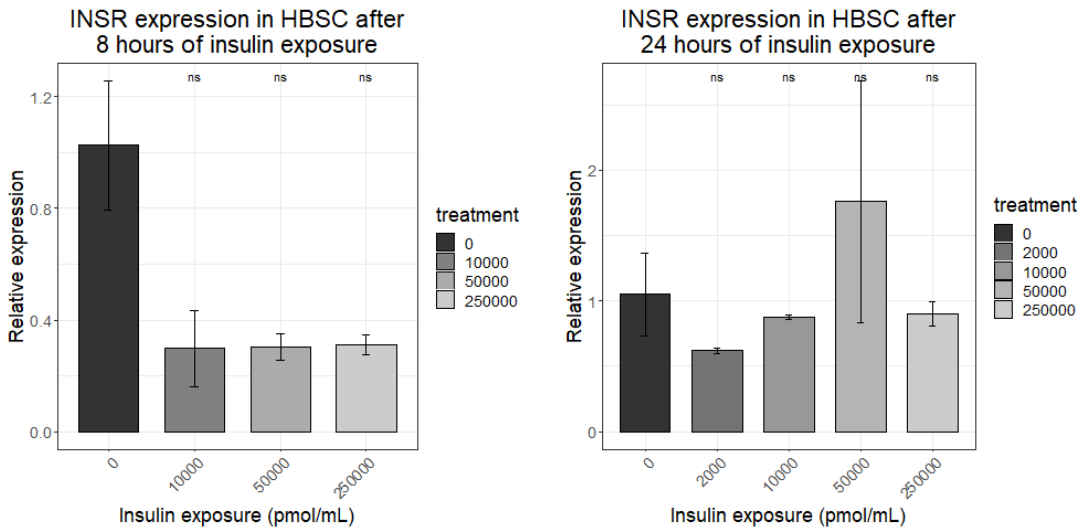

Figure 15. Expression of INSR after (a) 8 hours and (b) 24 hours of exposure to insulin. Significance based on paired Welch t-tests (p-value > 0.05, ns).

There appears to be a dose-dependent response to insulin exposure in the expression of FGFa, with a significant increase after exposure at a concentration of 250 000 pmol/mL (3-fold increase) (Figure 16). Exposure with insulin at a 50 000 pmol/mL concentration caused a 2-fold increase in FGFa expression but did not reach the threshold of significance. Expression of this growth factor was not assessed at the 24-hour time point and could thus not be evaluated.

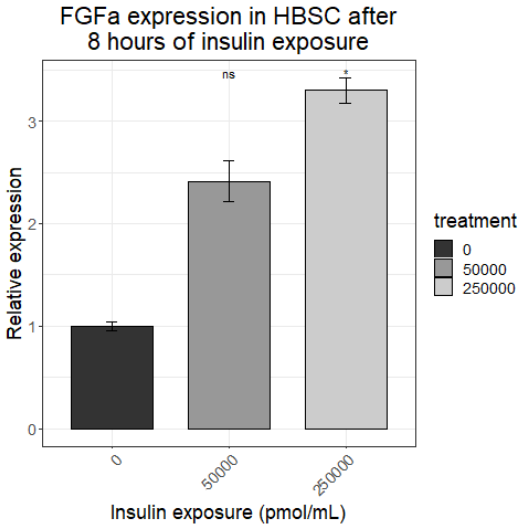

*Figure 16. Expression of FGFa after 8 hours of exposure to insulin. Significance based on paired Welch t- tests (p-value > 0.05, ns; <0.05, *).*

We observe a significant downregulation of NR3C1 expression after 8 hours of exposure to 10 000 pmol/mL insulin, with a similar downregulation after exposure to 50 000 pmol/mL (non-significant) (Figure 17). At the highest insulin concentration 250 000 pmol/mL there was no significant difference with the vehicle.

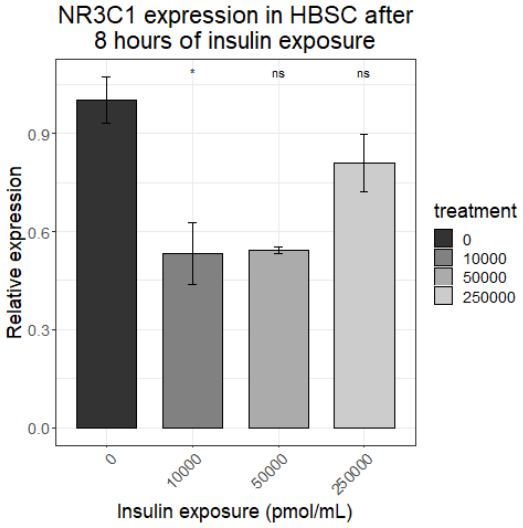

*Figure 17. Expression of NR3C1 after 8 hours of exposure to insulin. Significance based on paired Welch t- tests (p-value > 0.05, ns; <0.05, *).*

Insulin exposure elicited no changes in expression levels of LEPR compared to vehicle.

In **conclusion**, we observed an overall downregulation of HR1, IL13R, LEPR and NR3C1 after exposure to higher concentrations of leptin compared to control, which was first observed after 8 hours but persists at 24 hours. There was no effect of leptin on the expression of IL4R,

INSR or FGFA compared to control samples. After exposure to insulin, there is an initial drop in the expression of HR1, IL4R, IL13R, INSR and NR3C1 compared to control, at especially the lower concentrations of insulin. FGFa responded in a dose-dependent manner and its expression was significantly increased upon exposure to insulin. Further, we see a gradual increase in HR1 expression at 24 hours, with downregulation at lower concentrations, which gradually stabilizes to control expression levels at the highest concentration. IL4R, IL13R and INSR expression levels stabilize after 24 hours of exposure. There was no effect of insulin on the expression of LEPR compared to control samples.

2.2. Effect of IL-4 on HBSMCs in conjunction with exposure to obesity hormones

In order to understand the effects a Th2 mediated immune response may elicit in HBSMCs in combination with elevated obesity hormone concentrations, we exposed our cells to recombinant human IL-4 dissolved in HBSMC growth medium (30 ng/mL). Using IL-4 as opposed to house dust mite (HDM) extract allowed us to eliminate variability brought about by the varying lipopolysaccharide concentrations present in different HDM extracts. IL-4 is a key cytokine in the development of allergic inflammation in allergic asthma. RNA expression of HR1, IL4R, IL13R, LEPR, INSR and NR3C1 were assessed after 24 hours of exposure to the IL-4 medium together with insulin or leptin. To assess potential dose-dependent effects of obesity hormones, relative expression levels are compared to a vehicle only exposed to IL-4.

2.2.1. Immune receptor expression in response to IL-4 and obesity hormone leptin.

There were no patterns of dose-dependent differences detected between vehicle exposure and leptin, nor between the different leptin concentrations themselves. The distinctive expression patterns observed in HR1, IL13R and LEPR were no longer visible after the combined exposure to leptin and IL-4.

2.2.2. Immune receptor expression in response to IL-4 and obesity hormone insulin.

We did not perceive any expression patterns in any of the investigated immune receptors after 24 hours of combined exposure to insulin and IL-4.

In **conclusion**, treatment with IL-4 medium together with exposure to leptin or insulin did not show any patterns in the expression of HR1, IL4R, IL13R, LEPR, INSR and NR3C1.

2.3. Comparison of immune receptor expression between control- and IL-4 treated cells after exposure to obesity hormones.

We were interested in observing any effect the exposure to IL-4 may have had in HBSMC in addition to the obesity hormones leptin and insulin, comparatively to exposure to the hormones in control medium. To properly assess this effect, we opted to focus our investigation on two supraphysiological concentrations of the obesity hormones representing the most extreme cases of hyperleptinemia and hyperinsulinemia in serum; namely 200 ng/mL of leptin and 10 000 pmol/mL of insulin.

2.3.1. Immune receptor expression after exposure to 200 ng/mL leptin, comparison between control- and IL-4 treated HBSMC.

While we have a significant downregulation of HR1 expression after exposure to leptin in control medium, the opposite of this trend appears to be true in IL-4 medium-exposed HBSMC (Figure 18). Where IL-4 medium displayed a downregulation in HR1 expression compared to control, levels re-adjust to the control-medium expression levels after exposure to both IL-4 and leptin.

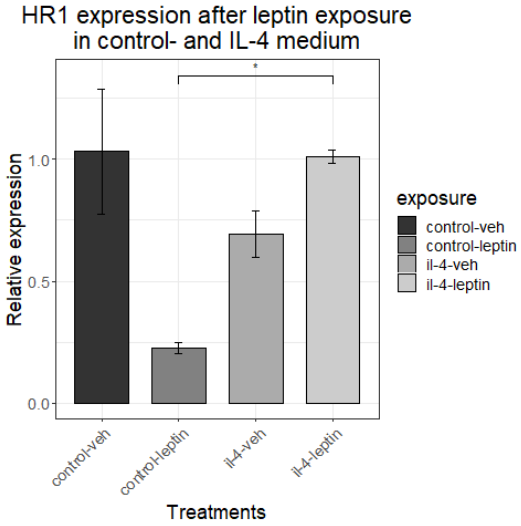

*Figure 18. Expression of HR1 in control and IL-4 treated medium, after 24 hours of exposure to vehicle (veh) or leptin. Significance based on paired Welch t- tests (p-value > 0.05, ns; p-value <0.05, *).*

IL13R expression was downregulated in control medium in response to leptin. We observed the same downregulation compared to the control-vehicle treatment, in response to IL-4 exposure, with and without additional leptin exposure. No significant differences were observed between the control- and leptin exposed samples within the IL-4 exposed group

(Figure 19). We saw the same trends in LEPR expression, with the IL-4+leptin treatment causing a significant downregulation of LEPR expression compared to the control-vehicle treatment (Figure 20).

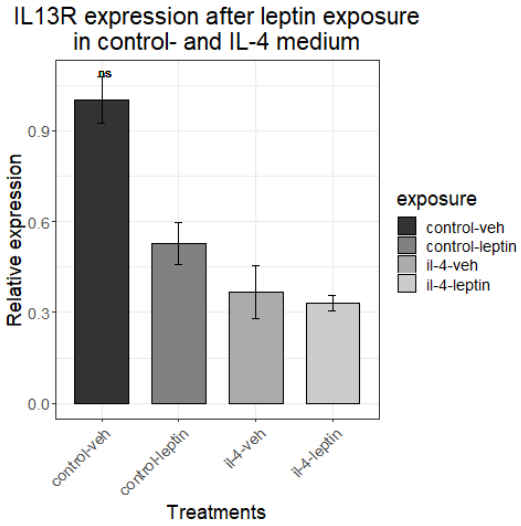

Figure 19. Expression of IL13R in control and IL-4 treated medium, after 24 hours of exposure to vehicle (veh) or leptin. Significance based on paired Welch t- tests (p-value > 0.05, ns).

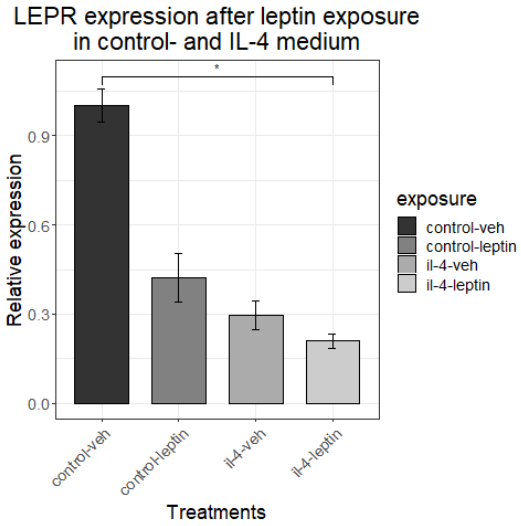

*Figure 20. Expression of LEPR in control and IL-4 treated medium, after 24 hours of exposure to vehicle (veh) or leptin. Significance based on paired Welch t- tests (p-value > 0.05, ns; p-value <0.05, *).*

Leptin exposure did not have an effect on INSR expression in neither control nor IL-4 exposed HBSMC but there was a clear trend of elevated INSR expression in the IL-4 treated groups compared to the control medium samples (Figure 21).

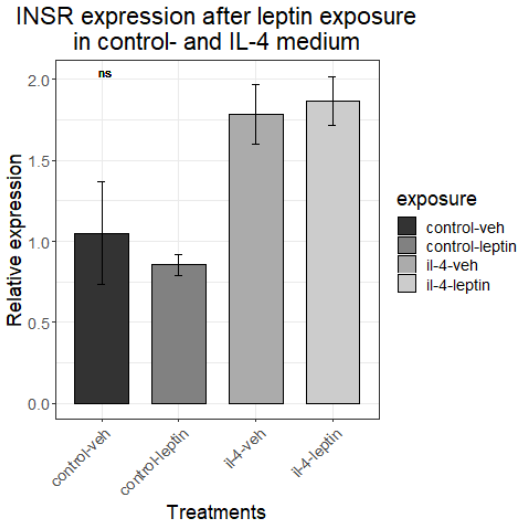

Figure 21. Expression of INSR in control and IL-4 treated medium, after 24 hours of exposure to vehicle (veh) or leptin. Significance based on paired Welch t- tests (p-value > 0.05, ns).

We did not observe any differences in IL4R expression between IL-4 and control treated samples. Exposure to leptin did not alter IL4R levels.

2.3.2. Immune receptor expression after exposure to 10 000 pmol/mL of insulin, comparison between control- and IL-4 treated HBSMC.

There appears to be a slight reduction in HR1 expression when comparing vehicle samples to insulin-exposed HBSMCs in control medium. Within IL-4 treated samples, there is a significant increase in HR1 expression after exposure to insulin, though neither of the IL-4 treatments differed significantly from the control medium grown vehicle (Figure 22).

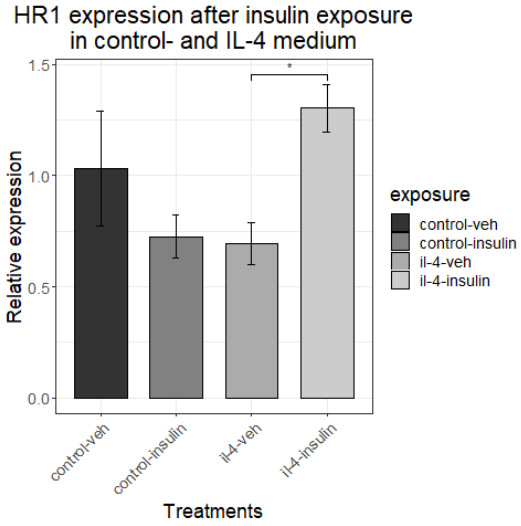

*Figure 22. Expression of HR1 in control and IL-4 treated medium, after 24 hours of exposure to vehicle (veh) or insulin. Significance based on paired Welch t- tests (p-value > 0.05, ns; p-value <0.05, *).*

IL13R expression levels stayed constant in response to the insulin exposure in both control as well as IL-4 treated HBSMC. We did spot an effect of IL-4 medium on IL13R expression, with apparent downregulation of the receptor in all IL-4 treatments compared to controls (Figure 23). Further, we observe a significant reduction in IL13R expression between the insulin treated samples over the two different media.

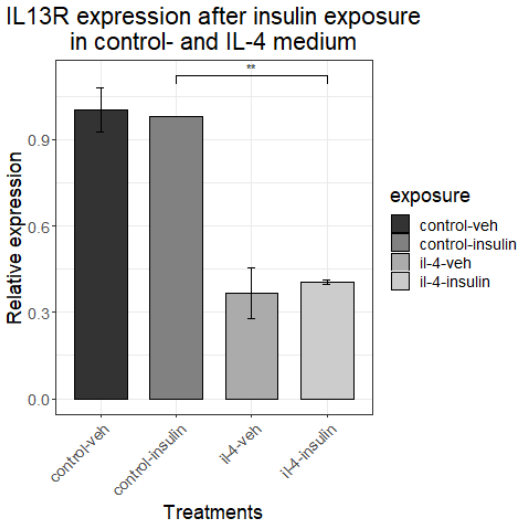

*Figure 23. Expression of IL13R in control and IL-4 treated medium, after 24 hours of exposure to vehicle (veh) or insulin. Significance based on paired Welch t- tests (p-value > 0.05, ns; p-value <0.05, *; < 0.01 **).*

Similarly, we did not detect an effect of insulin on INSR expression within the control and IL-4 treated HBSMC (Figure 24). Here we observed an opposite trend compared to the IL13R expression, with IL4 exposure causing near doubling in INSR expression compared to the control vehicle treated HBSMC.

Figure 24. Expression of INSR in control and IL-4 treated medium, after 24 hours of exposure to vehicle (veh) or insulin. Significance based on paired Welch t- tests (p-value > 0.05, ns).

We observed a visible effect of insulin treatment on IL4R expression in both the control and IL-4 exposed samples. In both groups insulin exposure resulted in a downregulation of IL4R, though this change in expression was not significant (Figure 25). While in both vehicle samples IL4R expression stays equal, the reduction in expression after IL-4 exposure appears greater than in vehicle.

Figure 25. Expression of IL4R in control and IL-4 treated medium, after 24 hours of exposure to vehicle (veh) or insulin. Significance based on paired Welch t- tests (p-value > 0.05, ns).

There was a slight reduction in LEPR expression in the control medium in response to insulin exposure (non-significant). This was not visible in the IL-4 medium treated HBSMC, were relative expression remained similar between vehicle and insulin exposed HBSMCs. There was

a significant threefold reduction in LEPR expression in both the IL-4 treated samples compared to control medium insulin exposed samples. Though this reduction is not significant compared to the control vehicle treatment, it is clearly also present here (Figure 26).

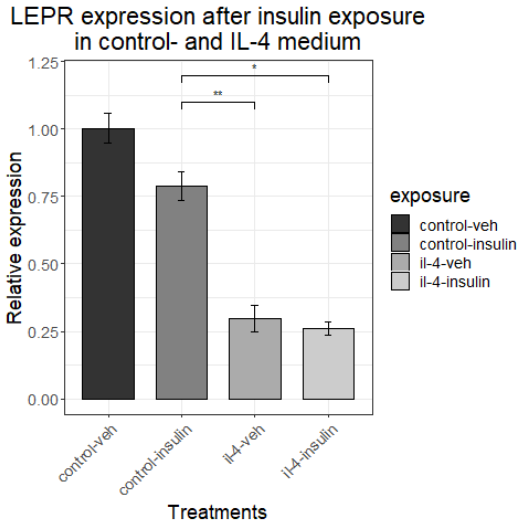

*Figure 26. Expression of LEPR in control and IL-4 treated medium, after 24 hours of exposure to vehicle (veh) or insulin. Significance based on paired Welch t- tests (p-value > 0.05, ns; p-value <0.05, *; < 0.01 **).*

In **conclusion**, HR1 expression was reduced after exposure to leptin in control medium, but was restored upon exposure to leptin in IL-4 medium. Upon exposure to leptin in control medium, both LEPR and IL13R expression were reduced, which perpetuated in HBSMC grown in IL-4 medium. Exposure to leptin caused a downregulation in IL4R expression in both media, though more pronounced in IL-4 medium. There was no effect of leptin on INSR expression, while we did observe a near two-fold increase of its expression after exposure to IL-4 medium. There was no visible effect of insulin on IL13R and INSR expression, though in response to IL-4 exposure, RNA-levels were down- and upregulated compared to control medium, respectively. HR1 expression was slightly down in insulin-exposed control samples compared to vehicle, though exposure to both IL-4 medium and insulin lead to a significant increase in HR1 expression. LEPR expression was significantly decreased when grown in IL-4 medium compared to control medium, regardless of insulin exposure.

3. Protein levels in response to obesity hormones

We investigated protein levels in HBSMCs after exposure to different leptin concentrations, namely 50, 100, 200 and 1 000 ng/mL, and after exposure to insulin, at 2 000, 10 000, 50 000 and 250 000 pmol/mL. For this we used two different flow cytometry assays. We assessed

protein levels of cytokines IL-5, IL-13, IL-2, IL-6, IL-9, IL-10, TNF, IL-17a, IL-17b, IL-4 and IL-22 with a Th Cytokine kit (Biolegend LEGENDplex, San Diego, USA). Protein levels of adipokines adipsin, RBP4, MCP-1, IL-1β, IP-10, IL-10, IL-8, leptin, IL-6, IFN-γ and resistin were assessed using the adipokine kit (Biolegend LEGENDplex, San Diego, USA). Adipokine and Th cytokine samples were collected after 8 and 24 hours of exposure to leptin or insulin, in control, IL-4 and IFN-γ medium. The relative protein levels are compared to the vehicle exposed HBSMCs at the relevant time point. As at the 8-hour time point only one biological sample (technical replicas measured in duplo) of the vehicle exposure in control medium was obtained, statistical analysis with comparisons to the vehicle was here not possible.

3.1 Production of Th cytokines in response to leptin concentrations

Detection of IL-5, IL-13, IL-2, IL-6, IL-9, IL-10, TNF, IL-17a, IL-17b, IL-4 and IL-22 using the Th cytokine kit yielded only results regarding the IL-6. While other cytokines were detected (IL-2, IL-5, IL-9, IL-13), these only just reached the minimum of the limit of detection (LOD). They will thus not be discussed in this document.

3.2 Production of adipokines in response to leptin concentrations

Of the investigated adipokines present within the Biolegend LEGENDplex kit, IFN-γ, resistin, IL-10, IP-10 and IL-1β were not detected in any of the tested samples, and will thus not be discussed any further in this section.

At the 8-hour time point, we observed a staggering downregulation of MCP-1 compared to control at the lower concentrations of leptin, with MCP-1 levels increasing as the leptin concentrations increased (Figure 27a). At the highest leptin concentration MCP-1 levels were 25% higher than in vehicle. We observed the same plummet in MCP-1 levels at low leptin concentrations after 24 hours, with significant differences between vehicle and 50 ng/mL, and vehicle and 100 ng/mL leptin (Figure 27b). At 1000 ng/mL leptin MCP-1 levels were similar to vehicle. Overall, MCP-1 levels increased in all leptin treatments at 24 hours compared to 8 hours.

a. b.

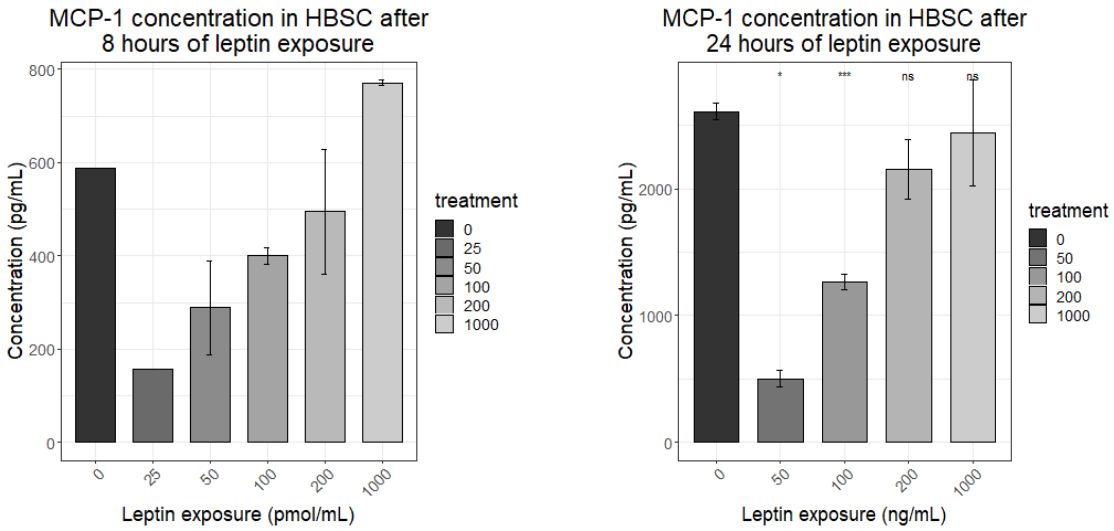

*Figure 27. MCP-1 concentration after (a) 8 hours and (b) 24 hours of exposure to leptin. Significance based on paired Welch t- tests (p-value > 0.05, ns; < 0.05, *; < 0.01, **; < 0.005, ***).*

There was an apparent reduction in IL-8 protein levels at all concentrations after 8 hours of leptin exposure (Figure 28a). After 24 hours this reduction was no longer visible, with IL-8 levels having increased in all leptin treated groups (Figure 28b). At the highest concentration however, there is still a significant decrease in IL-8 expression compared to vehicle. Overall, we see an increase in IL-8 at 24 hours compared to the 8-hour time point.

a. b.

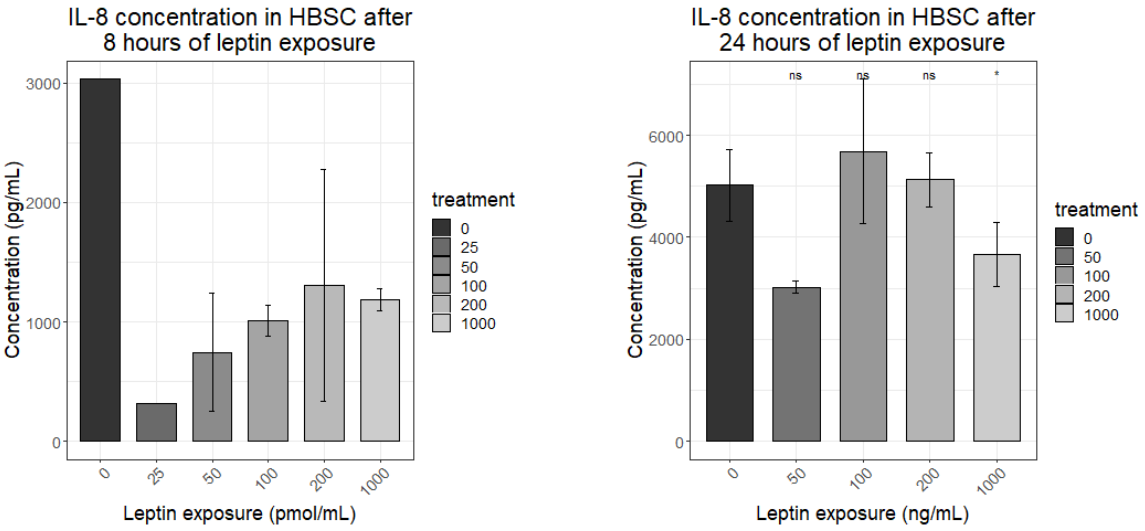

*Figure 28. IL-8 concentration after (a) 8 hours and (b) 24 hours of exposure to leptin. Significance based on paired Welch t-tests (p-value > 0.05, ns; < 0.05, *).*

IL-6 levels appear to go down in response to leptin after 8 hours of exposure, with the highest reduction occurring at the highest leptin concentrations (200 ng/mL and 1 000 ng/mL) (Figure 29a). After 24 hours, IL-6 levels were similar to vehicle in all leptin treatments, except for the 50 ng/mL exposure (Figure 29b). Though not statistically significant, IL-6 concentrations after exposure to this lowest concentration seems to have quadrupled.

a. b.

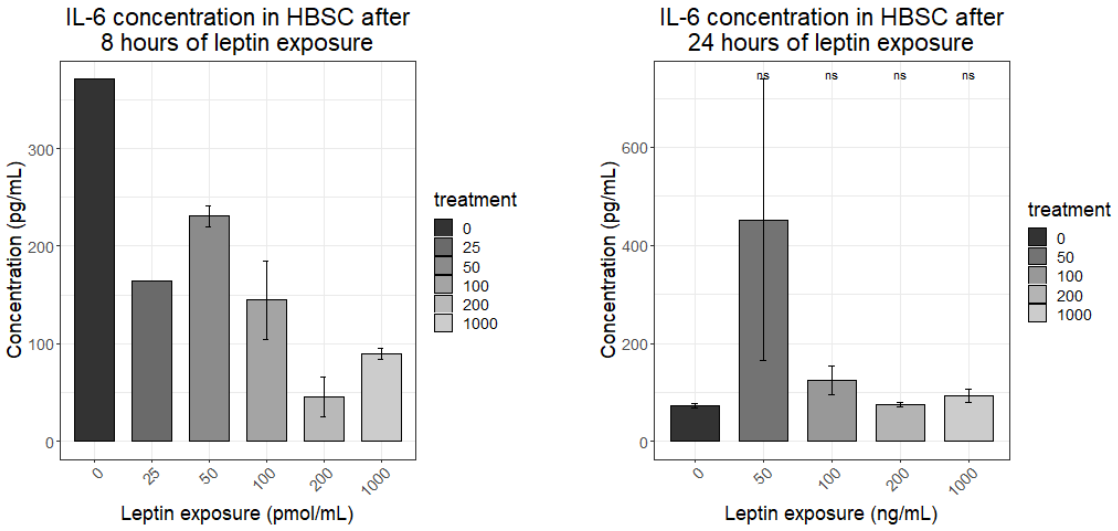

Figure 29. IL-6 concentration after (a) 8 hours and (b) 24 hours of exposure to leptin. Significance based on paired Welch t-tests (p-value > 0.05, ns).

Adipsin and RBP4 production did not show any significant changes over time in response to any leptin concentration compared to the control samples.

3.3. Production of adipokines in response to insulin concentrations

MCP-1 was increased after exposure to insulin at 250 000 pmol/mL, with no differences being observed at the lower concentrations (Figure 30a). After 24 hours this increase was no longer visible, with vehicle concentrations being level with concentrations after exposure wiht 250 000 pmol/mL insulin. There was a significant decrease at 50 000 pmol/mL compared to vehicle, also visible at 2 000 pmol/mL (non-significant) (Figure 30b).

a. b.

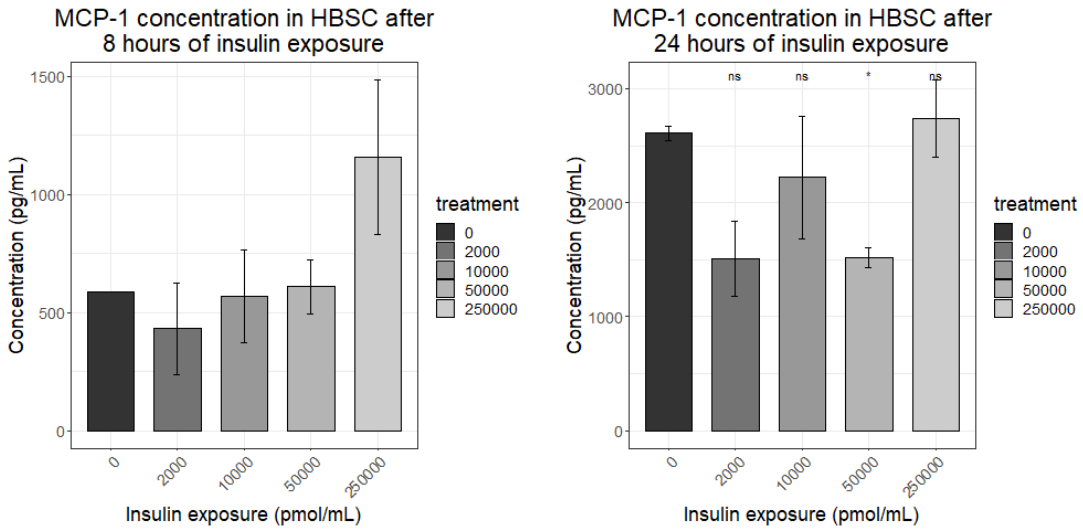

*Figure 30. MCP-1 concentration after (a) 8 hours and (b) 24 hours of exposure to insulin. Significance based on paired Welch t- tests (p-value > 0.05, ns; < 0.05, *).*

There is a large reduction in IL-6 and IL-8 production at the 8-hour time point in response to insulin exposure of all concentrations (Figure 31a, Figure 32a). After 24 hours this effect was no longer visible in neither IL-6 nor IL-8, with exposure of all insulin concentrations being similar to vehicle (Figure 31b, Figure 32b). Overall IL-8 production increased over time in all samples to concentrations floating around 4000 pg/mL, whereas the levelling in IL-6 production was largely due to a decrease in vehicle concentrations between 8 and 24 hours.

a. b.

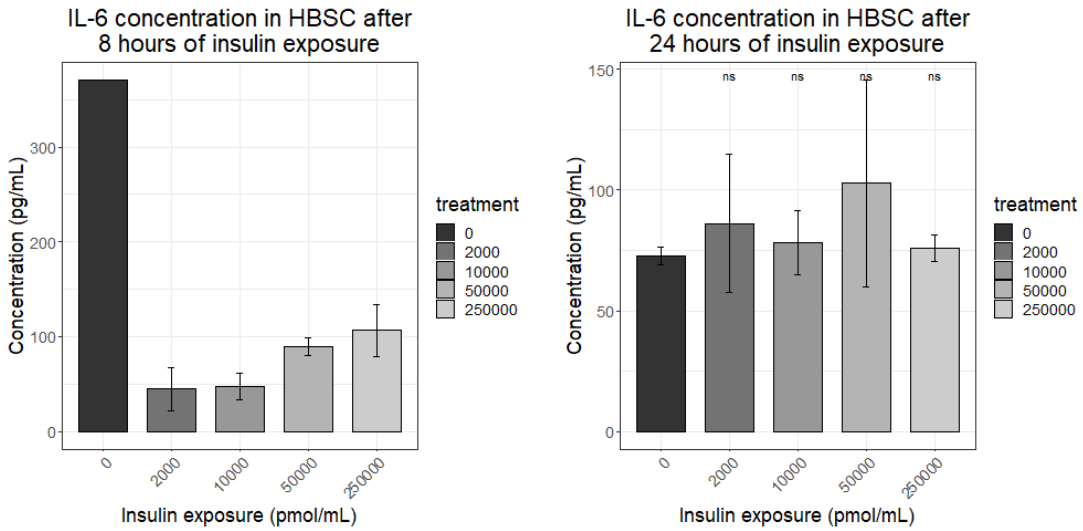

Figure 31 IL-6 concentration after (a) 8 hours and (b) 24 hours of exposure to insulin. Significance based on paired Welch t-tests (p-value > 0.05, ns).

a. b.

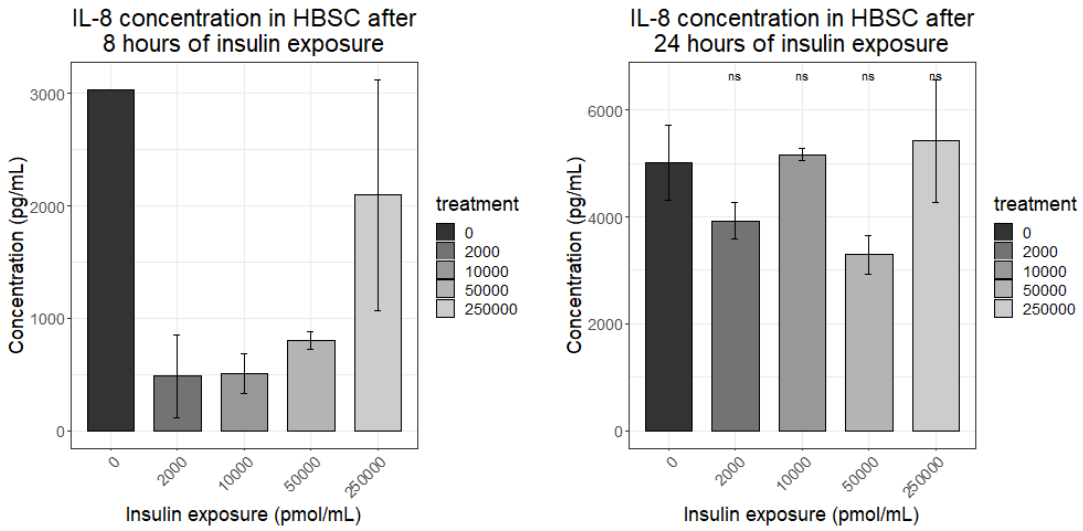

Figure 32. IL-8 concentration after (a) 8 hours and (b) 24 hours of exposure to insulin. Significance based on paired Welch t-tests (p-value > 0.05, ns).

We did not detect any changes in adipsin or RPB4 levels after exposure to any insulin concentration, at 8 hours nor after 24 hours.

In **conclusion**, there was a dose-dependent response to the exposure of leptin in the production of MCP-1, which disappeared after 24 hours. Overall, IL-6 and IL-8 were both reduced after exposure to leptin for 8 hours, regardless of their concentrations. IL-8 remained downregulated at the highest concentration after 24 hours, while other concentrations did not cause any changing effect. In IL-6, the lowest leptin concentration caused an upregulation while there was no difference between higher concentrations and control. There appears to be an increase in MCP-1 production in response to the highest concentration of insulin at the 8-hour time point, though this is no longer visible after 24 hours. IL-6 and IL-8 are both downregulated after exposure to each insulin concentration compared to control at the 8-hour time point, with concentrations stabilizing again after 24 hours.

3.4. General effect of IL-4 on adipokine production in HBSMCs during exposure to obesity hormones

We exposed our cells to human IL-4 dissolved in HBSMC growth medium at a concentration of 50 ng/mL, to assess the cumulative effect of exposure to elevated levels of leptin or insulin together with an inflammatory cytokine such as IL-4. Adipokine concentrations were checked using the aforementioned Biolegend LEGENDplex kits. To assess a dose-dependent effect of

obesity hormones, relative expression levels are compared to the vehicle, also exposed to IL-4. Samples were taken after 24 hours of exposure to both IL-4 and the obesity hormone.

3.4.1. Adipokine levels in response to IL-4 and the obesity hormone leptin

No significant differences were detected between our IL-4 exposed vehicle sample and any concentrations of leptin, in adipsin, RBP4, MCP-1, IL-8 or IL-6.

We did observe an apparent dose-dependent peak in IL-6 expression after exposure to leptin, with a peak in IL-6 levels after exposure to 100 ng/mL of leptin (Figure 33). At 200 and 1000 ng/mL IL-6 concentrations dropped again to levels on par and below the vehicle treated samples, respectively. However, large margins of error prohibit us from making any steadfast conclusions here.

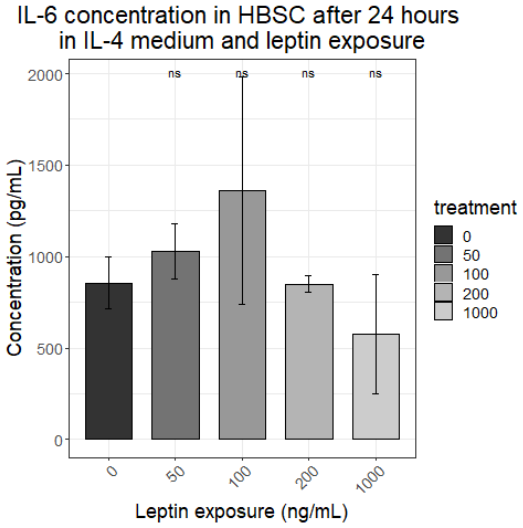

Figure 33. IL-6 levels after 24 hours of leptin exposure in IL-4 medium. Significance based on paired Welch t- tests (p-value >0.05, ns).

3.4.2. Adipokine levels in response to IL-4 and the obesity hormone insulin

We did not observe any significant changes in adipsin nor RBP4 expression after exposure to the different insulin concentrations. In both proteins however we did notice a decline in production compared to IL-4 exposed vehicle after exposure to the lower concentrations of insulin, from 2 000 pmol/mL to 50 000 pmol/mL (Figure 34). At the highest insulin concentration 250 000 pmol/mL, adipsin and RBP4 concentrations did not differ from the control.

a. b.

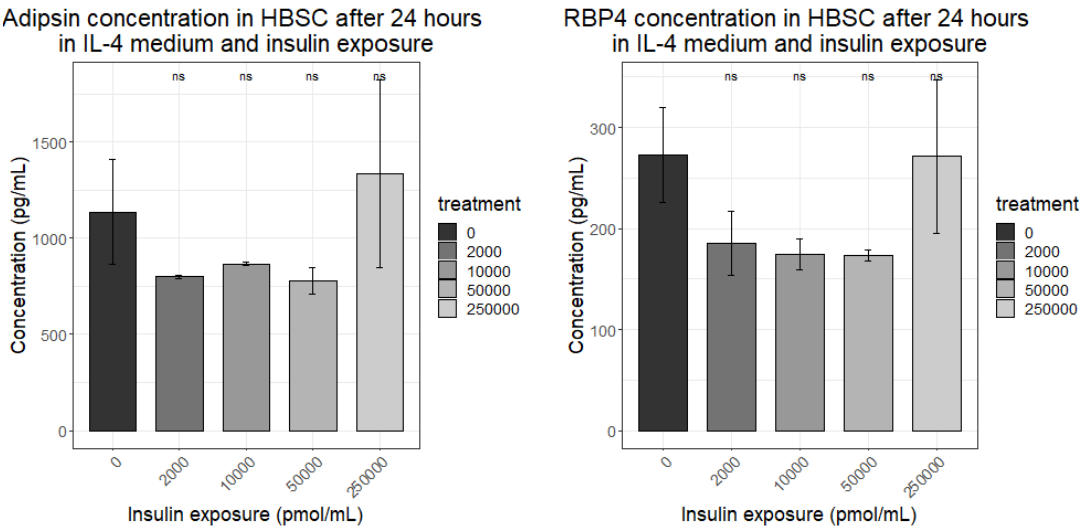

Figure 34. (a) Adipsin and (b) RPB4 levels after 24 hours of insulin exposure in IL-4 medium. Significance based on paired Welch t- tests (p-value > 0.05, ns).

MCP-1 levels decreased after exposure to all insulin concentrations compared to all vehicle (Figure 35).

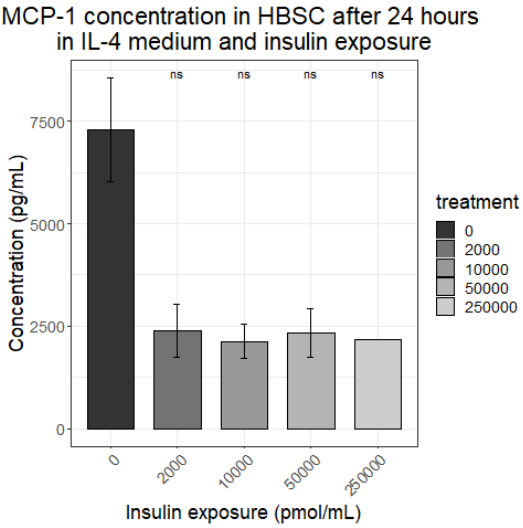

Figure 35. MCP-1 levels after 24 hours of insulin exposure in IL-4 medium. Significance based on paired Welch t- tests (p-value >0.05, ns).

We did not detect any significant differences in IL-8 or IL-6 expression in response to IL-4 at any insulin concentration.

In **conclusion**, we observed a dose-dependent peak in IL-6 production after 100 ng/mL of leptin exposure in IL-4 medium. After insulin exposure in IL-4 medium, there was an overall

downregulation in MCP-1, adipsin and RBP4 concentrations, though exposure with 250 000 pmol/mL of insulin did not cause a chance compared to control IL-4 medium in the latter two.

3.5. Comparison of adipokine production between control- and IL-4 treated cells after exposure to obesity hormones.

We were interested in observing any effect the exposure to IL-4 may have had on HBSMCs in addition to the obesity hormones leptin and insulin, comparatively to exposure to the hormones in control medium.

As we had done during our investigation of immune receptors, we opted to focus our investigation on two supraphysiological concentrations of the obesity hormones, namely 200 ng/mL of leptin and 10 000 pmol/mL of insulin.

3.5.1. Adipokine production after exposure to 200 ng/mL leptin, comparison between control- and IL-4 treated HBSMC.

Leptin did not affect adipsin production in HBSMCs when these were grown in control medium (Figure 36). After exposure to IL-4, we observed an overall increase in adipsin concentrations (non-significant), in vehicle-IL-4 medium.

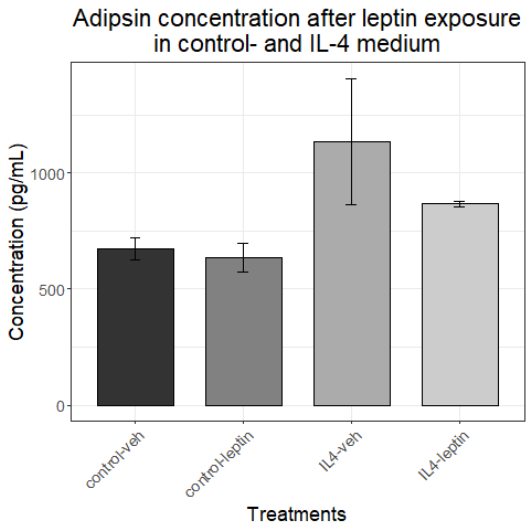

Figure 36. Adipsin concentrations in control and IL-4 treated medium, after 24 hours of exposure to vehicle (veh) or leptin. Significance based on paired Welch t- tests (p-value >0.05, ns).

There was no effect of leptin on RBP4 nor MCP-1 concentrations in control medium HBSMC (Figure 37). When exposed to IL-4, there was an increase in production of RBP4 and MCP-1

though non-significant. Further, exposure to both IL-4 and leptin together reduced this production back to control levels.

a. b.

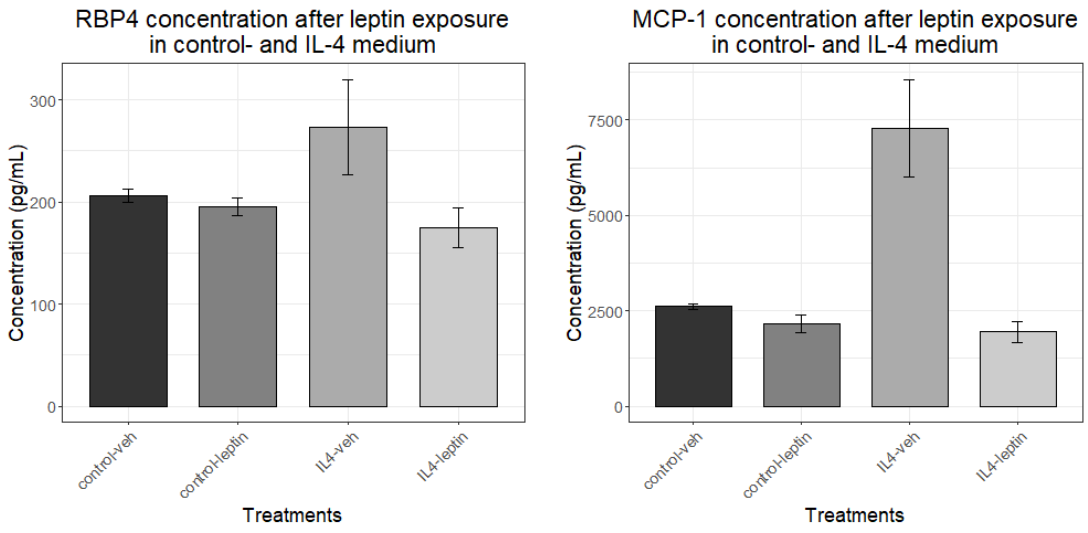

Figure 37. (a) Adipsin and (b) RBP4 concentrations in control and IL-4 treated medium, after 24 hours of exposure to vehicle (veh) or leptin. Significance based on paired Welch t- tests (p-value >0.05, ns).

IL-6 production stayed level in control medium after exposure to leptin. In IL-4 medium however, there was a significant increase when exposed to leptin together with IL-4 medium as well as a large increase when only exposed to IL-4 medium (Figure 38).

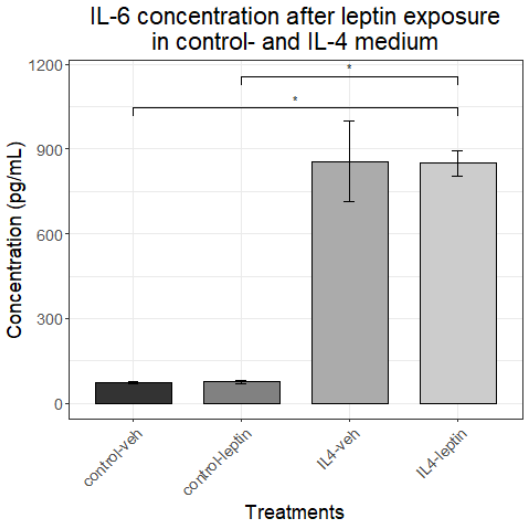

*Figure 38. IL-6 concentrations in control and IL-4 treated medium, after 24 hours of exposure to vehicle (veh) or leptin. Significance based on paired Welch t- tests (p-value <0.05, *).*

Contrarily, IL-8 levels were relatively high in control medium, and dropped substantially after exposure to IL-4 (Figure 39). No effect of leptin could be observed in either of the two media.

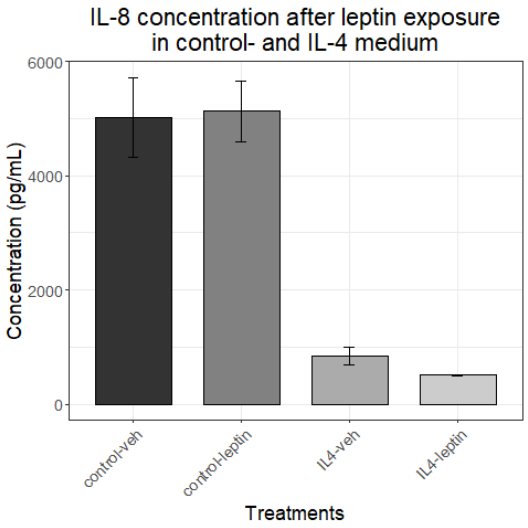

Figure 39. IL-8 concentrations in control and IL-4 treated medium, after 24 hours of exposure to vehicle (veh) or leptin. Significance based on paired Welch t- tests (p-value >0.05, ns).

3.5.2. Adipokin production after exposure to 1000 pmol/mL insulin, comparison between control- and IL-4 treated HBSMC.

There was no effect of insulin in control medium on the production of adipsin by HBSMCs. Upon exposure to IL-4, there was a significant increase in adipsin production in control samples exposed to insulin and IL-4 samples exposed to insulin (Figure 40). Though the increase in adipsin concentration appears larger in cells grown in IL-4 without insulin, due to the greater variability within the samples it did not reach our threshold of significance.

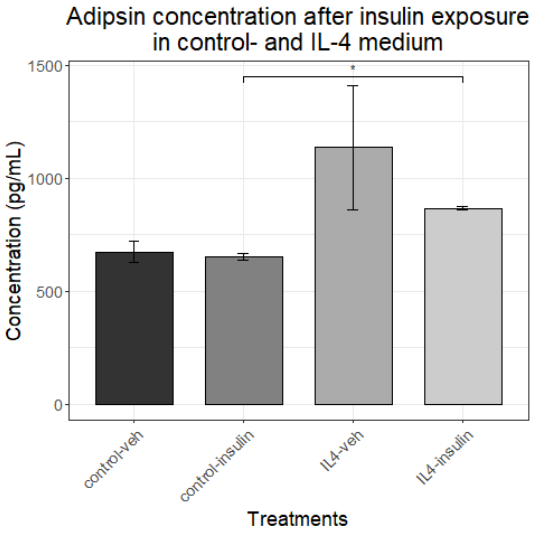

*Figure 40. Adipsin concentrations in control and IL-4 treated medium, after 24 hours of exposure to vehicle (veh) or insulin. Significance based on paired Welch t- tests (p-value <0.05, *; < 0.01 **).*

There was no effect of insulin in control medium on the production of RPB4 nor MCP-1 (Figure 41). Exposure to insulin did appear to induce an increase in the production of these proteins, especially in MCP-1. However, after exposure to both insulin and IL-4 together, protein concentrations returned to the levels of the control samples.

a. b.

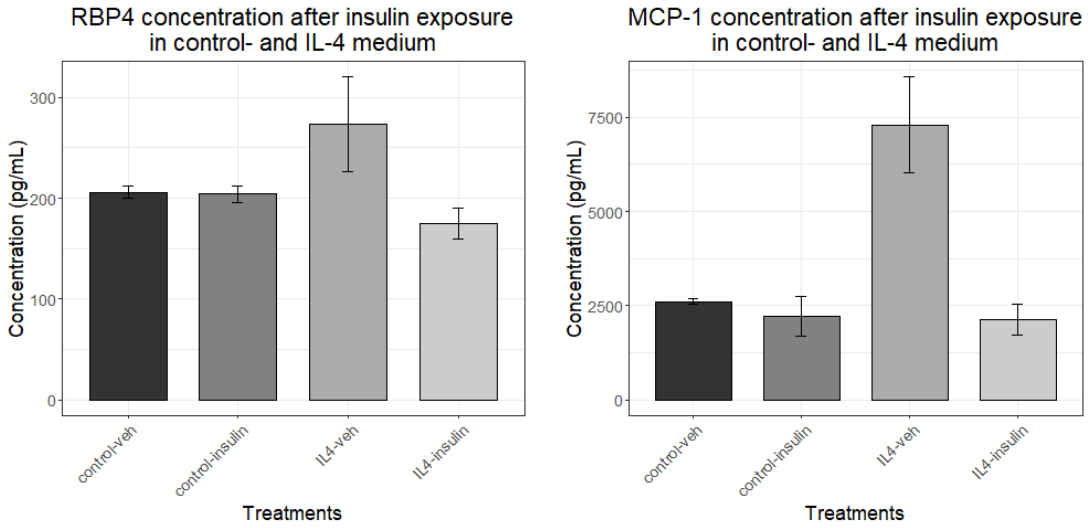

Figure 41.(a) RBP4 and (b) MCP-1 concentrations in control and IL-4 treated medium, after 24 hours of exposure to vehicle (veh) or insulin. Significance based on paired Welch t- tests (p-value >0.05, ns).

We saw a clear effect of exposure to IL-4 in the HBSMC's production of IL-6. Concentrations were increased (non-significant) after exposure to IL-4, both with and without exposure to

insulin (Figure 42). We did not detect any clear differences between vehicle and leptin treated samples in control nor IL-4 medium.

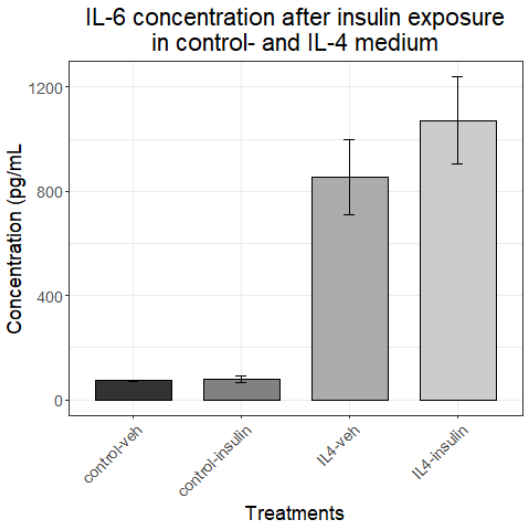

Figure 42. IL-6 concentrations in control and IL-4 treated medium, after 24 hours of exposure to vehicle (veh) or insulin. Significance based on paired Welch t- tests (p-value >0.05, ns).

In control medium, IL-8 was produced in high concentrations (± 5000 pg/mL). No significant difference in the production of IL-8 was detected upon treatment with insulin in control or IL-4 medium (Figure 43). Samples grown in IL-4 medium showed a drastic reduction in IL-8 expression, with significant reductions when comparing IL-4 medium samples with the control-insulin grown cells.

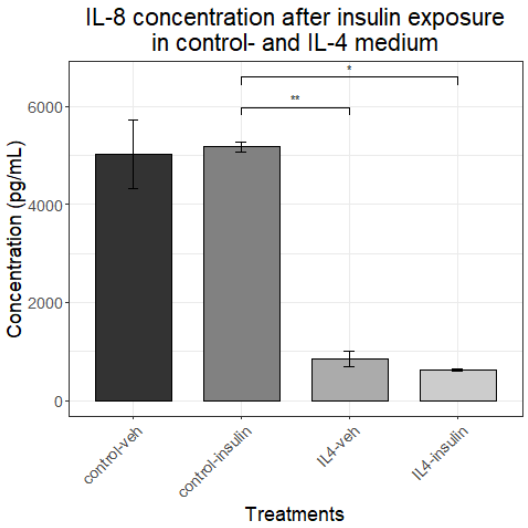

*Figure 43. IL-8 concentrations in control and IL-4 treated medium, after 24 hours of exposure to vehicle (veh) or insulin. Significance based on paired Welch t- tests (p-value <0.05, *; < 0.01 **).*

In **conclusion**, insulin nor leptin appear to have an influence on the production of IL-6 or IL-8. Exposure to IL-4 does cause a change in production of these cytokines, namely an upregulation of IL-6 and downregulation of IL-8, observed both with and without added exposure to leptin and insulin. Further, adipsin, RBP4 and MCP-1 were upregulated in response to IL-4 compared to the control samples. However, additional exposure to leptin or insulin appears to negate this upregulation and restores production of these adipokines back to the control levels.

3.6. General effect of IFN-γ on adipokine production in HBSMCs during exposure to obesity hormones.

We were interested in observing the effect that the exposure to IFN-γ may have had in HBSMCs on adipokine production, in addition to the obesity hormones leptin and insulin. In order to investigate the tandem effect of IFN-γ and the obesity hormones, we exposed HBSMCs to a concentration of 30 ng/mL IFN-γ for 24 hours. The HBSMC were also exposed to different leptin concentrations, namely 100, 200 and 1000 ng/mL, and to different insulin concentrations, 10 000, 50 000 and 250 000 pmol/mL. However, we did not observe a trend in the production of any of the investigated proteins after exposure to neither insulin nor leptin.

3.7. Comparison of adipokine production between control- and IFN-γ treated cells after exposure to obesity hormones.

Next, we focussed on the two supraphysiological concentrations of leptin (200 ng/mL) and insulin (10 000 pmol/mL). We were only able to obtain one sample for our control sample grown in IFN-γ medium, resulting in a lack of statistical testing compared with this study group. The rest of the samples were compared with each other as stipulated in the materials and methods, using paired Welch *t*-tests with a cut-off for significance set at p-value < 0.05.

3.7.1. Adipokin production after exposure to 200 ng/mL leptin, comparison between control- and IFN-γ treated HBSMC.

While there was no effect of leptin in control-medium grown HBSMCs, we did see an overall increase in adipsin concentration in IFN-γ medium grown cells. The increase was significant when comparing control vehicle to IFN-γ HBSMC exposed to leptin (Figure 44). The IFN-γ

grown control also appears to be increased greatly compared to control samples (non-significant).

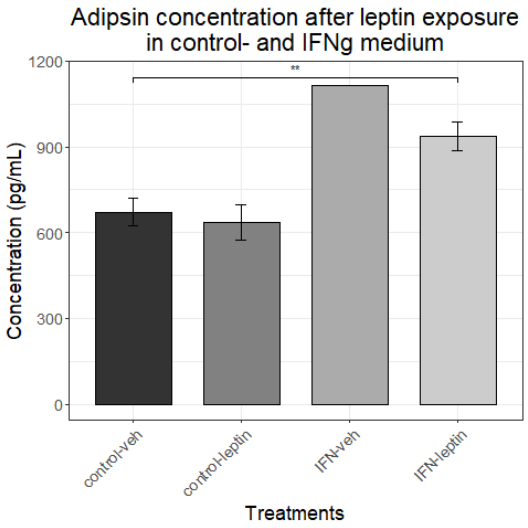

*Figure 44. Adipsin concentrations in control and IFN-γ treated medium, after 24 hours of exposure to vehicle (veh) or leptin. Significance based on paired Welch t- tests (p-value <0.05, *; < 0.01 **).*

MCP-1 concentrations did not differ between control medium vehicle and leptin-exposed control samples. However, after exposure to IFN-γ, MCP-1 production increased massively (Figure 47). When exposure to IFN-γ was combined with leptin, levels lowered again to be relatively even with control medium, suggesting a role for leptin in the suppression of IFN-γ induced MCP-1 production.

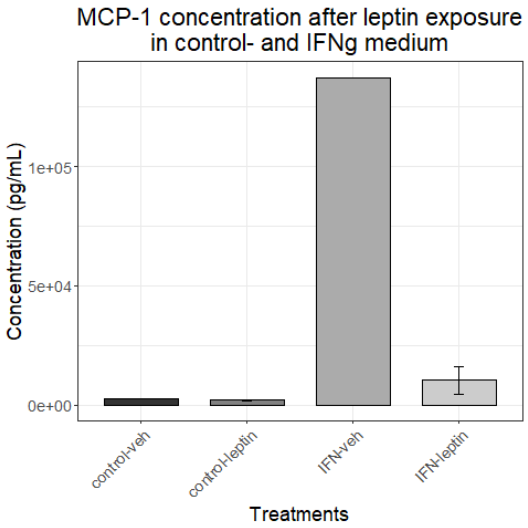

Figure 45. MCP-1 concentrations in control and IFN-γ treated medium, after 24 hours of exposure to vehicle (veh) or leptin. Significance based on paired Welch t- tests (p-value >0.05, ns).

Similar to what we saw in IL-4 medium, IFN-γ medium exposure caused an increase in IL-6 production (Figure 46a) and a decrease in IL-8 production (Figure 46b). There was no effect of leptin on the production of either cytokine, in control nor in IFN-γ medium.

a. b.

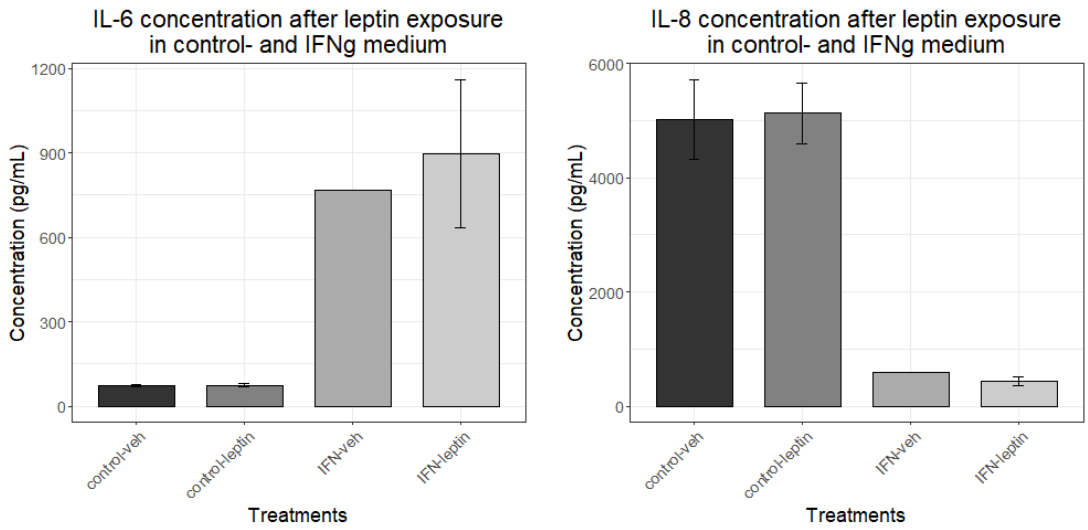

Figure 46. (a) IL-6 and (b) IL-8 concentrations in control and IFN-γ treated medium, after 24 hours of exposure to vehicle (veh) or leptin. Significance based on paired Welch t- tests (p-value >0.05, ns).

We did not see any effect of medium nor leptin exposure on RBP4 concentrations in any of the samples.

3.7.2. Adipokin production after exposure to 1000 pmol/mL insulin, comparison between control- and IFN-γ treated HBSMCs.

There was no effect of insulin on adipsin production in control-medium grown HBSMCs. MCP-1 concentration increased after exposure to IFN-γ, in both vehicle and insulin exposed samples (non-significant) (Figure 47). The increase was markedly lower after exposure to IFN-γ together with insulin.

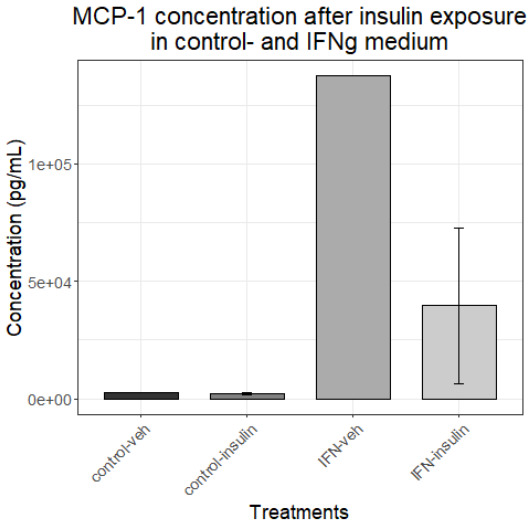

Figure 47. MCP-1 concentrations in control and IFN-γ treated medium, after 24 hours of exposure to vehicle (veh) or insulin. Significance based on paired Welch t- tests (p-value >0.05, ns).

IL-6 concentrations were increased after exposure to the IFN-γ medium (Figure 48). Where insulin did not have any effect in the control medium, in IFN-γ medium there seems to be a reducing effect of insulin on IL-6 production, though the large margin of error prevents us from making reliable conclusions.

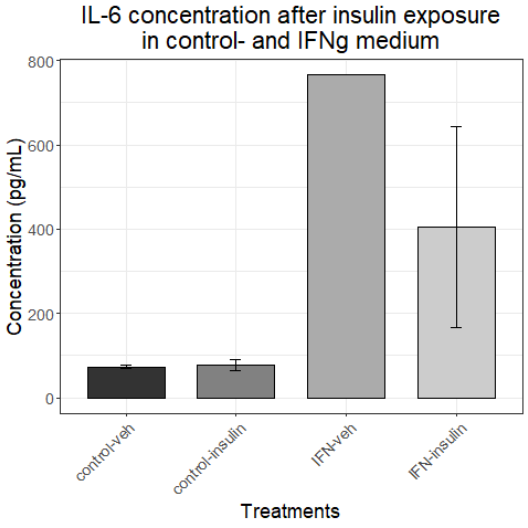

Figure 48. IL-6 concentrations in control and IFN-γ treated medium, after 24 hours of exposure to vehicle (veh) or insulin. Significance based on paired Welch t- tests (p-value >0.05, ns).

We observed an overall decrease of IL-8 concentrations after exposure to IFN-γ medium (Figure 49). No discernible effect of insulin on either of the insulin exposed samples was detected.

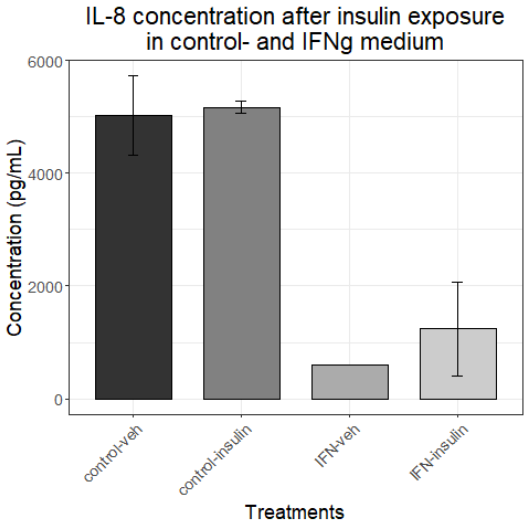

Figure 49. IL-8 concentrations in control and IFN-γ treated medium, after 24 hours of exposure to vehicle (veh) or insulin. Significance based on paired Welch t- tests (p-value >0.05, ns).

We were not able to detect any effect brought about by insulin exposure nor IFN-γ medium in the production of RBP4.

In **conclusion**, we observed the same effect of IFN-γ on IL-6 and IL-8 production as we did after exposure to IL-4, with an increase in IL-6 and reduction in IL-8 upon exposure. MCP-1 production increased after exposure to IFN-γ compared to control samples, but was reduced after exposure to both IFN-γ and one of the obesity hormones, leptin or insulin.

Summary of results

- Leptin nor insulin had an effect on cell death or proliferation rate in HBSMC.
- Leptin exposure caused a downregulation in expression of immune receptors HR1, IL13R, LEPR and NR3C1 (GR). There was no effect of leptin on IL4R, INSR or FGFa expression.
- Insulin exposure caused a dose-dependent increase in FGFa expression, and a decrease in HR1, IL4R, IL13R, INSR and NR3C1 (GR).
- Stimulation with IL-4, with and without leptin or insulin, reduced IL13R and LEPR expression.
- After 8 hours of stimulation with leptin concentrations, IL-6 and IL-8 production was reduced. MCP-1 production was stimulated in a dose-response manner. After 24 hours, cytokine production reached control levels again.
- After IL-4 stimulation, IL-6 production increased and IL-8 production decreased, regardless of added leptin or insulin exposure. Adipsin, RBP4 and MCP-1 production increased in response to IL-4, but reduced back to control levels after combined stimulation with leptin or insulin
- After IFN-γ stimulation, again IL-6 production increased and IL-8 production decreased, regardless of added leptin or insulin exposure.

Discussion

Metabolic syndrome and asthma are both diseases that have seen an astounding global increase in incidence over the last decades. Moreover, either disease appears to aggravate the other, with metabolic syndrome being associated with low-grade systemic inflammation and disturbed mechanics of lung ventilation, inducing an environment where asthma exacerbations are more severe. On the other hand, fear of asthma exacerbations leads to reduced levels of activity and generally a more sedentary life style[258], [259]. Obese asthma has been acknowledged as a distinct disease phenotype, characterized by worse response to therapy with inhaled corticosteroids, more frequent exacerbations and neutrophilic infiltration of the airways. The research I undertook was an attempt to elucidate the link between obesity hormones and inflammation, in order to uncover their role in allergic immune responses and pave the road towards novel therapies. To accomplish these goals, I used a cell culture model of human bronchial smooth muscle cells (HBSMCs) exposed to supraphysiological levels of leptin and insulin, as seen in obesity. I selected this model due to smooth muscle tissue's integral role in the pathophysiology of severe asthma, being responsible for bronchoconstriction and airway remodelling[43]. I investigated the expression of different immune receptors in a lysate of cultured HBSMCs by means of RT-PCR. Additionally, using flow cytometry, I determined soluble adipokine- and inflammatory markers in cell culture supernatants.

Our interest in leptin derives from recent publications pointing towards this obesity hormone being partially responsible for the induced state of chronic, systemic low-grade inflammation typical in patients suffering from metabolic disease[231], [260], [261]. Reportedly during acute infections, leptin levels increase and are capable of activating innate immune cells as a first defence response[262]. We found that exposure to leptin induced an overall reduction in the expression of immune response-modulating receptors, among which HR1, IL13R, LEPR and NR3C1. At higher leptin concentrations, downregulation of these receptors in response to leptin was apparent especially after 24 hours of exposure. Chief among our receptors of interest was HR1, a well-known mediator of histamine-induced bronchoconstriction, by direct activation of lung smooth muscle tissue[263]. In mice it was demonstrated that histamine attenuates serum leptin levels. Histidine decarboxylase (HDC) knock-out mice, lacking this enzyme essential in histamine biosynthesis, showed elevated

levels of leptin in serum together with decreased LEPR expression. Further, HR1 knock-out mice also displayed diet-induced fat tissue accumulation and increased serum leptin levels[264]. Studies have also shown a complete absence of an anorexigenic effect of leptin in HR1 knock-out mice[265]–[267]. To the best of our knowledge, no studies have focused on the effect of leptin on histamine signalling pathways. Our findings suggest a reciprocal negative regulation between these two mediators[268]. It is plausible that in obese asthma patients, in whom higher levels of circulating leptin were reported, airway hyperreactivity is not chiefly mediated by histamine. However, further research is necessary to uncover the nature of the leptin-HR1 interactions *in vivo*.

LEPR shows structural similarities to the class I cytokine receptor family, and is ubiquitously distributed over different cell types. It facilitates the transport and degradation of leptin, as well as signal transduction via the activation of the mitogen-activated protein kinase (MAPK) pathway[269]. Increased levels of leptin have been observed in the serum of obese individuals and asthmatic patients. Some studies reported an association between higher serum leptin and asthma severity[221]. However, it is not clear how leptin affects different cell types in the lung tissue. On one hand, it may contribute to worsening of asthma in obese patients via stimulation of inflammatory chemokine and cytokine production like MCP-1, eotaxin, IL-8 and IL-6 through LEPR signalling[231]. In our study, however, exposure of HBSMCs to leptin did not result in an increase in aforementioned chemo- and cytokines. Another study found that the expression of LEPR was lower in the bronchial epithelial cells of patients with severe asthma, and associated it with an increased thickness of the epithelial basement membrane, a marker of tissue remodelling[270]. The expression of LEPR in HBSMCs was downregulated after exposure to leptin and Th2 type cytokines, namely IL-4. Exposure to leptin, however, did not affect the proliferation rate of HBSMCs. The physiological role of leptin is to promote insulin sensitivity and maintain metabolic homeostasis. Decrease in leptin signalling, as suggested by the reduced expression of LEPR after exposure to supraphysiological level of the adipokine, could lead to metabolic dysfunction in a glucose-rich environment, as seen in obesity. Longitudinal experiments assessing not only proliferation rates, but also healing responses of HBSMCs, are necessary to establish whether leptin contributes to smooth muscle cell hyperplasia in obese asthma.

The IL13R mediates inflammatory responses in the lung during Th2 inflammation via the IL-13 and IL-4 signalling pathway. It regulates several key features of asthmatic inflammation, including airway hyperreactivity, excessive mucus secretion and, in long-term, airway remodelling[271]. IL-13R expression is upregulated in bronchial smooth muscle tissue murine models of allergic bronchial asthma[272]. Our observation of IL13R downregulation in response to leptin appears contradictory with the role both IL13R and leptin play in mediating airway hyperresponsiveness. It could be speculated that at higher concentrations, leptin signalling becomes disrupted due to the development of leptin resistance, also observed in obese patients[157], [273]. This could also explain the reduction in LEPR expression, which has been observed before in leptin resistance in controls and asthmatics[270].

NR3C1 is a gene encoding the glucocorticoid receptor (GR), capable of binding both natural and synthetic glucocorticoids. NR3C1 functions as a nuclear transcription factor. As displayed in Figure 3, glucocorticoids are the cornerstone and the gold standard of asthma management. Their efficiency in asthma relies mostly on the inhibition of eosinophilic inflammation, but also repression of neutrophil recruitment to the sites of inflammation and induction of apoptosis in proinflammatory T cell subsets, while maintaining the survival of T regulatory cells (Tregs)[274]–[276]. Chang et al. were able to show a reduction in GR expression in lung biopsy tissue samples from subjects with severe and non-severe asthma, as compared to healthy controls. Further, they confirmed that dexamethasone-induced nuclear translocation is reduced in severe asthmatics compared to non-severe asthma patients and healthy control individuals[277]. We observed leptin-induced downregulation in NR3C1 expression in HBSMC, which is in line with the reduced response to inhaled corticosteroid treatment in obese asthmatics. This finding could also be translated into an overall decline in immunosuppressive activity. This would fit well into the role of leptin as a pro-inflammatory mediator in obesity and exacerbator of allergic immune responses.

We exposed HBSMCs to a range of increasing insulin concentrations and observed a dose-dependent upregulation of FGFa expression. Fan et al. [278] investigated the effect of FGFa supplementation on high fat diet-induced obesity in mice and saw an improvement in insulin sensitivity upon treatment. Further, FGFa treatment also exerted anti-inflammatory effects, inhibiting expression of anti-inflammatory genes such as MCP-1, TNF, IL-6 and IL-

1β[278]. Another factor in the FGFa induced amelioration of insulin resistance was a decrease in the number of adipose-tissue macrophages[279]. This effect was attributed to reduced peripheral blood monocyte recruitment to the adipose tissue. On the other hand, FGFa has also been established as a human adipogenic molecule, displaying a proliferative effect on human preadipocytes in cells from subcutaneous and intra-abdominal sites[280]. The observed dose-dependent increase in FGFa expression in response to insulin could be a form of counteracting systemic inflammation associated with obesity and IR[281], [282].

HR1 expression after exposure to leptin in supraphysiological concentrations showed a reduction compared to control. Exposure with IL-4 recovered HR1 expression, with combined IL-4 and leptin exposure returning HR1 expression back to control levels. This finding is surprising, given that previous studies have reported IL-4 stimulating expression of HR1[283], [284]. The difference in effect here could lay in the different IL-4 concentrations used during exposure[284] as well as the usage of different cell types[283]. Even though IL-4 and leptin did restore HR1 expression, it did not exceed control levels. This suggests that pro-inflammatory responses in obese asthma are not induced via HR1 signalling. This notion is further supported by the reported reduced impact of allergy therapeutics like antihistamines in obese patients compared to controls.

IL-4 and IL-13 signalling plays a prominent role in the pathogenesis of allergic airway diseases by modulating immune cell differentiation, promoting mucus hypersecretion and airway fibrosis. The function of IL-4 and IL-13 is mediated by two heterodimeric transmembrane complexes: type I receptor comprised of IL4Rα and IL4Rγ-c subunits, and type II receptor comprised of IL4Rα and IL13Rα subunits[285]. While IL-4 is capable of binding both receptors, IL-13 only activates type II receptor complex. Therefore, the effect of IL-4 and IL-13 signalling only partially overlaps. HBSMCs have functional expression of both receptor types. IL-13 and IL-4 have been shown to increase the contractile potency of histamine on *in vitro* cultured human organoids, as well as HBSMCs[284]. We have observed a significant downregulation of both IL4Rα (IL4R) and IL13Rα (IL13R) subunit expression in HBSMCs after exposure to IL-4, suggesting a negative feedback loop shared between the two cytokines. Simultaneous stimulation with leptin did not reverse the effect. In fact, IL-4 and leptin exposure resulted in the largest reduction of the expression of type II receptor subunits. Therefore, exposure to supraphysiological levels of leptin does not disrupt the negative

feedback loop to IL-4 signalling in HBSMCs. Additionally, we did not observe any effect of insulin exposure on the HBSMCs expression of IL13R and LEPR. As we had observed after leptin exposure, there was a downregulation in the expression of both receptors caused by IL-4 supplemented medium. Given that there were no differences within the control medium nor IL-4 medium caused by insulin, we can conclude that insulin has no effect on the expression of IL13R nor LEPR in HBSMCs at supraphysiological concentrations. A plethora of anti-inflammatory properties has been ascribed to insulin, from the ability to suppress TNF production to the suppression of pro-inflammatory chemokines like MCP-1 and inflammatory intracellular adhesion molecule 1 (ICAM-1)[281], [282]. However, it appears here that both obesity metabolites have limited impact on the expression of LEPR and IL13R. It is plausible that airway hyperresponsiveness described in obese asthmatic patients is primarily mediated via different pathways. This is in line with comparable levels of IL-4 in the serum of obese and non-obese individuals[286].

While we did not observe a shift in INSR expression in response to leptin or insulin in control nor IL-4 medium, there was an increase in response to IL-4 exposure. Chang et al. [287] investigated the effect of IL-4 on glucose and lipid metabolism, and found a novel role for IL-4 as a mediator of glucose tolerance and insulin sensitivity. Our observation confirms this finding for HBSMCs. Interestingly, IL4R expression was downregulated after exposure to insulin.

HR1 expression increased after exposure to insulin and IL-4 supplemented medium, while HBSMCs stimulation with each of these mediators alone led to a decrease in expression. H1R and IL-4 are both important drivers of type 2 immune responses[288]–[291]. HR1 signalling promotes T cell differentiation towards the Th2 phenotype; Th2 cells further produce IL-4. Downregulation of HR1 receptor expression after IL-4 stimulation is part of the negative feedback loop, which we observed could be interrupted by simultaneous exposure to supraphysiological levels of insulin. This suggests that IR could be linked to promoting a type 2 immune responses.

Leptin has previously been shown to affect cytokine production and release by immune and non-immune cell types. We investigated the effect of different leptin concentrations on cytokine production by HBSMCs. We found that leptin stimulated MCP-1 production in a dose-dependent manner. MCP-1, also known as chemokine (C-C motif) ligand

2 (CCL2), is a potent chemoattractant, capable of recruiting a multitude of immune cells, among which monocytes, macrophages, basophils, T lymphocytes and NK cells[292], [293]. Furthermore, MCP-1 released by HBSMC induces fibroblast migration and, as a consequence, airway remodelling. In leptin deficient mice, who spontaneously develop obesity, exogenous administration of leptin induced a decrease in the levels of circulating MCP-1 and IL-6. The authors of this study conclude that subphysiological to physiological levels of leptin counteract adipose tissue associated inflammation[294]. Our findings are in line with this hypothesis, as HBSMCs stimulated with lower leptin concentrations (subphysiological) produced less MCP-1 in comparison to the control conditions. However, MCP-1 production increased with the increase in leptin concentration (up to supraphysiological level). At a leptin concentration of 1000 ng/mL, MCP-1 production exceeded the baseline level in control samples, switching to a pro-inflammatory environment. It should be noted that the opposite effect of leptin has also been observed, with leptin injection into isolated epididymal adipose tissue of ob/ob mice resulting in a dose-dependent increase in MCP-1 secretion[295]. The minimum leptin concentration used in this experiment was at minimum a 10-fold increase from the concentration used by Hoffmann et al. [294], further emphasising the importance of leptin dosage on the provocation of inflammatory immune responses. We also observed a downregulation in IL-6 production immediately after leptin exposure (8 hours), though this effect was lost after 24 hours. As we have discussed previously, IL-6 is a pro-inflammatory cytokine overproduced in patients with obesity, but it also has a homeostatic role inducing leptin and insulin release[218], [232], [296]. The initial decrease in IL-6 production in response to leptin is therefore part of the physiological regulation between these mediators. However, patients with obesity are exposed to elevated concentrations of leptin over an extensive period of time, which leads to leptin desensitization and loss of the physiological regulation. We observed normalization of IL-6 production after 24 hours of exposure. Longer observation is needed to establish the effect of chronic exposure to supraphysiological levels of leptin.

We observed the same trend in IL-8 production after exposure to leptin, with an initial decrease and return to control levels after 24 hours. Production of IL-8 is regulated by leptin binding to LEPR, and it is possible that the reduction in IL-8 is caused by the reduced LEPR-expression we observed after leptin exposure. A literature search was not able to confirm our findings. Moreover, the majority of studies describe an upregulation in IL-8 upon exposure to

leptin[231], [297]–[300]. The majority of these studies however used animal- or fibroblast models, which may account for variation in the results. Further, Watanabe et al. [231] measured cytokine production by leptin-exposed lung fibroblasts after 48 hours of exposure, which perhaps provides a better window into the effects of chronic leptin exposure in obesity and the associated low-grade inflammatory environment.

In response to insulin, we observed an immediate reduction in IL-6 and IL-8 production after 8 hours. Production of these cytokines is regulated by, among others, Toll-like receptor 4 (TLR4), whose expression in turn, is regulated by insulin. In immune mononuclear cells, insulin blocks TLR4 transcription and decreases TNF-a, IL-1B and IL-6 expression[301]. We suspect the same pathway may be activated in HBSMCs. The initial reduction in IL-6 and IL-8 production, however, was no longer visible after 24 hours. Varying effects of insulin over time have been described by Iwasaki et al. [302], who showed a short-term inhibitory effect after 6 hours and long term stimulatory effect on NF-kB-mediated transcription in hepatic cells. NF-kB is a transcription factor that plays an essential role in the transcription of inflammation-related genes such as CRP, serum amyloid A and coagulation factor VIII[302]. The transcription factor is also activated by TLR4 signalling, adding further credibility to our proposed mechanism of insulin activity[303].

Production of adipokines and cytokines of interest were similar between exposure to leptin and insulin. We were unable to detect any effect of leptin nor insulin on the production of IL-6 and IL-8, regardless of presence of IL-4 in the cell culture medium. There was however a clear but opposite effect of IL-4 stimulation: IL-8 was downregulated in response to IL-4, while IL-6 production was upregulated. Regulation of the chemokine IL-8 by IL-4 has been well-described for human monocytes and keratinocytes[304], [305]. On the other hand, IL-6 was upregulated in response to IL-4. IL-4, a classical driver of type 2 immunity, promotes proinflammatory state in HBSMCs. Thus, these findings are within the expectations.

Leptin nor insulin exposure had any effect on the production of retinol binding protein 4 (RBP4) or adipsin in control medium. Briefly, RBP4 and adipsin are both adipokines. RBP4, as its name suggests, transports retinol and is implicated in dysregulation of glucose and lipid homeostasis[306]. Adipsin, also known under the name complement factor D, catalyses the alternative pathway of complement activation. Other functions of the molecule are formation of the C5-C9 membrane attack complex and formation of complement peptides C3a and C5a.

Importantly, it also plays a role in lipid homeostasis and increases insulin secretion in response to glucose[307], [308]. The production of both adipokines was upregulated after exposure to IL-4 supplemented medium. Studies have outlined potential relationships between adipsin and Th2-driven allergic diseases before. Adipsin serum levels were found to be upregulated in patients with seasonal allergic rhinitis[309]. In a study evaluating cows' milk oral immunotherapy efficacy, serum adipsin was elevated in patients who did not respond to therapy[306]. Given the role of IL-4 in the pathology of allergic diseases, this cytokine could be a driving factor in the production of adipsin. Though no such link between RBP4 and IL-4 could be found in literature, based on our data we hypothesize that a regulatory relationship between the two exists.

Both insulin and leptin exposure combined with IL-4 reduce adipsin and RBP4 levels back to control conditions. Interestingly, RBP4 and adipsin appear to influence insulin metabolism in a contrasting manner. In obese patients RBP4 causes adipose tissue inflammation and induces IR[310], [311], whereas increased adipsin concentrations are associated with lower fasting glucose levels and reduced IR[310]. It was surprising to find both RBP4 and adipsin downregulated in response to both leptin and insulin in IL-4 medium.

Lastly, while IL-4 is a key mediator of type 2 immunity response, IFN-γ is a major component of the type 1 immune responses and is aimed primarily at host defence against viral and bacterial infections. Of note, type 1 immune responses have been associated with neutrophilic inflammation in asthma, with IFN-γ as one of the main drivers of the pathology. Supplementation of medium with IFN-γ resulted in the same production trend as we observed after IL-4 stimulation. This shows that both type 2 and type 1 immune responses can converge to the establishment of a proinflammatory environment in the airways of asthmatic patients.

Conclusion

We summarize our most important findings below;

- Leptin initiates a reciprocal negative feedback loop between leptin and HR1 in HBSMCs. Additional exposure to IL-4 together with leptin restores HR1 expression to control levels.
- Leptin and insulin downregulate NR3C1, the gene encoding a glucocorticoid receptor. We suspect the disruption of this transcription factor signalling may play an important role in the reduced effectiveness of glucocorticoids' treatment in obese asthmatics.
- FGFa expression is upregulated in a dose-dependent manner after contact with insulin. We propose this may be one of the mechanisms used to counteract systemic inflammation in obesity, while also advancing airway remodelling as observed in asthmatic patients.
- Exposure to IL-4 reduced expression of IL13R and LEPR in HBSMCs, regardless of additional exposure to insulin or leptin. We propose airway hyperresponsiveness in obese asthmatics is likely perpetuated via other immune receptors.
- Leptin influenced MCP-1, IL-6 and IL-8 production by HBSMCs in a dose-dependent manner, with subphysiological concentrations greatly reducing production, and supraphysiological concentrations restoring production to control levels. We thus conclude that the effect of insulin and leptin on HBSMC production of cytokines is dependent on the hormone concentration. In supraphysiological concentration the anti-inflammatory effect of insulin and leptin signalling is lost.
- The production of IL-6 is increased and of IL-8 decreased in response to IL-4 and IFN-γ regardless of leptin or insulin exposure.
- IFN-γ and IL-4 elicit largely the same immune response in HBSMCs exposed to obesity hormones, showing that Type 1 and Type 2 immune responses can converge in the production of a pro-inflammatory environment in obese asthmatics.

Abstract

Background: Asthma is a chronic, immune-mediated inflammatory disease of the respiratory system, affecting more than 300 million people worldwide. Asthma is a heterogenous disease and several clinical and immune phenotypes have been distinguished. One of them is obesity-associated asthma, characterized by neutrophilic infiltration of the airways, frequent exacerbations and poor response to therapy with inhaled corticosteroids. With steady increase in the prevalence of both diseases, obesity-associated asthma is a major socioeconomical burden.

Aim: We aimed to uncover the role of obesity hormones leptin and insulin in their capacity to influence the immune responses in asthma. We specifically focused on their impact on human bronchial smooth muscle cells (HBSMCs) as the determinants of long-term disease outcomes.

Material and methods: We exposed HBSMCs *in vitro* to a range of sub- and supraphysiological concentrations of leptin and insulin. Next, in order to mimic the pro-inflammatory environment present during allergic hypersensitivity reactions, we exposed HBSMCs to a combination of interleukin 4 (IL-4) or interferon γ (INF-γ) and an obesity hormone. We measured immune receptor expression via RT-PCR and adipokine and cytokine production using cytometric bead arrays.

Results and discussion: The expression of histamine receptor 1 (HR1), IL-13 receptor and glucocorticoid receptor NRC31 by HBSMCs decreased upon exposure to both leptin and insulin. Furthermore, exposure to insulin stimulated fibroblast growth factor-1 (FGFa) expression in a dose-dependent manner. Exposure to obesity hormones caused a transient reduction in production of IL-6 and IL-8. Exposure to IL-4 and IFN-γ caused an upregulation in IL-6, and downregulation in IL-8 production, regardless of additional insulin or leptin exposure.

Conclusion: Leptin and insulin affect the expression of immune receptors and cytokine production by HBSMCs. In physiological concentrations these hormones have an anti-inflammatory effect, which disappears at supraphysiological concentrations, as seen in obese patients.

Abstrakt w języku polskim

Wstęp: Astma to przewlekła choroba zapalna układu oddechowego o podłożu immunologicznym. Zróżnicowana patofizjologia i symptomatologia schorzenia doprowadziła do wyróżnienia kilku fenotypów astmy. Jednym z nich jest astma związana z otyłością. Fenotyp ten charakteryzuje się infiltracją dróg oddechowych przez neutrofile, częstymi zaostrzeniami choroby oraz gorszą odpowiedzią na terapię z wykorzystaniem wziewnych glikokortykosteroidów. Dokładne mechanizmy immunologiczne propagujące stan zapalny w astmie związanej z otyłością nie są jeszcze poznane.

Cele: Celem niniejszej pracy było ustalenie wpływu leptyny oraz insuliny na regulację odpowiedzi immunologicznej w astmie, że szczególnym naciskiem na działanie tych hormonów na komórki mięśniowe gładkie oskrzeli (ang. *human bronchial smooth muscle cells (HBSMCs)*). Mięśniówka gładka dróg oddechowych odgrywa kluczową rolę w nagłych zaostrzeniach astmy, jak również definiuje najistotniejsze odległe powikłanie choroby, tj. przebudowę dróg oddechowych.

Materiały i metody: HBSMCs w warunkach hodowli komórkowej *in vitro* zostały poddane stymulacji leptyną oraz insuliną w stężeniach odpowiadających fizjologicznym oraz powyżej fizjologicznych, odpowiadających stężeniu tych hormonów we krwi osób otyłych. Następnie HBSMCs zostały poddane stymulacji leptyną oraz insuliną w połączeniu z interleukiną 4 (IL-4) oraz interferonem gamma (IFN-γ). Efekty stymulacji na poziom ekspresji genów dla receptorów immunologicznych oraz produkcji mediatorów stanu zapalnego został oceniony odpowiednio za pomocą reakcji łańcuchowej polimerazy z odwrotną transkrypcją oraz cytometrii przepływowej.

Wyniki badań: Stymulacja HBSMCs zarówno leptyną jak i insuliną doprowadziła do zmniejszenia ekspresji genów dla receptora histaminy 1, receptora IL-13 oraz receptora glukokortykoidowego NRC31. Ekspozycja HBSMCs na rosnące stężenie insuliny spowodowała stopniowy wzrost ekspresji genu dla czynnika wzrostu fibroblastów. Stymulacja leptyną oraz insuliną zredukowała produkcję IL-6 oraz IL-8 przez HBSMCs. Stymulacja IL-4 oraz IFN-γ doprowadziła do zwiększenia produkcji IL-6 oraz zmniejszenia produkcji IL-8 przez HBSMCs, niezależnie od jednoczesnej stymulacji leptyną i insuliną.

Wnioski: Stymulacja komórek mięśni gładkich oskrzeli leptyną oraz insuliną powoduje zmiany w ekspresji genów dla receptorów immunologicznych oraz produkcji mediatorów stanu zapalnego. W fizjologicznych stężeniach leptyna i insulina wykazują działanie przeciwzapalne poprzez zmniejszenie produkcji cytokin prozapalnych; w stężeniach suprafizjologicznych efekt ten zanika.

References

[1] S. G. O. Johansson *et al.*, "A revised nomenclature for allergy: An EAACI position statement from the EAACI nomenclature task force," *Allergy*, vol. 56, no. 9, pp. 813–824, Jul. 2008, doi: 10.1111/j.1398-9995.2001.00002.x-i1.

[2] C. Traidl-Hoffmann, T. Zuberbier, and T. Werfel, *Allergic Diseases – From Basic Mechanisms to Comprehensive Management and Prevention*. 2022.

[3] T. Zheng, "The Atopic March: Progression from Atopic Dermatitis to Allergic Rhinitis and Asthma," *Journal of Clinical & Cellular Immunology*, vol. 05, no. 02, 2014, doi: 10.4172/2155-9899.1000202.

[4] D. A. Hill and J. M. Spergel, "The atopic march: Critical evidence and clinical relevance," *Annals of Allergy, Asthma and Immunology*, vol. 120, no. 2. American College of Allergy, Asthma and Immunology, pp. 131–137, Feb. 01, 2018. doi: 10.1016/j.anai.2017.10.037.

[5] L. K. Tanno *et al.*, "Constructing a classification of hypersensitivity/allergic diseases for ICD-11 by crowdsourcing the allergist community," *Allergy: European Journal of Allergy and Clinical Immunology*, vol. 70, no. 6, pp. 609–615, Jun. 2015, doi: 10.1111/all.12604.

[6] L. K. Tanno, M. Calderon, N. G. Papadopoulos, and P. Demoly, "Mapping hypersensitivity/allergic diseases in the International Classification of Diseases (ICD)-11: Cross-linking terms and unmet needs," *Clinical and Translational Allergy*, vol. 5, no. 1, Jun. 2015, doi: 10.1186/s13601-015-0063-x.

[7] "Worldwide variation in prevalence of symptoms of asthma, allergic rhinoconjunctivitis, and atopic eczema: ISAAC. The International Study of Asthma and Allergies in Childhood (ISAAC) Steering Committee," *The Lancet*, 1998.

[8] J. Mallol, J. Crane, E. von Mutius, J. Odhiambo, U. Keil, and A. Stewart, "The International Study of Asthma and Allergies in Childhood (ISAAC) Phase Three: A global synthesis," *Allergologia et Immunopathologia*, vol. 41, no. 2, pp. 73–85, Mar. 2013, doi: 10.1016/j.aller.2012.03.001.

[9] R. Pawankar, "Allergic diseases and asthma: A global public health concern and a call to action," *World Allergy Organization Journal*, vol. 7, no. 1, pp. 1–3, 2014, doi: 10.1186/1939-4551-7-12.

[10] S. S. Braman, "The global burden of asthma," in *Chest*, 2006, vol. 130, no. 1 SUPPL., pp. 4S-12S. doi: 10.1378/chest.130.1_suppl.4S.

[11] P. J. Turner, D. E. Campbell, M. S. Motosue, and R. L. Campbell, "Global Trends in Anaphylaxis Epidemiology and Clinical Implications," *Journal of Allergy and Clinical Immunology: In Practice*, vol. 8, no. 4, pp. 1169–1176, Apr. 2020, doi: 10.1016/j.jaip.2019.11.027.

[12] A. Baseggio Conrado, D. Ierodiakonou, M. H. Gowland, R. J. Boyle, and P. J. Turner, "Food anaphylaxis in the United Kingdom: Analysis of national data, 1998-2018," *The BMJ*, vol. 372, Feb. 2021, doi: 10.1136/bmj.n251.

[13] E. Poirot, F. He, L. Hannah Gould, and J. L. Hadler, "Deaths, hospitalizations, and emergency department visits from food-related anaphylaxis, New York City, 2000-2014: Implications for

fatality prevention," *Journal of Public Health Management and Practice*, vol. 26, no. 6, pp. 548–556, Nov. 2020, doi: 10.1097/PHH.0000000000001137.

[14] J. Genuneit *et al.*, "Overview of systematic reviews in allergy epidemiology," *Allergy: European Journal of Allergy and Clinical Immunology*, vol. 72, no. 6. Blackwell Publishing Ltd, pp. 849–856, Jun. 01, 2017. doi: 10.1111/all.13123.

[15] A. Rodriguez, E. Brickley, L. Rodrigues, R. A. Normansell, M. Barreto, and P. J. Cooper, "Urbanisation and asthma in low-income and middle-income countries: a systematic review of the urban-rural differences in asthma prevalence," *Thorax*, vol. 74, no. 11. BMJ Publishing Group, pp. 1020–1030, Nov. 01, 2019. doi: 10.1136/thoraxjnl-2018-211793.

[16] L. Claudio, J. A. Stingone, and J. Godbold, "Prevalence of Childhood Asthma in Urban Communities: The Impact of Ethnicity and Income," *Annals of Epidemiology*, vol. 16, no. 5, pp. 332–340, May 2006, doi: 10.1016/j.annepidem.2005.06.046.

[17] R. S. Gupta, X. Zhang, L. K. Sharp, J. J. Shannon, and K. B. Weiss, "Geographic variability in childhood asthma prevalence in Chicago," *Journal of Allergy and Clinical Immunology*, vol. 121, no. 3, 2008, doi: 10.1016/j.jaci.2007.11.036.

[18] "https://www.cdc.gov/," May 28, 2022.

[19] E. Uphoff, B. Cabieses, M. Pinart, M. Valdés, J. Maria Antó, and J. Wright, "A systematic review of socioeconomic position in relation to asthma and allergic diseases," *European Respiratory Journal*, vol. 46, no. 2. European Respiratory Society, pp. 364–374, Aug. 01, 2015. doi: 10.1183/09031936.00114514.

[20] B. J. H. Dierick *et al.*, "Burden and socioeconomics of asthma, allergic rhinitis, atopic dermatitis and food allergy," *Expert Review of Pharmacoeconomics and Outcomes Research*, vol. 20, no. 5. Taylor and Francis Ltd., pp. 437–453, Sep. 02, 2020. doi: 10.1080/14737167.2020.1819793.

[21] A. M. Drucker, A. R. Wang, W. Q. Li, E. Sevetson, J. K. Block, and A. A. Qureshi, "The Burden of Atopic Dermatitis: Summary of a Report for the National Eczema Association," *Journal of Investigative Dermatology*, vol. 137, no. 1. Elsevier B.V., pp. 26–30, Jan. 01, 2017. doi: 10.1016/j.jid.2016.07.012.

[22] S. B. L. Barnett and T. A. Nurmagambetov, "Costs of asthma in the United States: 2002-2007," *Journal of Allergy and Clinical Immunology*, vol. 127, no. 1, pp. 145–152, Jan. 2011, doi: 10.1016/j.jaci.2010.10.020.

[23] C. Nunes, A. M. Pereira, and M. Morais-Almeida, "Asthma costs and social impact," *Asthma Research and Practice*, vol. 3, no. 1, Dec. 2017, doi: 10.1186/s40733-016-0029-3.

[24] S. C. Dharmage, J. L. Perret, and A. Custovic, "Epidemiology of Asthma in Children and Adults," *Frontiers in Pediatrics*, vol. 7, Jun. 2019, doi: 10.3389/fped.2019.00246.

[25] M. Masoli, D. Fabian, S. Holt, and R. Beasley, "The global burden of asthma: executive summary of the GINA Dissemination Committee Report," *Allergy*, vol. 59, no. 5, pp. 469–478, May 2004, doi: 10.1111/j.1398-9995.2004.00526.x.

[26] M. R. Laughter *et al.*, “The global burden of atopic dermatitis: lessons from the Global Burden of Disease Study 1990–2017*,” *British Journal of Dermatology*, vol. 184, no. 2, pp. 304–309, Feb. 2021, doi: 10.1111/bjd.19580.

[27] M. Guerriero, M. Caminati, S. Paiano, L. Assante, G. Senna, and C. Pomari, “The prevalence of asthma–chronic obstructive pulmonary disease overlap syndrome (ACOS) in a Northern Italy general population sample,” in *6.1 Epidemiology*, Sep. 2015, p. PA4064. doi: 10.1183/13993003.congress-2015.PA4064.

[28] Y. Xing and K. A. Hogquist, “T-Cell tolerance: Central and peripheral,” *Cold Spring Harbor Perspectives in Biology*, vol. 4, no. 6, pp. 1–15, Jun. 2012, doi: 10.1101/cshperspect.a006957.

[29] D. Nemazee, “Mechanisms of central tolerance for B cells,” *Nature Reviews Immunology*, vol. 17, no. 5. Nature Publishing Group, pp. 281–294, May 01, 2017. doi: 10.1038/nri.2017.19.

[30] L. Chen and D. B. Flies, “Molecular mechanisms of T cell co-stimulation and co-inhibition,” *Nature Reviews Immunology*, vol. 13, no. 4. pp. 227–242, Apr. 2013. doi: 10.1038/nri3405.

[31] M. Fukata and M. Arditi, “The role of pattern recognition receptors in intestinal inflammation,” *Mucosal Immunology*, vol. 6, no. 3. pp. 451–463, May 2013. doi: 10.1038/mi.2013.13.

[32] S. Rakoff-Nahoum, J. Paglino, F. Eslami-Varzaneh, S. Edberg, and R. Medzhitov, “Recognition of commensal microflora by toll-like receptors is required for intestinal homeostasis,” *Cell*, vol. 118, no. 2, pp. 229–241, Jul. 2004, doi: 10.1016/j.cell.2004.07.002.

[33] J. L. Kubinak and J. L. Round, “Toll-like receptors promote mutually beneficial commensal-host interactions,” *PLoS Pathogens*, vol. 8, no. 7, p. 1, Jul. 2012, doi: 10.1371/journal.ppat.1002785.

[34] T. Kawasaki and T. Kawai, “Toll-like receptor signaling pathways,” *Frontiers in Immunology*, vol. 5, no. SEP. Frontiers Media S.A., 2014. doi: 10.3389/fimmu.2014.00461.

[35] S. J. Koppelman, M. Wensing, M. Ertmann, A. C. Knulst, and E. F. Knol, “Relevance of Ara h1, Ara h2 and Ara h3 in peanut-allergic patients, as determined by immunoglobulin E Western blotting, basophil-histamine release and intracutaneous testing: Ara h2 is the most important peanut allergen,” *Clinical and Experimental Allergy*, vol. 34, no. 4, pp. 583–590, Apr. 2004, doi: 10.1111/j.1365-2222.2004.1923.x.

[36] S. Wünschmann, A. Gustchina, M. D. Chapman, and A. Pomés, “Cockroach allergen Bla g 2: An unusual aspartic proteinase,” *Journal of Allergy and Clinical Immunology*, vol. 116, no. 1, pp. 140–145, Jul. 2005, doi: 10.1016/j.jaci.2005.04.024.

[37] M. Reithofer and B. Jahn-Schmid, “Allergens with protease activity from house dust mites,” *International Journal of Molecular Sciences*, vol. 18, no. 7. MDPI AG, Jul. 01, 2017. doi: 10.3390/ijms18071368.

[38] T. Haahtela, “A biodiversity hypothesis,” *Allergy: European Journal of Allergy and Clinical Immunology*, vol. 74, no. 8. Blackwell Publishing Ltd, pp. 1445–1456, 2019. doi: 10.1111/all.13763.

[39] M. M. Stein *et al.*, “Innate Immunity and Asthma Risk in Amish and Hutterite Farm Children,” *New England Journal of Medicine*, vol. 375, no. 5, pp. 411–421, Aug. 2016, doi: 10.1056/nejmoa1508749.

[40] Z. Celebi Sozener *et al.*, “Epithelial barrier hypothesis: Effect of the external exposome on the microbiome and epithelial barriers in allergic disease,” *Allergy: European Journal of Allergy and Clinical Immunology*, vol. 77, no. 5. John Wiley and Sons Inc, pp. 1418–1449, May 01, 2022. doi: 10.1111/all.15240.

[41] M. Wang *et al.*, “Laundry detergents and detergent residue after rinsing directly disrupt tight junction barrier integrity in human bronchial epithelial cells,” *Journal of Allergy and Clinical Immunology*, vol. 143, no. 5, pp. 1892–1903, May 2019, doi: 10.1016/j.jaci.2018.11.016.

[42] P. K. Smith, M. Masilamani, X. M. Li, and H. A. Sampson, “The false alarm hypothesis: Food allergy is associated with high dietary advanced glycation end-products and proglycating dietary sugars that mimic alarmins,” *Journal of Allergy and Clinical Immunology*, vol. 139, no. 2, pp. 429–437, Feb. 2017, doi: 10.1016/j.jaci.2016.05.040.

[43] A. K. Abbas, A. H. Lichtman, and S. Pillai, *Cellular and Molecular Immunology*, 9th Edition. 2018.

[44] W. J. Pichler, “Delayed Drug Hypersensitivity Reactions,” *Annals of Internal Medicine*, vol. 139, no. 8, p. 683, Oct. 2003, doi: 10.7326/0003-4819-139-8-200310210-00012.

[45] M. Akdis and C. A. Akdis, “IgE class switching and cellular memory,” *Nature Immunology*, vol. 13, no. 4. pp. 312–314, Apr. 2012. doi: 10.1038/ni.2266.

[46] B. Sutton, A. Davies, H. Bax, and S. Karagiannis, “IgE Antibodies: From Structure to Function and Clinical Translation,” *Antibodies*, vol. 8, no. 1, p. 19, Feb. 2019, doi: 10.3390/antib8010019.

[47] C. J. Oliphant, J. L. Barlow, and A. N. J. Mckenzie, “Insights into the initiation of type 2 immune responses,” *Immunology*, vol. 134, no. 4. pp. 378–385, Dec. 2011. doi: 10.1111/j.1365-2567.2011.03499.x.

[48] M. Zhou and W. Ouyang, “The Function Role of GATA-3 in Th1 and Th2 Differentiation,” *Immunologic Research*, vol. 28, no. 1, pp. 25–38, 2003, doi: 10.1385/IR:28:1:25.

[49] Z. Shi *et al.*, “Inhibition of JAK/STAT pathway restrains TSLP-activated dendritic cells mediated inflammatory T helper type 2 cell response in allergic rhinitis,” *Molecular and Cellular Biochemistry*, vol. 430, no. 1–2, pp. 161–169, Jun. 2017, doi: 10.1007/s11010-017-2963-7.

[50] C. J. Oliphant, J. L. Barlow, and A. N. J. McKenzie, “Insights into the initiation of type 2 immune responses,” *Immunology*, vol. 134, no. 4, pp. 378–385, Dec. 2011, doi: 10.1111/j.1365-2567.2011.03499.x.

[51] P. Licona-Limón, L. K. Kim, N. W. Palm, and R. A. Flavell, “TH2, allergy and group 2 innate lymphoid cells,” *Nature Immunology*, vol. 14, no. 6, pp. 536–542, Jun. 2013, doi: 10.1038/ni.2617.

[52] S. T. Holgate, “A Brief History of Asthma and Its Mechanisms to Modern Concepts of Disease Pathogenesis,” *Allergy, Asthma and Immunology Research*, vol. 2, no. 3, p. 165, 2010, doi: 10.4168/aair.2010.2.3.165.

[53] W. Osler, *The Principles and Practice of Medicine.*, 1st edition. 1892.

[54] E. K. Persson *et al.*, "Protein crystallization promotes type 2 immunity and is reversible by antibody treatment," *Science*, vol. 364, no. 6442, May 2019, doi: 10.1126/science.aaw4295.

[55] R. Kaur and G. Chupp, "Phenotypes and endotypes of adult asthma: Moving toward precision medicine," *Journal of Allergy and Clinical Immunology*, vol. 144, no. 1, pp. 1–12, Jul. 2019, doi: 10.1016/j.jaci.2019.05.031.

[56] L. Pembrey *et al.*, "Understanding asthma phenotypes: The world asthma phenotypes (WASP) international collaboration," *ERJ Open Research*, vol. 4, no. 3, Jul. 2018, doi: 10.1183/23120541.00013-2018.

[57] M. E. Kuruvilla, F. E.-H. Lee, and G. B. Lee, "Understanding Asthma Phenotypes, Endotypes, and Mechanisms of Disease," *Clinical Reviews in Allergy & Immunology*, vol. 56, no. 2, pp. 219–233, Apr. 2019, doi: 10.1007/s12016-018-8712-1.

[58] S. T. Holgate, S. Wenzel, D. S. Postma, S. T. Weiss, H. Renz, and P. D. Sly, "Asthma," *Nature Reviews Disease Primers*, vol. 1, Sep. 2015, doi: 10.1038/nrdp.2015.25.

[59] T. F. Carr, A. A. Zeki, and M. Kraft, "Eosinophilic and Noneosinophilic Asthma," *American Journal of Respiratory and Critical Care Medicine*, vol. 197, no. 1, pp. 22–37, Jan. 2018, doi: 10.1164/rccm.201611-2232PP.

[60] S. E. Wenzel, "Asthma phenotypes: the evolution from clinical to molecular approaches," *Nature Medicine*, vol. 18, no. 5, pp. 716–725, May 2012, doi: 10.1038/nm.2678.

[61] M. A. G. E. Bannier, K. D. G. van de Kant, Q. Jöbsis, and E. Dompeling, "Biomarkers to predict asthma in wheezing preschool children," *Clinical & Experimental Allergy*, vol. 45, no. 6, pp. 1040–1050, Jun. 2015, doi: 10.1111/cea.12460.

[62] T. Jartti and J. E. Gern, "Role of viral infections in the development and exacerbation of asthma in children," *Journal of Allergy and Clinical Immunology*, vol. 140, no. 4, pp. 895–906, Oct. 2017, doi: 10.1016/j.jaci.2017.08.003.

[63] J. Schwarze and E. W. Gelfand, "Respiratory viral infections as promoters of allergic sensitization and asthma in animal models," *European Respiratory Journal*, vol. 19, no. 2, pp. 341–349, 2002, doi: 10.1183/09031936.02.00254302.

[64] N. W. Johnston *et al.*, "The September epidemic of asthma exacerbations in children: A search for etiology," *Journal of Allergy and Clinical Immunology*, vol. 115, no. 1, pp. 132–138, 2005, doi: 10.1016/j.jaci.2004.09.025.

[65] N. Khetsuriani *et al.*, "Prevalence of viral respiratory tract infections in children with asthma," *Journal of Allergy and Clinical Immunology*, vol. 119, no. 2, pp. 314–321, Feb. 2007, doi: 10.1016/j.jaci.2006.08.041.

[66] S. L. Johnston *et al.*, "The relationship between upper respiratory infections and hospital admissions for asthma: a time-trend analysis.," *American Journal of Respiratory and Critical Care Medicine*, vol. 154, no. 3, pp. 654–660, Sep. 1996, doi: 10.1164/ajrccm.154.3.8810601.

[67] S. D. Message *et al.*, “Rhinovirus-induced lower respiratory illness is increased in asthma and related to virus load and Th1/2 cytokine and IL-10 production,” *Proceedings of the National Academy of Sciences*, vol. 105, no. 36, pp. 13562–13567, Sep. 2008, doi: 10.1073/pnas.0804181105.

[68] M. R. Edwards, K. Strong, A. Cameron, R. P. Walton, D. J. Jackson, and S. L. Johnston, “Viral infections in allergy and immunology: How allergic inflammation influences viral infections and illness,” *Journal of Allergy and Clinical Immunology*, vol. 140, no. 4, pp. 909–920, Oct. 2017, doi: 10.1016/j.jaci.2017.07.025.

[69] E. J. Baan *et al.*, “Antibiotic use in children with asthma: Cohort study in UK and Dutch primary care databases,” *BMJ Open*, vol. 8, no. 11, Nov. 2018, doi: 10.1136/bmjopen-2018-022979.

[70] WICKENS, PEARCE, CRANE, and BEASLEY, “Antibiotic use in early childhood and the development of asthma,” *Clinical & Experimental Allergy*, vol. 29, no. 6, pp. 766–771, Jun. 1999, doi: 10.1046/j.1365-2222.1999.00536.x.

[71] D. M. Patrick *et al.*, “Decreasing antibiotic use, the gut microbiota, and asthma incidence in children: evidence from population-based and prospective cohort studies,” *The Lancet Respiratory Medicine*, vol. 8, no. 11, pp. 1094–1105, Nov. 2020, doi: 10.1016/S2213-2600(20)30052-7.

[72] V. Elenius *et al.*, “Lung function testing and inflammation markers for wheezing preschool children: A systematic review for the EAACI Clinical Practice Recommendations on Diagnostics of Preschool Wheeze,” *Pediatric Allergy and Immunology*, vol. 32, no. 3, pp. 501–513, Apr. 2021, doi: 10.1111/pai.13418.

[73] “GINA-Main-Report-2022-FINAL-22-05-03-WMS”.

[74] M. Sokolowska *et al.*, “Current perspective on eicosanoids in asthma and allergic diseases: EAACI Task Force consensus report, part I,” *Allergy: European Journal of Allergy and Clinical Immunology*, vol. 76, no. 1, pp. 114–130, Jan. 2021, doi: 10.1111/all.14295.

[75] M. Jutel *et al.*, “Histamine regulates T-cell and antibody responses by differential expression of H1 and H2 receptors,” *Nature*, vol. 413, no. 6854, pp. 420–425, 2001, doi: 10.1038/35096564.

[76] M. Jutel *et al.*, “Histamine Upregulates Th1 and Downregulates Th2 Responses due to Different Patterns of Surface Histamine 1 and 2 Receptor Expression,” *International Archives of Allergy and Immunology*, vol. 124, no. 1–3, pp. 190–192, 2001, doi: 10.1159/000053707.

[77] T. Shimamura *et al.*, “Structure of the human histamine H 1 receptor complex with doxepin,” *Nature*, vol. 475, no. 7354, pp. 65–72, Jul. 2011, doi: 10.1038/nature10236.

[78] M. Jutel, M. Akdis, and C. A. Akdis, “Histamine, histamine receptors and their role in immune pathology,” *Clinical & Experimental Allergy*, vol. 39, no. 12, pp. 1786–1800, Dec. 2009, doi: 10.1111/j.1365-2222.2009.03374.x.

[79] R. FADEL, N. HERPIN-RICHARD, J. P. RIHOUX, and E. HENOCQ, “Inhibitory effect of cetirizine 2HCl on eosinophil migration in vivo,” *Experimental Allergy*, vol. 17, no. 4, pp. 373–379, Jul. 1987, doi: 10.1111/j.1365-2222.1987.tb02027.x.

[80] R. A. Panettieri, P. A. Yadvish, A. M. Kelly, N. A. Rubinstein, and M. I. Kotlikoff, "Histamine stimulates proliferation of airway smooth muscle and induces c-fos expression," *American Journal of Physiology-Lung Cellular and Molecular Physiology*, vol. 259, no. 6, pp. L365–L371, Dec. 1990, doi: 10.1152/ajplung.1990.259.6.L365.

[81] P. J. Barnes, "Molecular mechanisms of corticosteroids in all_ergic diseases.," *Allergy*, vol. 56, no. 10, pp. 928–936, Oct. 2001, doi: 10.1034/j.1398-9995.2001.00001.x.

[82] M. C. McGregor, J. G. Krings, P. Nair, and M. Castro, "Role of biologics in asthma," *American Journal of Respiratory and Critical Care Medicine*, vol. 199, no. 4, pp. 433–445, Feb. 2019, doi: 10.1164/rccm.201810-1944CI.

[83] "The global burden of asthma: executive summary of the GINA Dissemination Committee Report." [Online]. Available: http://esa.un.org/unpp

[84] M. Engelkes, H. M. Janssens, J. C. de Jongste, M. C. J. M. Sturkenboom, and K. M. C. Verhamme, "Medication adherence and the risk of severe asthma exacerbations: a systematic review," *European Respiratory Journal*, vol. 45, no. 2, pp. 396–407, Feb. 2015, doi: 10.1183/09031936.00075614.

[85] R. W. H. Hui, "Inhaled corticosteroid-phobia and childhood asthma: Current understanding and management implications," *Paediatric Respiratory Reviews*, vol. 33. W.B. Saunders Ltd, pp. 62–66, Feb. 01, 2020. doi: 10.1016/j.prrv.2019.03.009.

[86] D. R. Taylor *et al.*, "A new perspective on concepts of asthma severity and control," *European Respiratory Journal*, vol. 32, no. 3. pp. 545–554, Sep. 2008. doi: 10.1183/09031936.00155307.

[87] A. I. Papaioannou, K. Kostikas, E. Zervas, L. Kolilekas, S. Papiris, and M. Gaga, "Control of asthma in real life: Still a valuable goal?," *European Respiratory Review*, vol. 24, no. 136, pp. 361–369, Jun. 2015, doi: 10.1183/16000617.00001615.

[88] K. Samitas, A. Carter, H. H. Kariyawasam, and G. Xanthou, "Upper and lower airway remodelling mechanisms in asthma, allergic rhinitis and chronic rhinosinusitis: The one airway concept revisited," *Allergy*, vol. 73, no. 5, pp. 993–1002, May 2018, doi: 10.1111/all.13373.

[89] I. Bara, A. Ozier, J.-M. Tunon de Lara, R. Marthan, and P. Berger, "Pathophysiology of bronchial smooth muscle remodelling in asthma," *European Respiratory Journal*, vol. 36, no. 5, pp. 1174–1184, Nov. 2010, doi: 10.1183/09031936.00019810.

[90] D. C. Doeing and J. Solway, "Airway smooth muscle in the pathophysiology and treatment of asthma," *J Appl Physiol*, vol. 114, pp. 834–843, 2013, doi: 10.1152/japplphysiol.00950.2012.-Airway.

[91] C. Pepe *et al.*, "Differences in airway remodeling between subjects with severe and moderate asthma," *Journal of Allergy and Clinical Immunology*, vol. 116, no. 3, pp. 544–549, Sep. 2005, doi: 10.1016/j.jaci.2005.06.011.

[92] M. Kaminska *et al.*, "Airway remodeling in subjects with severe asthma with or without chronic persistent airflow obstruction," *Journal of Allergy and Clinical Immunology*, vol. 124, no. 1, 2009, doi: 10.1016/j.jaci.2009.03.049.

[93] C. K. Oh, G. P. Geba, and N. Molfino, “Investigational therapeutics targeting the IL-4/IL-13/STAT-6 pathway for the treatment of asthma,” *European Respiratory Review*, vol. 19, no. 115, pp. 46–54, Mar. 2010, doi: 10.1183/09059180.00007609.

[94] S. M. Nur Husna, N. Md Shukri, N. S. Mohd Ashari, and K. K. Wong, “IL-4/IL-13 axis as therapeutic targets in allergic rhinitis and asthma,” *PeerJ*, vol. 10, p. e13444, May 2022, doi: 10.7717/peerj.13444.

[95] M. Panek *et al.*, “The NR3C1 Glucocorticoid Receptor Gene Polymorphisms May Modulate the TGF-beta mRNA Expression in Asthma Patients,” *Inflammation*, vol. 38, no. 4, pp. 1479–1492, Aug. 2015, doi: 10.1007/s10753-015-0123-3.

[96] P. J. Barnes, “Glucocorticosteroids: current and future directions,” *British Journal of Pharmacology*, vol. 163, no. 1, pp. 29–43, May 2011, doi: 10.1111/j.1476-5381.2010.01199.x.

[97] B. G. J. Dekkers, A. Pehlić, R. Mariani, I. S. T. Bos, H. Meurs, and J. Zaagsma, “Glucocorticosteroids and β2-adrenoceptor agonists synergize to inhibit airway smooth muscle remodeling,” *Journal of Pharmacology and Experimental Therapeutics*, vol. 342, no. 3, pp. 780–787, Sep. 2012, doi: 10.1124/jpet.112.195867.

[98] P. H. Howarth, A. J. Knox, Y. Amrani, O. Tliba, R. A. Panettieri, and M. Johnson, “Synthetic responses in airway smooth muscle,” in *Journal of Allergy and Clinical Immunology*, Aug. 2004, vol. 114, no. 2 SUPPL. doi: 10.1016/j.jaci.2004.04.041.

[99] Y. C. Xia *et al.*, “Pro-inflammatory and immunomodulatory functions of airway smooth muscle: Emerging concepts,” *Pulmonary Pharmacology and Therapeutics*, vol. 26, no. 1, pp. 64–74, Feb. 2013, doi: 10.1016/j.pupt.2012.05.006.

[100] J. L. Pype *et al.*, “Expression of Monocyte Chemotactic Protein (MCP)-1, MCP-2, and MCP-3 by Human Airway Smooth-Muscle Cells,” *American Journal of Respiratory Cell and Molecular Biology*, vol. 21, no. 4, pp. 528–536, Oct. 1999, doi: 10.1165/ajrcmb.21.4.3660.

[101] M. John *et al.*, “Expression and Release of Interleukin-8 by Human Airway Smooth Muscle Cells: Inhibition by Th-2 Cytokines and Corticosteroids,” *American Journal of Respiratory Cell and Molecular Biology*, vol. 18, no. 1, pp. 84–90, Jan. 1998, doi: 10.1165/ajrcmb.18.1.2813.

[102] M. Rincon and C. G. Irvin, “Role of IL-6 in Asthma and Other Inflammatory Pulmonary Diseases,” *International Journal of Biological Sciences*, vol. 8, no. 9, pp. 1281–1290, 2012, doi: 10.7150/ijbs.4874.

[103] S. Ravasi, S. Citro, B. Viviani, V. Capra, and G. E. Rovati, “CysLT1 receptor-induced human airway smooth muscle cells proliferation requires ROS generation, EGF receptor transactivation and ERK1/2 phosphorylation,” *Respiratory Research*, vol. 7, no. 1, p. 42, Dec. 2006, doi: 10.1186/1465-9921-7-42.

[104] K. Maruno, A. Absood, and S. I. Said, “VIP inhibits basal and histamine-stimulated proliferation of human airway smooth muscle cells,” *American Journal of Physiology-Lung Cellular and Molecular Physiology*, vol. 268, no. 6, pp. L1047–L1051, Jun. 1995, doi: 10.1152/ajplung.1995.268.6.L1047.

[105] C. da Huang *et al.*, “Human neutrophil-derived elastase induces airway smooth muscle cell proliferation,” *Life Sciences*, vol. 74, no. 20, pp. 2479–2492, Apr. 2004, doi: 10.1016/j.lfs.2003.07.059.

[106] A. G. Stewart, P. R. Tomlinson, D. J. Fernandes, J. W. Wilson, and T. Harris, “Tumor necrosis factor alpha modulates mitogenic responses of human cultured airway smooth muscle.,” *American Journal of Respiratory Cell and Molecular Biology*, vol. 12, no. 1, pp. 110–119, Jan. 1995, doi: 10.1165/ajrcmb.12.1.7529028.

[107] M. D. Cohen, V. Ciocca, and R. A. Panettieri, “TGF-beta 1 modulates human airway smooth-muscle cell proliferation induced by mitogens.,” *American Journal of Respiratory Cell and Molecular Biology*, vol. 16, no. 1, pp. 85–90, Jan. 1997, doi: 10.1165/ajrcmb.16.1.8998083.

[108] N. K. Malavia, C. B. Raub, S. B. Mahon, M. Brenner, R. A. Panettieri, and S. C. George, “Airway epithelium stimulates smooth muscle proliferation,” *American Journal of Respiratory Cell and Molecular Biology*, vol. 41, no. 3, pp. 297–304, Sep. 2009, doi: 10.1165/rcmb.2008-0358OC.

[109] N. Takeda *et al.*, “Epithelium-derived chemokines induce airway smooth muscle cell migration,” *Clinical and Experimental Allergy*, vol. 39, no. 7, pp. 1018–1026, Jul. 2009, doi: 10.1111/j.1365-2222.2009.03238.x.

[110] Y. Amrani and R. A. Panettieri, “Cytokines induce airway smooth muscle cell hyperresponsiveness to contractile agonists,” *Thorax*, vol. 53, no. 8, pp. 713–716, Aug. 1998, doi: 10.1136/thx.53.8.713.

[111] E. R. Davies *et al.*, “Soluble ADAM33 initiates airway remodeling to promote susceptibility for allergic asthma in early life,” *JCI Insight*, vol. 1, no. 11, Jul. 2016, doi: 10.1172/jci.insight.87632.

[112] Y. Jiang *et al.*, “Classification of non-acute bronchial asthma according to allergy and eosinophil characteristics: a retrospective study,” *Allergy, Asthma & Clinical Immunology*, vol. 17, no. 1, p. 45, Dec. 2021, doi: 10.1186/s13223-021-00546-1.

[113] S. Baos *et al.*, “Nonallergic asthma and its severity: Biomarkers for its discrimination in peripheral samples,” *Frontiers in Immunology*, vol. 9, no. JUN, Jun. 2018, doi: 10.3389/fimmu.2018.01416.

[114] J. Douwes, “Non-eosinophilic asthma: importance and possible mechanisms,” *Thorax*, vol. 57, no. 7, pp. 643–648, Jul. 2002, doi: 10.1136/thorax.57.7.643.

[115] B. Leynaert *et al.*, “Gender differences in prevalence, diagnosis and incidence of allergic and non-allergic asthma: a population-based cohort,” *Thorax*, vol. 67, no. 7, pp. 625–631, Jul. 2012, doi: 10.1136/thoraxjnl-2011-201249.

[116] J. Pakkasela *et al.*, “Age-specific incidence of allergic and non-allergic asthma,” *BMC Pulmonary Medicine*, vol. 20, no. 1, Jan. 2020, doi: 10.1186/s12890-019-1040-2.

[117] M. Terl *et al.*, “Asthma management: A new phenotype-based approach using presence of eosinophilia and allergy,” *Allergy*, vol. 72, no. 9, pp. 1279–1287, Sep. 2017, doi: 10.1111/all.13165.

[118] G. G. Brusselle, T. Maes, and K. R. Bracke, “Eosinophils in the Spotlight: Eosinophilic airway inflammation in nonallergic asthma,” *Nature Medicine*, vol. 19, no. 8, pp. 977–979, Aug. 2013, doi: 10.1038/nm.3300.

[119] D. STEVENSON and A. SZCZEKLIK, “Clinical and pathologic perspectives on aspirin sensitivity and asthma,” *Journal of Allergy and Clinical Immunology*, vol. 118, no. 4, pp. 773–786, Oct. 2006, doi: 10.1016/j.jaci.2006.07.024.

[120] T. Boonpiyathad, Z. C. Sözener, P. Satitsuksanoa, and C. A. Akdis, “Immunologic mechanisms in asthma,” *Seminars in Immunology*, vol. 46, p. 101333, Dec. 2019, doi: 10.1016/j.smim.2019.101333.

[121] H. A. Scott, P. G. Gibson, M. L. Garg, and L. G. Wood, “Airway inflammation is augmented by obesity and fatty acids in asthma,” *European Respiratory Journal*, vol. 38, no. 3, pp. 594–602, Sep. 2011, doi: 10.1183/09031936.00139810.

[122] G. W. Chalmers, K. J. MacLeod, L. Thomson, S. A. Little, C. McSharry, and N. C. Thomson, “Smoking and airway inflammation in patients with mild asthma,” *Chest*, vol. 120, no. 6, pp. 1917–1922, 2001, doi: 10.1378/chest.120.6.1917.

[123] E. D. Telenga *et al.*, “Obesity in asthma: More neutrophilic inflammation as a possible explanation for a reduced treatment response,” *Allergy: European Journal of Allergy and Clinical Immunology*, vol. 67, no. 8, pp. 1060–1068, Aug. 2012, doi: 10.1111/j.1398-9995.2012.02855.x.

[124] N. Turan *et al.*, “Neutrophilic Asthma Is Associated With Smoking, High Numbers of IRF5+, and Low Numbers of IL10+ Macrophages,” *Frontiers in Allergy*, vol. 2, Jun. 2021, doi: 10.3389/falgy.2021.676930.

[125] K. Håkansson, C. von Buchwald, S. F. Thomsen, J. P. Thyssen, V. Backer, and A. Linneberg, “Nonallergic rhinitis and its association with smoking and lower airway disease: A general population study,” *American Journal of Rhinology and Allergy*, vol. 25, no. 1, pp. 25–29, Jan. 2011, doi: 10.2500/ajra.2011.25.3556.

[126] H. T. T. Tan *et al.*, “Tight junction, mucin, and inflammasome-related molecules are differentially expressed in eosinophilic, mixed, and neutrophilic experimental asthma in mice,” *Allergy: European Journal of Allergy and Clinical Immunology*, vol. 74, no. 2, pp. 294–307, Feb. 2019, doi: 10.1111/all.13619.

[127] K. J. Baines *et al.*, “Sputum gene expression signature of 6 biomarkers discriminates asthma inflammatory phenotypes,” *Journal of Allergy and Clinical Immunology*, vol. 133, no. 4, pp. 997–1007, 2014, doi: 10.1016/j.jaci.2013.12.1091.

[128] W. Al-Ramli *et al.*, “TH17-associated cytokines (IL-17A and IL-17F) in severe asthma,” *Journal of Allergy and Clinical Immunology*, vol. 123, no. 5, pp. 1185–1187, May 2009, doi: 10.1016/j.jaci.2009.02.024.

[129] H. Ogawa *et al.*, “Neutrophils induce smooth muscle hyperplasia via neutrophil elastase-induced FGF-2 in a mouse model of asthma with mixed inflammation,” *Clinical and Experimental Allergy*, vol. 48, no. 12, pp. 1715–1725, Dec. 2018, doi: 10.1111/cea.13263.

[130] P. González-Muniesa *et al.*, "Obesity," *Nature Reviews Disease Primers*, vol. 3, no. 1, p. 17034, Dec. 2017, doi: 10.1038/nrdp.2017.34.

[131] K. G. M. M. Alberti *et al.*, "Harmonizing the Metabolic Syndrome," *Circulation*, vol. 120, no. 16, pp. 1640–1645, Oct. 2009, doi: 10.1161/CIRCULATIONAHA.109.192644.

[132] H.-M. Lakka, "The Metabolic Syndrome and Total and Cardiovascular Disease Mortality in Middle-aged Men," *JAMA*, vol. 288, no. 21, p. 2709, Dec. 2002, doi: 10.1001/jama.288.21.2709.

[133] R. H. Eckel, K. Alberti, S. M. Grundy, and P. Z. Zimmet, "The metabolic syndrome," *The Lancet*, vol. 375, no. 9710, pp. 181–183, Jan. 2010, doi: 10.1016/S0140-6736(09)61794-3.

[134] B. M. Brumpton, C. A. Camargo, P. R. Romundstad, A. Langhammer, Y. Chen, and X.-M. Mai, "Metabolic syndrome and incidence of asthma in adults: the HUNT study," *European Respiratory Journal*, vol. 42, no. 6, pp. 1495–1502, Dec. 2013, doi: 10.1183/09031936.00046013.

[135] Y. C. Chen, G. H. Dong, K. C. Lin, and Y. L. Lee, "Gender difference of childhood overweight and obesity in predicting the risk of incident asthma: a systematic review and meta-analysis," *Obesity Reviews*, vol. 14, no. 3, pp. 222–231, Mar. 2013, doi: 10.1111/j.1467-789X.2012.01055.x.

[136] E. Forno and J. C. Celedón, "The effect of obesity, weight gain, and weight loss on asthma inception and control," *Current Opinion in Allergy & Clinical Immunology*, vol. 17, no. 2, pp. 123–130, Apr. 2017, doi: 10.1097/ACI.0000000000000339.

[137] "ec.europa.eu/eurostat/statistics-explained/index.php?title=Overweight_and_obesity_-_BMI_statistics," May 2022.

[138] "https://www.cdc.gov/nchs/fastats/obesity-overweight.htm."

[139] "https://www.who.int/news-room/fact-sheets/detail/obesity-and-overweight accessed on 06/06/2022."

[140] J. Sobal and A. J. Stunkard, "Socioeconomic status and obesity: A review of the literature.," *Psychological Bulletin*, vol. 105, no. 2, pp. 260–275, 1989, doi: 10.1037/0033-2909.105.2.260.

[141] L. McLaren, "Socioeconomic Status and Obesity," *Epidemiologic Reviews*, vol. 29, no. 1, pp. 29–48, May 2007, doi: 10.1093/epirev/mxm001.

[142] C. Hawkes, "Uneven dietary development: linking the policies and processes of globalization with the nutrition transition, obesity and diet-related chronic diseases," *Globalization and Health*, vol. 2, no. 1, p. 4, Dec. 2006, doi: 10.1186/1744-8603-2-4.

[143] J. X. Moore, N. Chaudhary, and T. Akinyemiju, "Metabolic Syndrome Prevalence by Race/Ethnicity and Sex in the United States, National Health and Nutrition Examination Survey, 1988–2012," *Preventing Chronic Disease*, vol. 14, p. 160287, Mar. 2017, doi: 10.5888/pcd14.160287.

[144] G. Hirode and R. J. Wong, "Trends in the Prevalence of Metabolic Syndrome in the United States, 2011-2016," *JAMA*, vol. 323, no. 24, p. 2526, Jun. 2020, doi: 10.1001/jama.2020.4501.

[145] A. Scuteri *et al.*, “Metabolic syndrome across Europe: Different clusters of risk factors,” *European Journal of Preventive Cardiology*, vol. 22, no. 4, pp. 486–491, Apr. 2015, doi: 10.1177/2047487314525529.

[146] A. Rajca *et al.*, “Increase in the prevalence of the metabolic syndrome in Poland. Comparison of the results of the WOBASZ (2003–2005) and WOBASZ II (2013–2014) studies,” *Polish Archives of Internal Medicine*, Apr. 2021, doi: 10.20452/pamw.15975.

[147] A. Rajca *et al.*, “Increase in the prevalence of the metabolic syndrome in Poland. Comparison of the results of the WOBASZ (2003–2005) and WOBASZ II (2013–2014) studies,” *Polish Archives of Internal Medicine*, Apr. 2021, doi: 10.20452/pamw.15975.

[148] G. J. Nowicki *et al.*, “The Relationship Between the Metabolic Syndrome and the Place of Residence in the Local Community on the Example of the Janów Lubelski District in Eastern Poland: A Population-Based Study,” *Diabetes, Metabolic Syndrome and Obesity: Targets and Therapy*, vol. Volume 14, pp. 2041–2056, May 2021, doi: 10.2147/DMSO.S301639.

[149] G. Hu, “Prevalence of the Metabolic Syndrome and Its Relation to All-Cause and Cardiovascular Mortality in Nondiabetic European Men and Women,” *Archives of Internal Medicine*, vol. 164, no. 10, p. 1066, May 2004, doi: 10.1001/archinte.164.10.1066.

[150] M. G. Saklayen, “The Global Epidemic of the Metabolic Syndrome,” *Current Hypertension Reports*, vol. 20, no. 2, p. 12, Feb. 2018, doi: 10.1007/s11906-018-0812-z.

[151] J. J. Noubiap *et al.*, “Global, regional, and country estimates of metabolic syndrome burden in children and adolescents in 2020: a systematic review and modelling analysis,” *The Lancet Child & Adolescent Health*, vol. 6, no. 3, pp. 158–170, Mar. 2022, doi: 10.1016/S2352-4642(21)00374-6.

[152] K. D. Hall *et al.*, “Quantification of the effect of energy imbalance on bodyweight,” *The Lancet*, vol. 378, no. 9793, pp. 826–837, Aug. 2011, doi: 10.1016/S0140-6736(11)60812-X.

[153] R. de D. Mendonça *et al.*, “Ultraprocessed food consumption and risk of overweight and obesity: the University of Navarra Follow-Up (SUN) cohort study,” *The American Journal of Clinical Nutrition*, vol. 104, no. 5, pp. 1433–1440, Nov. 2016, doi: 10.3945/ajcn.116.135004.

[154] F. Rauber *et al.*, “ Ultra-processed food consumption and risk of obesity: a prospective cohort study of UK Biobank,” *European Journal of Nutrition*, vol. 60, no. 4, pp. 2169–2180, Jun. 2021, doi: 10.1007/s00394-020-02367-1.

[155] A. Ayton, A. Ibrahim, J. Dugan, E. Galvin, and O. W. Wright, “Ultra-processed foods and binge eating: A retrospective observational study,” *Nutrition*, vol. 84, p. 111023, Apr. 2021, doi: 10.1016/j.nut.2020.111023.

[156] M. D. Klok, S. Jakobsdottir, and M. L. Drent, “The role of leptin and ghrelin in the regulation of food intake and body weight in humans: a review,” *Obesity Reviews*, vol. 8, no. 1, pp. 21–34, Jan. 2007, doi: 10.1111/j.1467-789X.2006.00270.x.

[157] M. Obradovic *et al.*, “Leptin and Obesity: Role and Clinical Implication,” *Frontiers in Endocrinology*, vol. 12, May 2021, doi: 10.3389/fendo.2021.585887.

[158] M. G. Myers, R. L. Leibel, R. J. Seeley, and M. W. Schwartz, “Obesity and leptin resistance: distinguishing cause from effect,” *Trends in Endocrinology & Metabolism*, vol. 21, no. 11, pp. 643–651, Nov. 2010, doi: 10.1016/j.tem.2010.08.002.

[159] J. R. Vasselli, P. J. Scarpace, R. B. S. Harris, and W. A. Banks, “Dietary Components in the Development of Leptin Resistance,” *Advances in Nutrition*, vol. 4, no. 2, pp. 164–175, Mar. 2013, doi: 10.3945/an.112.003152.

[160] G. Fantuzzi and R. Faggioni, “Leptin in the regulation of immunity, inflammation, and hematopoiesis,” *Journal of Leukocyte Biology*, vol. 68, pp. 437–446, 2002.

[161] G. M. Lord, G. Matarese, J. K. Howard, R. J. Baker, S. R. Bloom, and R. I. Lechler, “Leptin modulates the T-cell immune response and reverses starvation-induced immunosuppression,” *Nature*, vol. 394, no. 6696, pp. 897–901, Aug. 1998, doi: 10.1038/29795.

[162] C. Martín-Romero, J. Santos-Alvarez, R. Goberna, and V. Sánchez-Margalet, “Human Leptin Enhances Activation and Proliferation of Human Circulating T Lymphocytes,” *Cellular Immunology*, vol. 199, no. 1, pp. 15–24, Jan. 2000, doi: 10.1006/cimm.1999.1594.

[163] S. Singh, Y. S. Prakash, A. Linneberg, and A. Agrawal, “Insulin and the Lung: Connecting Asthma and Metabolic Syndrome,” *Journal of Allergy*, vol. 2013, pp. 1–8, Sep. 2013, doi: 10.1155/2013/627384.

[164] L. L. N. Husemoen, C. Glümer, C. Lau, C. Pisinger, L. S. Mørch, and A. Linneberg, “Association of obesity and insulin resistance with asthma and aeroallergen sensitization,” *Allergy: European Journal of Allergy and Clinical Immunology*, vol. 63, no. 5, pp. 575–582, May 2008, doi: 10.1111/j.1398-9995.2007.01613.x.

[165] K. Esposito and D. Giugliano, “The metabolic syndrome and inflammation: association or causation?,” *Nutrition, Metabolism and Cardiovascular Diseases*, vol. 14, no. 5, pp. 228–232, Oct. 2004, doi: 10.1016/S0939-4753(04)80048-6.

[166] D. D. Sin and E. R. Sutherland, “Obesity and the lung: 4 {middle dot} Obesity and asthma,” *Thorax*, vol. 63, no. 11, pp. 1018–1023, Nov. 2008, doi: 10.1136/thx.2007.086819.

[167] L. M. Schachter, “Asthma and atopy in overweight children,” *Thorax*, vol. 58, no. 12, pp. 1031–1035, Dec. 2003, doi: 10.1136/thorax.58.12.1031.

[168] M. Willeboordse, D. L. C. M. van den Bersselaar, K. D. G. van de Kant, J. W. M. Muris, O. C. P. van Schayck, and E. Dompeling, “Sex Differences in the Relationship between Asthma and Overweight in Dutch Children: a Survey Study,” *PLoS ONE*, vol. 8, no. 10, p. e77574, Oct. 2013, doi: 10.1371/journal.pone.0077574.

[169] M. Nahhas, R. Bhopal, C. Anandan, R. Elton, and A. Sheikh, “Investigating the association between obesity and asthma in 6- to 8-year-old Saudi children: a matched case–control study,” *npj Primary Care Respiratory Medicine*, vol. 24, no. 1, p. 14004, Nov. 2014, doi: 10.1038/npjpcrm.2014.4.

[170] M. Gramss *et al.*, “Overweight Proxies Are Associated with Atopic Asthma: A Matched Case–Control Study,” *Hormone Research in Paediatrics*, vol. 91, no. 6, pp. 380–390, 2019, doi: 10.1159/000501685.

[171] K. D. Lu, J. Billimek, R. Bar-Yoseph, S. Radom-Aizik, D. M. Cooper, and H. Anton-Culver, “Sex Differences in the Relationship between Fitness and Obesity on Risk for Asthma in Adolescents,” *The Journal of Pediatrics*, vol. 176, pp. 36–42, Sep. 2016, doi: 10.1016/j.jpeds.2016.05.050.

[172] S. O. Shaheen, J. A. C. Sterne, S. M. Montgomery, and H. Azima, “Birth weight, body mass index and asthma in young adults,” *Thorax*, vol. 54, no. 5, pp. 396–402, May 1999, doi: 10.1136/thx.54.5.396.

[173] J. C. Seidell, L. C. de Groot, J. L. van Sonsbeek, P. Deurenberg, and J. G. Hautvast, “Associations of moderate and severe overweight with self-reported illness and medical care in Dutch adults.,” *American Journal of Public Health*, vol. 76, no. 3, pp. 264–269, Mar. 1986, doi: 10.2105/AJPH.76.3.264.

[174] E. Ronmark, “Obesity increases the risk of incident asthma among adults,” *European Respiratory Journal*, vol. 25, no. 2, pp. 282–288, Feb. 2005, doi: 10.1183/09031936.05.00054304.

[175] S. Y. N. Young, J. D. Gunzenhauser, K. E. Malone, and A. McTiernan, “Body Mass Index and Asthma in the Military Population of the Northwestern United States,” *Archives of Internal Medicine*, vol. 161, no. 13, p. 1605, Jul. 2001, doi: 10.1001/archinte.161.13.1605.

[176] J. von Behren *et al.*, “Obesity, waist size and prevalence of current asthma in the California Teachers Study cohort,” *Thorax*, vol. 64, no. 10, pp. 889–893, Oct. 2009, doi: 10.1136/thx.2009.114579.

[177] L. J. Akinbami and C. D. Fryar, “Current Asthma Prevalence by Weight Status Among Adults: United States, 2001-2014.,” *NCHS Data Brief*, no. 239, pp. 1–8, Mar. 2016.

[178] C. Koebnick *et al.*, “Interacting effects of obesity, race, ethnicity and sex on the incidence and control of adult-onset asthma,” *Allergy, Asthma & Clinical Immunology*, vol. 12, no. 1, p. 50, Dec. 2016, doi: 10.1186/s13223-016-0155-8.

[179] D. R. Gold, A. I. Damokosh, D. W. Dockery, and C. S. Berkey, “Body-mass index as a predictor of incident asthma in a prospective cohort of children,” *Pediatric Pulmonology*, vol. 36, no. 6, pp. 514–521, Dec. 2003, doi: 10.1002/ppul.10376.

[180] F. D. Gilliland, “Obesity and the Risk of Newly Diagnosed Asthma in School-age Children,” *American Journal of Epidemiology*, vol. 158, no. 5, pp. 406–415, Sep. 2003, doi: 10.1093/aje/kwg175.

[181] A. A. Mamun, D. A. Lawlor, R. Alati, M. J. O’Callaghan, G. M. Williams, and J. M. Najman, “Increasing body mass index from age 5 to 14 years predicts asthma among adolescents: evidence from a birth cohort study,” *International Journal of Obesity*, vol. 31, no. 4, pp. 578–583, Apr. 2007, doi: 10.1038/sj.ijo.0803571.

[182] D. M. Mannino *et al.*, “Boys with high body masses have an increased risk of developing asthma: findings from the National Longitudinal Survey of Youth (NLSY),” *International Journal of Obesity*, vol. 30, no. 1, pp. 6–13, Jan. 2006, doi: 10.1038/sj.ijo.0803145.

[183] Z. Zhang *et al.*, “Early childhood weight status in relation to asthma development in high-risk children,” *Journal of Allergy and Clinical Immunology*, vol. 126, no. 6, pp. 1157–1162, Dec. 2010, doi: 10.1016/j.jaci.2010.09.011.

[184] W.-C. Ho *et al.*, “Higher body mass index may induce asthma among adolescents with pre-asthmatic symptoms: a prospective cohort study,” *BMC Public Health*, vol. 11, no. 1, p. 542, Dec. 2011, doi: 10.1186/1471-2458-11-542.

[185] E. S. Ford, “Body mass index and asthma incidence among USA adults,” *European Respiratory Journal*, vol. 24, no. 5, pp. 740–744, Nov. 2004, doi: 10.1183/09031936.04.00088003.

[186] E. HUOVINEN, J. KAPRIO, and M. KOSKENVUO, “Factors associated to lifestyle and risk of adult onset asthma,” *Respiratory Medicine*, vol. 97, no. 3, pp. 273–280, Mar. 2003, doi: 10.1053/rmed.2003.1419.

[187] I. Romieu, “Body Mass Index, Change in Body Silhouette, and Risk of Asthma in the E3N Cohort Study,” *American Journal of Epidemiology*, vol. 158, no. 2, pp. 165–174, Jul. 2003, doi: 10.1093/aje/kwg131.

[188] M. I. Gunnbjornsdottir *et al.*, “Obesity and nocturnal gastro-oesophageal reflux are related to onset of asthma and respiratory symptoms,” *European Respiratory Journal*, vol. 24, no. 1, pp. 116–121, Jul. 2004, doi: 10.1183/09031936.04.00042603.

[189] C. A. Camargo, S. T. Weiss, S. Zhang, W. C. Willett, and F. E. Speizer, “Prospective Study of Body Mass Index, Weight Change, and Risk of Adult-onset Asthma in Women,” *Archives of Internal Medicine*, vol. 159, no. 21, p. 2582, Nov. 1999, doi: 10.1001/archinte.159.21.2582.

[190] Y. Chen, “Obesity May Increase the Incidence of Asthma in Women but Not in Men: Longitudinal Observations from the Canadian National Population Health Surveys,” *American Journal of Epidemiology*, vol. 155, no. 3, pp. 191–197, Feb. 2002, doi: 10.1093/aje/155.3.191.

[191] W. Nystad, “Body Mass Index in Relation to Adult Asthma among 135,000 Norwegian Men and Women,” *American Journal of Epidemiology*, vol. 160, no. 10, pp. 969–976, Nov. 2004, doi: 10.1093/aje/kwh303.

[192] D. A. Beuther and E. R. Sutherland, “Overweight, Obesity, and Incident Asthma,” *American Journal of Respiratory and Critical Care Medicine*, vol. 175, no. 7, pp. 661–666, Apr. 2007, doi: 10.1164/rccm.200611-1717OC.

[193] L.-S. Shan, Q.-L. Zhou, and Y.-X. Shang, “Bidirectional Association Between Asthma and Obesity During Childhood and Adolescence: A Systematic Review and Meta-Analysis,” *Frontiers in Pediatrics*, vol. 8, Oct. 2020, doi: 10.3389/fped.2020.576858.

[194] M. D. Althoff, A. Ghincea, L. G. Wood, F. Holguin, and S. Sharma, “Asthma and Three Colinear Comorbidities: Obesity, OSA, and GERD,” *The Journal of Allergy and Clinical Immunology: In Practice*, vol. 9, no. 11, pp. 3877–3884, Nov. 2021, doi: 10.1016/j.jaip.2021.09.003.

[195] W. C. Moore *et al.*, “Identification of Asthma Phenotypes Using Cluster Analysis in the Severe Asthma Research Program,” *American Journal of Respiratory and Critical Care Medicine*, vol. 181, no. 4, pp. 315–323, Feb. 2010, doi: 10.1164/rccm.200906-0896OC.

[196] P. Haldar *et al.*, “Cluster Analysis and Clinical Asthma Phenotypes,” *American Journal of Respiratory and Critical Care Medicine*, vol. 178, no. 3, pp. 218–224, Aug. 2008, doi: 10.1164/rccm.200711-1754OC.

[197] N. Leone *et al.*, “Lung Function Impairment and Metabolic Syndrome,” *American Journal of Respiratory and Critical Care Medicine*, vol. 179, no. 6, pp. 509–516, Mar. 2009, doi: 10.1164/rccm.200807-1195OC.

[198] P. Pelosi *et al.*, “The Effects of Body Mass on Lung Volumes, Respiratory Mechanics, and Gas Exchange During General Anesthesia,” *Anesthesia & Analgesia*, vol. 87, no. 3, pp. 654–660, Sep. 1998, doi: 10.1213/00000539-199809000-00031.

[199] G. Weinmayr, F. Forastiere, G. Büchele, A. Jaensch, D. P. Strachan, and G. Nagel, “Correction: Overweight/Obesity and Respiratory and Allergic Disease in Children: International Study of Asthma and Allergies in Childhood (ISAAC) Phase Two,” *PLOS ONE*, vol. 10, no. 4, p. e0126678, Apr. 2015, doi: 10.1371/journal.pone.0126678.

[200] M. Aydin *et al.*, “Interaction of Metabolic Syndrome with Asthma in Postmenopausal Women: Role of Adipokines,” *Inflammation*, vol. 36, no. 6, pp. 1232–1238, Dec. 2013, doi: 10.1007/s10753-013-9660-9.

[201] G. M. de Boer *et al.*, “Adult but not childhood onset asthma is associated with the metabolic syndrome, independent from body mass index,” *Respiratory Medicine*, vol. 188, p. 106603, Nov. 2021, doi: 10.1016/j.rmed.2021.106603.

[202] B. H. Thuesen, L. L. N. Husemoen, L.-G. Hersoug, C. Pisinger, and A. Linneberg, “Insulin resistance as a predictor of incident asthma-like symptoms in adults,” *Clinical & Experimental Allergy*, vol. 39, no. 5, pp. 700–707, May 2009, doi: 10.1111/j.1365-2222.2008.03197.x.

[203] B. E. Del-Rio-Navarro *et al.*, “Higher Metabolic Syndrome in Obese Asthmatic Compared to Obese Nonasthmatic Adolescent Males,” *Journal of Asthma*, vol. 47, no. 5, pp. 501–506, Jun. 2010, doi: 10.3109/02770901003702808.

[204] N. Assad *et al.*, “Body Mass Index Is a Stronger Predictor than the Metabolic Syndrome for Future Asthma in Women. The Longitudinal CARDIA Study,” *American Journal of Respiratory and Critical Care Medicine*, vol. 188, no. 3, pp. 319–326, Aug. 2013, doi: 10.1164/rccm.201303-0457OC.

[205] L.-P. Boulet, H. Turcotte, J. Martin, and P. Poirier, “Effect of bariatric surgery on airway response and lung function in obese subjects with asthma,” *Respiratory Medicine*, vol. 106, no. 5, pp. 651–660, May 2012, doi: 10.1016/j.rmed.2011.12.012.

[206] A. E. Dixon *et al.*, “Effects of obesity and bariatric surgery on airway hyperresponsiveness, asthma control, and inflammation,” *Journal of Allergy and Clinical Immunology*, vol. 128, no. 3, pp. 508-515.e2, Sep. 2011, doi: 10.1016/j.jaci.2011.06.009.

[207] S. D. Aaron *et al.*, “Overdiagnosis of asthma in obese and nonobese adults,” *Canadian Medical Association Journal*, vol. 179, no. 11, pp. 1121–1131, Nov. 2008, doi: 10.1503/cmaj.081332.

[208] L. L. Barros *et al.*, “Obesity and Poor Asthma Control in Patients with Severe Asthma,” *Journal of Asthma*, vol. 48, no. 2, pp. 171–176, Mar. 2011, doi: 10.3109/02770903.2011.554940.

[209] A. Youkou *et al.*, “Influence of Obesity on Control in Asthmatic Japanese Patients Defined by the Japanese Definition of Obesity,” *Internal Medicine*, vol. 50, no. 18, pp. 1911–1916, 2011, doi: 10.2169/internalmedicine.50.5474.

[210] R. H. Stanford, A. W. Gilsenan, R. Ziemiecki, X. Zhou, W. R. Lincourt, and H. Ortega, “Predictors of Uncontrolled Asthma in Adult and Pediatric Patients: Analysis of the Asthma Control Characteristics and Prevalence Survey Studies (ACCESS),” *Journal of Asthma*, vol. 47, no. 3, pp. 257–262, Apr. 2010, doi: 10.3109/02770900903584019.

[211] D. M. Mosen, M. Schatz, D. J. Magid, and C. A. Camargo, “The relationship between obesity and asthma severity and control in adults,” *Journal of Allergy and Clinical Immunology*, vol. 122, no. 3, pp. 507-511.e6, Sep. 2008, doi: 10.1016/j.jaci.2008.06.024.

[212] L. LAFOREST *et al.*, “Influence of patients’ characteristics and disease management on asthma control,” *Journal of Allergy and Clinical Immunology*, vol. 117, no. 6, pp. 1404–1410, Jun. 2006, doi: 10.1016/j.jaci.2006.03.007.

[213] M. Peters-Golden, “Influence of body mass index on the response to asthma controller agents,” *European Respiratory Journal*, vol. 27, no. 3, pp. 495–503, Mar. 2006, doi: 10.1183/09031936.06.00077205.

[214] D. Lefaudeux *et al.*, “U-BIOPRED clinical adult asthma clusters linked to a subset of sputum omics,” *Journal of Allergy and Clinical Immunology*, vol. 139, no. 6, pp. 1797–1807, Jun. 2017, doi: 10.1016/j.jaci.2016.08.048.

[215] S. A. Dias-Junior, M. Reis, R. M. de Carvalho-Pinto, R. Stelmach, A. Halpern, and A. Cukier, “Effects of weight loss on asthma control in obese patients with severe asthma,” *European Respiratory Journal*, vol. 43, no. 5, pp. 1368–1377, May 2014, doi: 10.1183/09031936.00053413.

[216] E. Selvin, “The Effect of Weight Loss on C-Reactive Protein,” *Archives of Internal Medicine*, vol. 167, no. 1, p. 31, Jan. 2007, doi: 10.1001/archinte.167.1.31.

[217] U. Peters, A. E. Dixon, and E. Forno, “Obesity and asthma,” *Journal of Allergy and Clinical Immunology*, vol. 141, no. 4, pp. 1169–1179, Apr. 2018, doi: 10.1016/j.jaci.2018.02.004.

[218] K. Eder, N. Baffy, A. Falus, and A. K. Fulop, “The major inflammatory mediator interleukin-6 and obesity,” *Inflammation Research*, vol. 58, no. 11, pp. 727–736, Nov. 2009, doi: 10.1007/s00011-009-0060-4.

[219] G. S. Hotamisligil, N. S. Shargill, and B. M. Spiegelman, “Adipose Expression of Tumor Necrosis Factor-α: Direct Role in Obesity-Linked Insulin Resistance,” *Science (1979)*, vol. 259, no. 5091, pp. 87–91, Jan. 1993, doi: 10.1126/science.7678183.

[220] J. G. Elliot, G. M. Donovan, K. C. W. Wang, F. H. Y. Green, A. L. James, and P. B. Noble, “Fatty airways: implications for obstructive disease,” *European Respiratory Journal*, vol. 54, no. 6, p. 1900857, Dec. 2019, doi: 10.1183/13993003.00857-2019.

[221] A. Tsaroucha *et al.*, “Leptin, Adiponectin, and Ghrelin Levels in Female Patients with Asthma during Stable and Exacerbation Periods,” *Journal of Asthma*, vol. 50, no. 2, pp. 188–197, Mar. 2013, doi: 10.3109/02770903.2012.747101.

[222] W. Liu, Q. Zeng, L. Zhou, R. Luo, and H. Dong, “Association of leptin with disease severity and inflammation indicators in Chinese obese children with allergic rhinitis,” *Pediatric Allergy and Immunology*, vol. 29, no. 2, pp. 186–193, Mar. 2018, doi: 10.1111/pai.12856.

[223] H. Zheng, X. Zhang, E. F. Castillo, Y. Luo, M. Liu, and X. O. Yang, “Leptin Enhances TH2 and ILC2 Responses in Allergic Airway Disease,” *Journal of Biological Chemistry*, vol. 291, no. 42, pp. 22043–22052, Oct. 2016, doi: 10.1074/jbc.M116.743187.

[224] H. Zheng *et al.*, “Leptin Promotes Allergic Airway Inflammation through Targeting the Unfolded Protein Response Pathway,” *Scientific Reports*, vol. 8, no. 1, p. 8905, Dec. 2018, doi: 10.1038/s41598-018-27278-4.

[225] S. A. Shore, I. N. Schwartzman, M. S. Mellema, L. Flynt, A. Imrich, and R. A. Johnston, “Effect of leptin on allergic airway responses in mice,” *Journal of Allergy and Clinical Immunology*, vol. 115, no. 1, pp. 103–109, Jan. 2005, doi: 10.1016/j.jaci.2004.10.007.

[226] M. D. Ihrie *et al.*, “Exogenous leptin enhances markers of airway fibrosis in a mouse model of chronic allergic airways disease,” *Respiratory Research*, vol. 23, no. 1, p. 131, Dec. 2022, doi: 10.1186/s12931-022-02048-z.

[227] F. Holguin, M. Rojas, L. anne Brown, and A. M. Fitzpatrick, “Airway and Plasma Leptin and Adiponectin in Lean and Obese Asthmatics and Controls,” *Journal of Asthma*, vol. 48, no. 3, pp. 217–223, Apr. 2011, doi: 10.3109/02770903.2011.555033.

[228] A. Lessard, J. St-Laurent, H. Turcotte, and L.-P. Boulet, “Leptin and adiponectin in obese and non-obese subjects with asthma,” *Biomarkers*, vol. 16, no. 3, pp. 271–273, May 2011, doi: 10.3109/1354750X.2010.550013.

[229] S. CONUS, A. BRUNO, and H. SIMON, “Leptin is an eosinophil survival factor,” *Journal of Allergy and Clinical Immunology*, vol. 116, no. 6, pp. 1228–1234, Dec. 2005, doi: 10.1016/j.jaci.2005.09.003.

[230] M. Suzukawa *et al.*, “Leptin enhances ICAM-1 expression, induces migration and cytokine synthesis, and prolongs survival of human airway epithelial cells,” *American Journal of Physiology-Lung Cellular and Molecular Physiology*, vol. 309, no. 8, pp. L801–L811, Oct. 2015, doi: 10.1152/ajplung.00365.2014.

[231] K. Watanabe *et al.*, “Leptin enhances cytokine/chemokine production by normal lung fibroblasts by binding to leptin receptor,” *Allergology International*, vol. 68, pp. S3–S8, Sep. 2019, doi: 10.1016/j.alit.2019.04.002.

[232] S. Wueest and D. Konrad, “The role of adipocyte-specific IL-6-type cytokine signaling in FFA and leptin release,” *Adipocyte*, vol. 7, no. 3, pp. 226–228, Jul. 2018, doi: 10.1080/21623945.2018.1493901.

[233] J. Spranger *et al.*, “Inflammatory Cytokines and the Risk to Develop Type 2 Diabetes,” *Diabetes*, vol. 52, no. 3, pp. 812–817, Mar. 2003, doi: 10.2337/diabetes.52.3.812.

[234] J. Braune *et al.*, “IL-6 Regulates M2 Polarization and Local Proliferation of Adipose Tissue Macrophages in Obesity,” *The Journal of Immunology*, vol. 198, no. 7, pp. 2927–2934, Apr. 2017, doi: 10.4049/jimmunol.1600476.

[235] F. O. Martinez and S. Gordon, “The M1 and M2 paradigm of macrophage activation: time for reassessment,” *F1000Prime Reports*, vol. 6, Mar. 2014, doi: 10.12703/P6-13.

[236] I. TILLIE-LEBLOND *et al.*, “Balance between Proinflammatory Cytokines and Their Inhibitors in Bronchial Lavage from Patients with Status Asthmaticus,” *American Journal of Respiratory and Critical Care Medicine*, vol. 159, no. 2, pp. 487–494, Feb. 1999, doi: 10.1164/ajrccm.159.2.9805115.

[237] A. Yokoyama *et al.*, “Circulating interleukin-6 levels in patients with bronchial asthma.,” *American Journal of Respiratory and Critical Care Medicine*, vol. 151, no. 5, pp. 1354–1358, May 1995, doi: 10.1164/ajrccm.151.5.7735584.

[238] W. A. Neveu *et al.*, “Elevation of IL-6 in the allergic asthmatic airway is independent of inflammation but associates with loss of central airway function,” *Respiratory Research*, vol. 11, no. 1, p. 28, Dec. 2010, doi: 10.1186/1465-9921-11-28.

[239] M. C. Peters *et al.*, “Plasma interleukin-6 concentrations, metabolic dysfunction, and asthma severity: a cross-sectional analysis of two cohorts,” *The Lancet Respiratory Medicine*, vol. 4, no. 7, pp. 574–584, Jul. 2016, doi: 10.1016/S2213-2600(16)30048-0.

[240] M. C. Peters *et al.*, “Evidence for Exacerbation-Prone Asthma and Predictive Biomarkers of Exacerbation Frequency,” *American Journal of Respiratory and Critical Care Medicine*, vol. 202, no. 7, pp. 973–982, Oct. 2020, doi: 10.1164/rccm.201909-1813OC.

[241] N. Akar-Ghibril, P. Permaul, C. Petty, and W. Phipatanakul, “Association of Systemic IL-6 Inflammation with Body Mass Index, Asthma Morbidity, and Metabolic Dysfunction in the School Inner-City Asthma Study,” *Journal of Allergy and Clinical Immunology*, vol. 145, no. 2, p. AB11, Feb. 2020, doi: 10.1016/j.jaci.2019.12.862.

[242] W. A. Neveu *et al.*, “IL-6 Is Required for Airway Mucus Production Induced by Inhaled Fungal Allergens,” *The Journal of Immunology*, vol. 183, no. 3, pp. 1732–1738, Aug. 2009, doi: 10.4049/jimmunol.0802923.

[243] G. S. Marijsse *et al.*, “Obese Individuals with Asthma Preferentially Have a High IL-5/IL-17A/IL-25 Sputum Inflammatory Pattern,” *American Journal of Respiratory and Critical Care Medicine*, vol. 189, no. 10, pp. 1284–1285, May 2014, doi: 10.1164/rccm.201311-2011LE.

[244] M. G. Duvall *et al.*, “Natural killer cell–mediated inflammation resolution is disabled in severe asthma,” *Science Immunology*, vol. 2, no. 9, Mar. 2017, doi: 10.1126/sciimmunol.aam5446.

[245] L. Dejager *et al.*, “Neutralizing TNFα restores glucocorticoid sensitivity in a mouse model of neutrophilic airway inflammation,” *Mucosal Immunology*, vol. 8, no. 6, pp. 1212–1225, Nov. 2015, doi: 10.1038/mi.2015.12.

[246] M. BERRY, C. BRIGHTLING, I. PAVORD, and A. WARDLAW, “TNF-α in asthma,” *Current Opinion in Pharmacology*, vol. 7, no. 3, pp. 279–282, Jun. 2007, doi: 10.1016/j.coph.2007.03.001.

[247] G. S. Hotamisligil, P. Arner, J. F. Caro, R. L. Atkinson, and B. M. Spiegelman, “Increased adipose tissue expression of tumor necrosis factor-alpha in human obesity and insulin resistance.,” *Journal of Clinical Investigation*, vol. 95, no. 5, pp. 2409–2415, May 1995, doi: 10.1172/JCI117936.

[248] P. A. Kern, M. Saghizadeh, J. M. Ong, R. J. Bosch, R. Deem, and R. B. Simsolo, “The expression of tumor necrosis factor in human adipose tissue. Regulation by obesity, weight loss, and relationship to lipoprotein lipase.,” *Journal of Clinical Investigation*, vol. 95, no. 5, pp. 2111–2119, May 1995, doi: 10.1172/JCI117899.

[249] K. Popko *et al.*, “Proinflammatory cytokines IL-6 and TNF-α and the development of inflammation in obese subjects,” *European Journal of Medical Research*, vol. 15, no. S2, p. 120, Dec. 2010, doi: 10.1186/2047-783X-15-S2-120.

[250] F. J. OVERVELD, Ph. G. JORENS, M. RAMPART, W. BACKER, and P. A. VERMEIRE, “Tumour necrosis factor stimulates human skin mast cells to release histamine and tryptase,” *Clinical <html_ent glyph="@amp;" ascii="&"/> Experimental Allergy*, vol. 21, no. 6, pp. 711–714, Nov. 1991, doi: 10.1111/j.1365-2222.1991.tb03200.x.

[251] G. K. Griffin *et al.*, “IL-17 and TNF-α Sustain Neutrophil Recruitment during Inflammation through Synergistic Effects on Endothelial Activation,” *The Journal of Immunology*, vol. 188, no. 12, pp. 6287–6299, Jun. 2012, doi: 10.4049/jimmunol.1200385.

[252] N. Krishnamoorthy *et al.*, “Neutrophil cytoplasts induce T $_{H}$ 17 differentiation and skew inflammation toward neutrophilia in severe asthma,” *Science Immunology*, vol. 3, no. 26, Aug. 2018, doi: 10.1126/sciimmunol.aao4747.

[253] H. Y. Kim *et al.*, “Interleukin-17–producing innate lymphoid cells and the NLRP3 inflammasome facilitate obesity-associated airway hyperreactivity,” *Nature Medicine*, vol. 20, no. 1, pp. 54–61, Jan. 2014, doi: 10.1038/nm.3423.

[254] M. Kudo *et al.*, “IL-17A produced by αβ T cells drives airway hyper-responsiveness in mice and enhances mouse and human airway smooth muscle contraction,” *Nature Medicine*, vol. 18, no. 4, pp. 547–554, Apr. 2012, doi: 10.1038/nm.2684.

[255] “Health Effects of Overweight and Obesity in 195 Countries over 25 Years,” *New England Journal of Medicine*, vol. 377, no. 1, pp. 13–27, Jul. 2017, doi: 10.1056/NEJMoa1614362.

[256] L. G. Wood, “Asthma in the Obese: A Big and Growing Problem,” *American Journal of Respiratory and Critical Care Medicine*, vol. 195, no. 1, pp. 4–5, Jan. 2017, doi: 10.1164/rccm.201608-1582ED.

[257] E. Forno, Y.-Y. Han, J. Mullen, and J. C. Celedón, “Overweight, Obesity, and Lung Function in Children and Adults—A Meta-analysis,” *The Journal of Allergy and Clinical Immunology: In Practice*, vol. 6, no. 2, pp. 570-581.e10, Mar. 2018, doi: 10.1016/j.jaip.2017.07.010.

[258] X. Zhang *et al.*, “Systemic inflammation mediates the detrimental effects of obesity on asthma control,” *Allergy and Asthma Proceedings*, vol. 39, no. 1, pp. 43–50, Jan. 2018, doi: 10.2500/aap.2018.39.4096.

[259] S. Mohanan, H. Tapp, A. McWilliams, and M. Dulin, “Obesity and asthma: Pathophysiology and implications for diagnosis and management in primary care,” *Experimental Biology and Medicine*, vol. 239, no. 11, pp. 1531–1540, Nov. 2014, doi: 10.1177/1535370214525302.

[260] C. Procaccini, E. Jirillo, and G. Matarese, “Leptin as an immunomodulator,” *Molecular Aspects of Medicine*, vol. 33, no. 1, pp. 35–45, Feb. 2012, doi: 10.1016/j.mam.2011.10.012.

[261] X. Wang, Y. Shen, X. Ke, H. Kang, and S. Hong, “Role of leptin/ILC2 axis in allergic rhinitis in obese children,” *International Journal of Pediatric Otorhinolaryngology*, vol. 157, p. 111127, Jun. 2022, doi: 10.1016/j.ijporl.2022.111127.

[262] T. Trinh and H. E. Broxmeyer, “Role for Leptin and Leptin Receptors in Stem Cells During Health and Diseases,” *Stem Cell Reviews and Reports*, vol. 17, no. 2, pp. 511–522, Apr. 2021, doi: 10.1007/s12015-021-10132-y.

[263] K. Yamauchi and M. Ogasawara, “The role of histamine in the pathophysiology of asthma and the clinical efficacy of antihistamines in asthma therapy,” *International Journal of Molecular Sciences*, vol. 20, no. 7. MDPI AG, Apr. 01, 2019. doi: 10.3390/ijms20071733.

[264] K. Hegyi, K. A. Fülöp, K. J. Kovács, A. Falus, and S. Tóth, “High leptin level is accompanied with decreased long leptin receptor transcript in histamine deficient transgenic mice,” *Immunology Letters*, vol. 92, no. 1–2, pp. 193–197, Mar. 2004, doi: 10.1016/j.imlet.2003.11.029.

[265] T. Masaki *et al.*, “Involvement of Hypothalamic Histamine H1 Receptor in the Regulation of Feeding Rhythm and Obesity,” *Diabetes*, vol. 53, no. 9, pp. 2250–2260, Sep. 2004, doi: 10.2337/diabetes.53.9.2250.

[266] T. Masaki, H. Yoshimatsu, S. Chiba, T. Watanabe, and T. Sakata, “Targeted Disruption of Histamine H1-Receptor Attenuates Regulatory Effects of Leptin on Feeding, Adiposity, and UCP Family in Mice,” *Diabetes*, vol. 50, no. 2, pp. 385–391, Feb. 2001, doi: 10.2337/diabetes.50.2.385.

[267] T. Morimoto *et al.*, “Involvement of the Histaminergic System in Leptin-Induced Suppression of Food Intake,” *Physiology & Behavior*, vol. 67, no. 5, pp. 679–683, Nov. 1999, doi: 10.1016/S0031-9384(99)00123-7.

[268] E. A. Jørgensen, U. Knigge, J. Warberg, and A. Kjær, “Histamine and the Regulation of Body Weight,” *Neuroendocrinology*, vol. 86, no. 3, pp. 210–214, 2007, doi: 10.1159/000108341.

[269] A. Pérez-Pérez, F. Sánchez-Jiménez, T. Vilariño-García, and V. Sánchez-Margalet, “Role of Leptin in Inflammation and Vice Versa,” *International Journal of Molecular Sciences*, vol. 21, no. 16, p. 5887, Aug. 2020, doi: 10.3390/ijms21165887.

[270] A. Bruno *et al.*, “Leptin and leptin receptor expression in asthma,” *Journal of Allergy and Clinical Immunology*, vol. 124, no. 2, pp. 230-237.e4, Aug. 2009, doi: 10.1016/j.jaci.2009.04.032.

[271] A. Munitz, E. B. Brandt, M. Mingler, F. D. Finkelman, and M. E. Rothenberg, “Distinct roles for IL-13 and IL-4 via IL-13 receptor α1 and the type II IL-4 receptor in asthma pathogenesis,” *Proceedings of the National Academy of Sciences*, vol. 105, no. 20, pp. 7240–7245, May 2008, doi: 10.1073/pnas.0802465105.

[272] Y. Chiba, S. Onoda, M. Todoroki, Y. Nishida, and M. Misawa, “Upregulation of interleukin-13 receptor chains in bronchial smooth muscle tissues of mouse experimental asthma,” *Journal of Smooth Muscle Research*, vol. 46, no. 1, pp. 49–55, 2010, doi: 10.1540/jsmr.46.49.

[273] A. G. Izquierdo, A. B. Crujeiras, F. F. Casanueva, and M. C. Carreira, “Leptin, Obesity, and Leptin Resistance: Where Are We 25 Years Later?,” *Nutrients*, vol. 11, no. 11, p. 2704, Nov. 2019, doi: 10.3390/nu11112704.

[274] M. Kadmiel and J. A. Cidlowski, “Glucocorticoid receptor signaling in health and disease,” *Trends in Pharmacological Sciences*, vol. 34, no. 9, pp. 518–530, Sep. 2013, doi: 10.1016/j.tips.2013.07.003.

[275] J. M. Busillo and J. A. Cidlowski, “The five Rs of glucocorticoid action during inflammation: ready, reinforce, repress, resolve, and restore,” *Trends in Endocrinology & Metabolism*, vol. 24, no. 3, pp. 109–119, Mar. 2013, doi: 10.1016/j.tem.2012.11.005.

[276] M. Zen *et al.*, “The kaleidoscope of glucorticoid effects on immune system,” *Autoimmunity Reviews*, vol. 10, no. 6, pp. 305–310, Apr. 2011, doi: 10.1016/j.autrev.2010.11.009.

[277] P.-J. Chang *et al.*, “Impaired Nuclear Translocation of the Glucocorticoid Receptor in Corticosteroid-Insensitive Airway Smooth Muscle in Severe Asthma,” *American Journal of Respiratory and Critical Care Medicine*, vol. 191, no. 1, pp. 54–62, Jan. 2015, doi: 10.1164/rccm.201402-0314OC.

[278] L. Fan, L. Ding, J. Lan, J. Niu, Y. He, and L. Song, “Fibroblast Growth Factor-1 Improves Insulin Resistance via Repression of JNK-Mediated Inflammation,” *Frontiers in Pharmacology*, vol. 10, Dec. 2019, doi: 10.3389/fphar.2019.01478.

[279] L. Zhao *et al.*, “Fibroblast growth factor 1 ameliorates adipose tissue inflammation and systemic insulin resistance via enhancing adipocyte mTORC2/Rictor signal,” *Journal of Cellular and Molecular Medicine*, vol. 24, no. 21, pp. 12813–12825, Nov. 2020, doi: 10.1111/jcmm.15872.

[280] L. Hutley *et al.*, “Fibroblast Growth Factor 1,” *Diabetes*, vol. 53, no. 12, pp. 3097–3106, Dec. 2004, doi: 10.2337/diabetes.53.12.3097.

[281] P. Dandona, A. Chaudhuri, P. Mohanty, and H. Ghanim, “Anti-inflammatory effects of insulin,” *Current Opinion in Clinical Nutrition and Metabolic Care*, vol. 10, no. 4, pp. 511–517, Jul. 2007, doi: 10.1097/MCO.0b013e3281e38774.

[282] P. Dandona, A. Chaudhuri, H. Ghanim, and P. Mohanty, “Insulin as an Anti-Inflammatory and Antiatherogenic Modulator,” *J Am Coll Cardiol*, vol. 53, no. 5, pp. S14–S20, Feb. 2009, doi: 10.1016/j.jacc.2008.10.038.

[283] T. Wierzbicki, S. M. Iqbal, S. L. Cuvelier, G. Awong, L. A. Tibbles, and K. D. Patel, “IL-4 primes human endothelial cells for secondary responses to histamine,” *Journal of Leukocyte Biology*, vol. 74, no. 3, pp. 420–427, Sep. 2003, doi: 10.1189/jlb.1102571.

[284] M. L. Manson *et al.*, “IL-13 and IL-4, but not IL-5 nor IL-17A, induce hyperresponsiveness in isolated human small airways,” *Journal of Allergy and Clinical Immunology*, vol. 145, no. 3, pp. 808-817.e2, Mar. 2020, doi: 10.1016/j.jaci.2019.10.037.

[285] N. Gour and M. Wills-Karp, “IL-4 and IL-13 signaling in allergic airway disease,” *Cytokine*, vol. 75, no. 1, pp. 68–78, Sep. 2015, doi: 10.1016/j.cyto.2015.05.014.

[286] M. Azizian *et al.*, “Cytokine profiles in overweight and obese subjects and normal weight individuals matched for age and gender,” *Annals of Clinical Biochemistry: International Journal of Laboratory Medicine*, vol. 53, no. 6, pp. 663–668, Nov. 2016, doi: 10.1177/0004563216629997.

[287] Y. H. Chang, K. T. Ho, S. H. Lu, C. N. Huang, and M. Y. Shiau, “Regulation of glucose/lipid metabolism and insulin sensitivity by interleukin-4,” *International Journal of Obesity*, vol. 36, no. 7, pp. 993–998, Jul. 2012, doi: 10.1038/ijo.2011.168.

[288] E. B. Thangam *et al.*, “The Role of Histamine and Histamine Receptors in Mast Cell-Mediated Allergy and Inflammation: The Hunt for New Therapeutic Targets,” *Frontiers in Immunology*, vol. 9, Aug. 2018, doi: 10.3389/fimmu.2018.01873.

[289] L. O’Mahony, M. Akdis, and C. A. Akdis, “Regulation of the immune response and inflammation by histamine and histamine receptors,” *Journal of Allergy and Clinical Immunology*, vol. 128, no. 6, pp. 1153–1162, 2011, doi: 10.1016/j.jaci.2011.06.051.

[290] K. Botturi, Y. Lacoeuille, D. Vervloet, and A. Magnan, “Histamine induces Th2 activation through the histamine receptor 1 in house dust mite rhinitic but not asthmatic patients,” *Clinical & Experimental Allergy*, Feb. 2010, doi: 10.1111/j.1365-2222.2010.03457.x.

[291] S. Chapoval, P. Dasgupta, N. J. Dorsey, and A. D. Keegan, “Regulation of the T helper cell type 2 (Th2)/T regulatory cell (Treg) balance by IL-4 and STAT6,” *Journal of Leukocyte Biology*, vol. 87, no. 6, pp. 1011–1018, Jun. 2010, doi: 10.1189/jlb.1209772.

[292] L. M. Arendt *et al.*, “Obesity Promotes Breast Cancer by CCL2-Mediated Macrophage Recruitment and Angiogenesis,” *Cancer Research*, vol. 73, no. 19, pp. 6080–6093, Oct. 2013, doi: 10.1158/0008-5472.CAN-13-0926.

[293] S. Y. Lim, A. E. Yuzhalin, A. N. Gordon-Weeks, and R. J. Muschel, “Targeting the CCL2-CCR2 signaling axis in cancer metastasis,” *Oncotarget*, vol. 7, no. 19, pp. 28697–28710, May 2016, doi: 10.18632/oncotarget.7376.

[294] A. Hoffmann *et al.*, “Leptin decreases circulating inflammatory IL-6 and MCP-1 in mice,” *BioFactors*, vol. 45, no. 1, pp. 43–48, Jan. 2019, doi: 10.1002/biof.1457.

[295] Z. Zhou *et al.*, “Leptin differentially regulate STAT3 activation in ob/ob mouse adipose mesenchymal stem cells,” *Nutrition and Metabolism*, vol. 9, no. 1, Dec. 2012, doi: 10.1186/1743-7075-9-109.

[296] L. Kern, M. Mittenbühler, A. Vesting, A. Ostermann, C. Wunderlich, and F. Wunderlich, “Obesity-Induced TNFα and IL-6 Signaling: The Missing Link between Obesity and Inflammation—Driven Liver and Colorectal Cancers,” *Cancers (Basel)*, vol. 11, no. 1, p. 24, Dec. 2018, doi: 10.3390/cancers11010024.

[297] L. Wei, Y. Chen, C. Zhang, M. Liu, and H. Xiong, “Leptin induces IL-6 and IL-8 expression through leptin receptor Ob-Rb in human dental pulp fibroblasts,” *Acta Odontologica Scandinavica*, vol. 77, no. 3, pp. 205–212, Apr. 2019, doi: 10.1080/00016357.2018.1536280.

[298] K.-M. Tong *et al.*, “Leptin induces IL-8 expression via leptin receptor, IRS-1, PI3K, Akt cascade and promotion of NF-κB/p300 binding in human synovial fibroblasts,” *Cellular Signalling*, vol. 20, no. 8, pp. 1478–1488, Aug. 2008, doi: 10.1016/j.cellsig.2008.04.003.

[299] R. Gomez, M. Scotece, J. Conde, J. J. Gomez-Reino, F. Lago, and O. Gualillo, “Adiponectin and leptin increase IL-8 production in human chondrocytes,” *Annals of the Rheumatic Diseases*, vol. 70, no. 11, pp. 2052–2054, Nov. 2011, doi: 10.1136/ard.2010.145672.

[300] R. Tazawa *et al.*, “Elevated leptin levels induce inflammation through IL-6 in skeletal muscle of aged female rats,” *BMC Musculoskeletal Disorders*, vol. 20, no. 1, p. 199, Dec. 2019, doi: 10.1186/s12891-019-2581-5.

[301] Q. Sun, J. Li, and F. Gao, “New insights into insulin: The anti-inflammatory effect and its clinical relevance,” *World Journal of Diabetes*, vol. 5, no. 2, p. 89, 2014, doi: 10.4239/wjd.v5.i2.89.

[302] Y. Iwasaki *et al.*, “Insulin exhibits short-term anti-inflammatory but long-term proinflammatory effects in vitro,” *Molecular and Cellular Endocrinology*, vol. 298, no. 1–2, pp. 25–32, Jan. 2009, doi: 10.1016/j.mce.2008.09.030.

[303] C. Küper, F.-X. Beck, and W. Neuhofer, “Toll-like receptor 4 activates NF-κB and MAP kinase pathways to regulate expression of proinflammatory COX-2 in renal medullary collecting duct cells,” *American Journal of Physiology-Renal Physiology*, vol. 302, no. 1, pp. F38–F46, Jan. 2012, doi: 10.1152/ajprenal.00590.2010.

[304] J. Raingeaud and J. Pierre, “Interleukin-4 downregulates TNFα-induced IL-8 production in keratinocytes,” *FEBS Letters*, vol. 579, no. 18, pp. 3953–3959, Jul. 2005, doi: 10.1016/j.febslet.2005.06.019.

[305] T. Standiford, R. Strieter, S. Chensue, J. Westwick, K. Kasahara, and S. Kunkel, “IL-4 inhibits the expression of IL-8 from stimulated human monocytes ,” *Journal of Immunology*, vol. 145, no. 5, pp. 1435–1439, 1990.

[306] S. Salmivesi, M. Paassilta, H. Huhtala, R. Nieminen, E. Moilanen, and M. Korppi, “Elevated serum adipsin may predict unsuccessful treatment for cows’ milk allergy but other biomarkers do not,” *Acta Paediatrica*, vol. 107, no. 2, pp. 328–332, Feb. 2018, doi: 10.1111/apa.14117.

[307] J. C. Lo *et al.*, “Adipsin is an adipokine that improves β cell function in diabetes,” *Cell*, vol. 158, no. 1, pp. 41–53, Jul. 2014, doi: 10.1016/j.cell.2014.06.005.

[308] N. Gómez-Banoy *et al.*, “Adipsin preserves beta cells in diabetic mice and associates with protection from type 2 diabetes in humans,” *Nature Medicine*, vol. 25, no. 11, pp. 1739–1747, Nov. 2019, doi: 10.1038/s41591-019-0610-4.

[309] G. Ciprandi, M. de Amici, and G. Marseglia, “Serum adipsin levels in patients with seasonal allergic rhinitis: Preliminary data,” *International Immunopharmacology*, vol. 9, no. 12, pp. 1460–1463, Nov. 2009, doi: 10.1016/j.intimp.2009.08.004.

[310] G. G. Tafere, D. Z. Wondafrash, K. A. Zewdie, B. T. Assefa, and M. A. Ayza, “ Plasma Adipsin as a Biomarker and Its Implication in Type 2 Diabetes Mellitus ,” *Diabetes, Metabolic Syndrome and Obesity: Targets and Therapy*, vol. Volume 13, pp. 1855–1861, May 2020, doi: 10.2147/DMSO.S253967.

[311] P. M. Moraes-Vieira, A. Castoldi, P. Aryal, K. Wellenstein, O. D. Peroni, and B. B. Kahn, “Antigen Presentation and T-Cell Activation Are Critical for RBP4-Induced Insulin Resistance,” *Diabetes*, vol. 65, no. 5, pp. 1317–1327, May 2016, doi: 10.2337/db15-1696.