Paediatric Obesity, Inflammation & Asthma

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BNutrDiet(Hons)

A thesis submitted for the degree of Doctor of Philosophy

University of Newcastle, Australia

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Statement of Originality

This thesis contains no material which has been accepted for the award of any other degree or diploma in any university or tertiary institution and, to my knowledge and belief, contains no material previously published or written by another person, except where due reference has been given in text. I give consent to the final version of my thesis being made available worldwide when deposited in the University’s Digital Repository, subject to the provisions of the Copyright Act 1968.

Acknowledgement of Authorship

I hereby certify that the work embodied in this thesis contains published papers/scholarly work of which I am a joint author. I have included as part of the thesis a written statement, endorsed by my supervisor, attesting to my contribution to the joint publications/scholarly work.

............................................................

Megan E Jensen
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**Peer-reviewed Manuscripts**

   
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   **Statement of contribution:** Involved in the review concept design; review of the literature; manuscript preparation.

   
   **Statement of contribution:** Involved in generating the review question and the design; protocol development and preparation.

   
   **Statement of contribution:** Hypothesis generation; data search, entry and management; statistical analysis and interpretation; manuscript preparation.

   
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Statement of contribution: Involved in the literature search; critical analysis of literature; data extraction; manuscript preparation.


Statement of contribution: Design and implementation of the dietary intervention; data collection, entry and management; statistical analysis and interpretation; manuscript preparation.


Statement of contribution: Hypothesis generation; data collection, entry and management; statistical analysis and interpretation; manuscript preparation.

**Peer-Reviewed Abstracts**


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### Abbreviations

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
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<tbody>
<tr>
<td>ACQ</td>
<td>Asthma Control Questionnaire</td>
</tr>
<tr>
<td>AHR</td>
<td>Airway hyperresponsiveness</td>
</tr>
<tr>
<td>ANOVA</td>
<td>Analysis of variance</td>
</tr>
<tr>
<td>APARQ</td>
<td>Adolescent Physical Activity Questionnaire</td>
</tr>
<tr>
<td>ASAQ</td>
<td>Adolescent Sedentary Activity Questionnaire</td>
</tr>
<tr>
<td>BAL</td>
<td>Bronchoalveolar lavage</td>
</tr>
<tr>
<td>BHR</td>
<td>Bronchial hyperresponsiveness</td>
</tr>
<tr>
<td>BMI</td>
<td>Body mass index</td>
</tr>
<tr>
<td>CAMP</td>
<td>Childhood Asthma Management Program</td>
</tr>
<tr>
<td>CRP</td>
<td>C-Reactive Protein</td>
</tr>
<tr>
<td>CVD</td>
<td>Cardiovascular disease</td>
</tr>
<tr>
<td>DEXA</td>
<td>Dual-energy x-ray absorptiometry</td>
</tr>
<tr>
<td>eNO</td>
<td>Exhaled nitric oxide</td>
</tr>
<tr>
<td>ERV</td>
<td>Expiratory reserve volume</td>
</tr>
<tr>
<td>FEV₁</td>
<td>Forced expiratory volume in one second</td>
</tr>
<tr>
<td>FRC</td>
<td>Functional residual capacity</td>
</tr>
<tr>
<td>FCI-II</td>
<td>Food Cravings Index-II</td>
</tr>
<tr>
<td>FVC</td>
<td>Forced vital capacity</td>
</tr>
<tr>
<td>GINA</td>
<td>Global Initiative for Asthma</td>
</tr>
<tr>
<td>HDL</td>
<td>High density lipoprotein</td>
</tr>
<tr>
<td>HOMA-IR</td>
<td>Homeostasis model assessment of insulin resistance</td>
</tr>
<tr>
<td>ICS</td>
<td>Inhaled corticosteroid</td>
</tr>
<tr>
<td>IgE</td>
<td>Immunoglobulin E</td>
</tr>
<tr>
<td>IL</td>
<td>Interleukin</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Full Form</td>
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<tr>
<td>--------------</td>
<td>-----------</td>
</tr>
<tr>
<td>IQR</td>
<td>Interquartile range</td>
</tr>
<tr>
<td>LDL</td>
<td>Low density lipoprotein</td>
</tr>
<tr>
<td>LPS</td>
<td>Lipopolysaccharide</td>
</tr>
<tr>
<td>MCP</td>
<td>Monocyte chemotactic protein</td>
</tr>
<tr>
<td>MEF</td>
<td>Maximum expiratory flow</td>
</tr>
<tr>
<td>METS</td>
<td>Metabolic Equivalent</td>
</tr>
<tr>
<td>MUFA</td>
<td>Monounsaturated fatty acid</td>
</tr>
<tr>
<td>NF-kB</td>
<td>Nuclear factor-kappa B</td>
</tr>
<tr>
<td>PAI</td>
<td>Plasminogen activator inhibitor</td>
</tr>
<tr>
<td>PAQLQ</td>
<td>Pediatric Asthma Quality of Life Questionnaire</td>
</tr>
<tr>
<td>PEF</td>
<td>Peak expiratory flow</td>
</tr>
<tr>
<td>PD$_{15}$</td>
<td>Provocation dose required to induce a drop in FEV1 of 15%</td>
</tr>
<tr>
<td>PDSS</td>
<td>Pediatric Daytime Sleepiness Scale</td>
</tr>
<tr>
<td>PUFA</td>
<td>Polyunsaturated fatty acid</td>
</tr>
<tr>
<td>REM</td>
<td>Rapid eye movement</td>
</tr>
<tr>
<td>RV</td>
<td>Residual volume</td>
</tr>
<tr>
<td>SD</td>
<td>Standard deviation</td>
</tr>
<tr>
<td>SDS</td>
<td>Standard deviation score</td>
</tr>
<tr>
<td>Th</td>
<td>T-helper</td>
</tr>
<tr>
<td>TLC</td>
<td>Total lung capacity</td>
</tr>
<tr>
<td>TLR</td>
<td>Toll-like receptor</td>
</tr>
<tr>
<td>TNF-(\alpha)</td>
<td>Tumour necrosis factor-alpha</td>
</tr>
<tr>
<td>TST</td>
<td>Total sleep time</td>
</tr>
<tr>
<td>TTA</td>
<td>Total time awake</td>
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</table>
Synopsis

Obesity and asthma are the most common conditions affecting the paediatric population worldwide, with obesity being more prevalent in the population with asthma. Obesity in children with asthma is associated with increased asthma symptoms, increased number and severity of exacerbations, and increased use of medications, including inhaled corticosteroids. With the advent of obese asthma, occurring in parallel with Westernisation, the role of obesity and associated metabolic and lifestyle factors in the development and/or pathogenesis of asthma, and in asthma management, have been called into question. Although obese asthma has been described in the adult population as a distinct clinical phenotype, characterized by neutrophilic airway inflammation, reduced static lung function and corticosteroid resistance, there has been minimal research on obese asthma in the paediatric population.

The current thesis aims to characterise the inflammatory, physiological and clinical aspects of obese asthma in children; to understand the prevalence of risk factors for weight gain in children with asthma; and to investigate the feasibility and efficacy of dietary intervention to induce weight loss and improve asthma outcomes in paediatric obese asthma.

Chapter III presents the airway and systemic inflammatory profile, dynamic and static lung function, and clinical asthma outcomes in obese and non-obese children, with and without asthma. In this cross-sectional study, we found a poorer quality of life and reduced static lung function (expiratory reserve volume (ERV)) in obese asthmatic children. Sputum %eosinophils and the prevalence of eosinophilic asthma was lower in obese females compared to obese males, indicating that the female
gender may be associated with a different pattern of airway inflammation in obese asthma. This is important to asthma management and requires further investigation. However, the overall airway and systemic inflammatory profile did not differ between obese and non-obese asthmatic children.

In Chapter IV, the associations between lung function and body composition in children, with and without asthma, were explored. Body weight, fat mass and lean mass were inversely associated with static lung function (functional residual capacity (FRC) and ERV), suggesting that obesity, regardless of composition, is associated with reduced static lung function. Conversely, lean mass was positively associated with improvements in dynamic lung function. This study indicates that it is important to consider body composition as fat and lean mass, which both increase with obesity, may have differential effects on lung function. Chapter III and IV demonstrate that obesity is associated with lung deficits that are not detectable through routine spirometry. This suggests that in clinical practice static lung function needs to be routinely measured in obese asthmatic children.

In Chapter V, the presence of key modifiable risk factors for weight gain were compared in a cross-sectional study of non-obese children, with and without asthma, including sleep architecture, appetite and dietary intake, and physical and sedentary behaviour. Sleep latency was extended, and triglyceride levels were higher, in children with controlled asthma compared to non-asthmatic children. This study did not detect differences in plasma appetite hormone concentrations, food cravings, dietary intake or physical activity levels. However, in this group of asthmatic and non-asthmatic children, daytime sleepiness and reduced sleep duration were associated with adverse changes in plasma lipids, dietary patterns and sedentary
behaviour, which can potentially lead to positive energy balance and warrants further investigation.

In Chapter VI, the feasibility and efficacy of a ten week dietary intervention to induce acute weight loss in a group of obese children with asthma was demonstrated in a pilot randomised controlled trial. Dietary intervention induced statistically significant acute weight loss in asthmatic children, with improvements in asthma control and static lung function. This indicates that dietetic consultation is beneficial and should be integrated as part of the management of the obese child with asthma.

The research conducted as part of this thesis has contributed to the understanding of paediatric obese asthma; investigated the prevalence of key lifestyle risk factors for obesity in asthmatic and non-asthmatic children; and provided pilot data to support the efficacy of dietary-induced weight loss to improve asthma outcomes in obese asthmatic children.
1. Chapter I: Introduction

Excerpts of this chapter have been published:


1.1 Obesity in children and adolescents

1.1.1 Prevalence & Manifestation

Childhood overweight and obesity rates have been climbing globally for the past three decades\(^1\). Worldwide, an estimated 22 million children under 5 years of age\(^2\) and approximately 10% of children 5-17 years old are overweight or obese\(^3\), with this number increasing in Westernised countries to more than 30%\(^3,4\). The upward trend in childhood obesity rates has become apparent over the past 20-30 years\(^5\), with an additional 1% of children becoming overweight each year in some countries, such as Australia and Canada, during the 1990s\(^3\). Notably, epidemiological evidence suggests that in the past decade, the rise in overweight and obesity prevalence rates in children may have slowed and even reached a plateau in some countries\(^6,7\). However, the prevalence rates of overweight and obesity still remain alarmingly high in developed countries, including Australia. Approximately one in four Australian children, aged 2-16 years, are above the 85\(^{th}\) body mass index (BMI) percentile\(^6\), with obesity affecting approximately 6%\(^6\) of the paediatric population.

Obesity results from chronic excess energy intake in susceptible individuals\(^8\). It presents following persistent negative changes in lifestyle practices, energy expenditure and food consumption\(^9\). Indeed, the advent of Westernisation has seen large societal, cultural, and physical changes that have shaped the modern environment and potentially ‘primed’ susceptible individuals for excess weight gain. Such societal and environmental influences potentially surpass the innate control of appetite and dietary intake\(^3\). Both endogenous and exogenous factors, from conception through to adulthood, may increase the risk for overweight and obesity such as the intra-uterine environment, including the influence of maternal nutrition,
gestational diabetes and smoking\textsuperscript{10}; genetic predisposition/ heritability\textsuperscript{10} and gene-environment interactions\textsuperscript{3}; infant birth weight and growth\textsuperscript{10}, including the timing of adiposity rebound\textsuperscript{11}; dietary composition and portion sizes, within and outside the home\textsuperscript{3}; reduction in active transport and physical labour, within the home and work setting\textsuperscript{3}; the family environment, including socio-economic status, and parental activity and dietary habits\textsuperscript{3}; the psychological health of the child and family members\textsuperscript{3, 9}; parental obesity\textsuperscript{12}; infant-feeding\textsuperscript{10, 13} and child-rearing practices\textsuperscript{9}; disease states and certain medications\textsuperscript{3}; and additional lifestyle factors throughout childhood including sleep duration\textsuperscript{14}, eating patterns\textsuperscript{3}, and sedentary and physical activity patterns\textsuperscript{3}. There is no doubt that the origin of childhood obesity is as multi-faceted and complex as the factors that sustain and perpetuate excess weight gain in this population. This epidemic of childhood obesity has the potential to impact on the short-term and long-term health of the child, and eventually have adverse consequences for adult health.

1.1.2 Health impact

Emerging with the high prevalence of childhood obesity is the onset of chronic diseases, notably Type-II diabetes mellitus and cardiovascular disease (CVD), previously thought to be limited to the adult age group. Risk factors for CVD and diabetes reported in obese children\textsuperscript{3} include dyslipidemia\textsuperscript{15, 16}, elevated levels of homocysteine\textsuperscript{15}, C-reactive protein (CRP)\textsuperscript{16} and coagulation factors (plasminogen activating inhibitor (PAI)-1 and fibrinogen\textsuperscript{16}), impaired glucose tolerance\textsuperscript{9}, hyperinsulinemia and insulin resistance\textsuperscript{17}, and higher systolic and diastolic blood pressure\textsuperscript{16}. In addition, hypertrophy of the left ventricle\textsuperscript{3}, and advancing atherosclerosis\textsuperscript{3, 17} have been reported, demonstrating that cardiovascular damage can occur early in life.
However, the health consequences of obesity are not limited to cardiovascular and metabolic complications. Obesity is a systemic disease, affecting multiple systems and organs within the body. The obese child may present with a myriad of physical, psychological and social complaints: gynaecological problems, including early puberty, polycystic ovarian syndrome and dysmenorrhoea; renal and liver dysfunction, indicated by elevated urinary levels of serum proteins (albumin and beta-2-microglobulin\textsuperscript{9}) and uric acid levels\textsuperscript{16}, raised liver enzymes\textsuperscript{16} and non-alcoholic fatty liver disease\textsuperscript{3}; increased oxidative stress as measured by reduced activity of endogenous antioxidants, superoxide dismutase and glutathione peroxidase\textsuperscript{15}; respiratory complaints, including obesity hypoventilation syndrome, sleep-disordered breathing, and asthma-like symptoms e.g. dyspnoea\textsuperscript{3}; skeletal, muscular and orthopaedic issues\textsuperscript{17}; and severe psychosocial consequences including depression, poor self-esteem\textsuperscript{17} and poor quality of life\textsuperscript{18}. Unfortunately, these conditions can limit physical capacity and mobility, compounding their condition to the further detriment of their physical and psycho-social health. The statistics do not improve with aging, with at least 50% of obese children >13 years of age estimated to become obese adults\textsuperscript{9}. This highlights the myriad of disease that can be prevented if the progression of overweight and obesity is intervened at a young age. Obesity-related health effects are primarily related to the presence of excess adiposity, specifically associated with its function as an endocrine organ and involvement in the promotion of chronic low-grade systemic inflammation\textsuperscript{19}.

1.1.3 Adipose tissue dysfunction & systemic inflammation in obesity

The main cell in adipose tissue is the adipocyte, encompassed in a connective tissue matrix with pre-adipocytes, vascular and nerve tissue, fibroblasts and macrophages\textsuperscript{19, 20}. Endocrine, autocrine and paracrine signalling factors derived from tissue-resident
macrophages and adipocytes within adipose tissue, including non-esterified fatty acids, cytokines, acute phase proteins, hormones and alternative pathway of complement components, allow adipose tissue to communicate with other organs such as the pancreas, endothelium, hypothalamus, skeletal muscle, liver and the central nervous system\textsuperscript{20-22}. Receptors located in adipose tissue receive afferent signals from these organs\textsuperscript{20}. The role of adipose tissue as an endocrine organ is demonstrated through this communication network and involvement in many regulatory systems within the body including the pituitary-adrenal axis, pituitary-gonadal axis and the sympathetic nervous system\textsuperscript{19}. This function is essential to the main role of adipose tissue, which is the mobilisation and storage of energy in response to physiological needs of the individual\textsuperscript{19}.

However, with sustained positive energy balance, the number and volume of adipocytes increase to accommodate excess energy stores, and an influx of innate immune cells, predominantly macrophages, infiltrate adipose tissue at a rate proportional to the level of adiposity\textsuperscript{23}. Macrophages infiltrating adipose tissue in obesity are polarized towards the pro-inflammatory M1 (classically activated) phenotype, which increases the adipose tissue inflammatory process\textsuperscript{24}. Adipocytes possess many functions common to macrophages including direct release of cytokines, activation of the cytokine cascade, and release of acute phase proteins\textsuperscript{19}. Important mediators involved in the inflammation process produced and secreted by both macrophages and adipocytes in adipose tissue include cytokines, such as tumour necrosis factor-\(\alpha\) (TNF-\(\alpha\)) and interleukins (IL-1, IL-6, IL-10, IL-18); as well as endocrine hormones, such as resistin, leptin and adiponectin\textsuperscript{22, 25}. With increases in adiposity, there is an associated increase in the production of pro-inflammatory mediators\textsuperscript{25}, triggering an imbalance in important regulatory systems and creating a
pro-inflammatory environment\textsuperscript{20} (Figure 1.1). Furthermore, adipose-derived inflammatory mediators subsequently activate hepatocyte and liver macrophage production of inflammatory factors and acute phase proteins resulting in a secondary inflammatory response\textsuperscript{19}.

\textbf{Figure 1.1.} Adipose tissue is a source of inflammation.
With excessive increases in adipose tissue there is an increase in the unregulated production of pro-inflammatory mediators (including interleukin (IL)-6, leptin, C-Reactive Protein (CRP), and tumour necrosis factor (TNF)-\(\alpha\)) and a reduction in the anti-inflammatory adipokine, adiponectin. With a reduction in adiposity, there is a decrease in the dysregulated production of pro-inflammatory mediators.

Notably, fat storage is an important factor determining the metabolic activity of adipose tissue. Visceral adipose tissue has a greater macrophage infiltration\textsuperscript{23} and secretes an array of mediators into the hepatic portal vein\textsuperscript{26} as compared to subcutaneous adipose tissue, which may account for the increased metabolic activity of visceral fat and increased disease risk associated with visceral obesity\textsuperscript{26}.

In effect, the presence of excess adiposity provides a constant stimulus for chronic, low grade systemic inflammation. This adiposity-related inflammatory response may be implicated in the localised inflammation and dysfunction of peripheral organs and
tissues\textsuperscript{27}, thereby contributing to the pathogenesis of obesity-related chronic disease. Key inflammatory mediators produced and secreted by adipose tissue are highlighted below.

1.1.3.1 **Leptin**

Leptin is a 16kDa polypeptide that, under normal conditions, acts via the hypothalamus as an appetite suppressant and metabolic stimulant\textsuperscript{20, 28}. The amount of leptin secreted by adipose tissue is directly proportional to the level of adiposity\textsuperscript{20}. The levels of leptin also fluctuate in response to caloric intake, with circulating levels of insulin and glucose suggested as a signal for leptin production\textsuperscript{29}. The structure of leptin resembles that of the cytokine structure\textsuperscript{20} and allows leptin to function as a pro-inflammatory hormone, inducing innate inflammation via lipopolysaccharide (LPS) stimulation of signalling pathways in macrophages and monocytes that lead to the production of additional pro-inflammatory cytokines (IL-1, IL-6, TNF-\(\alpha\))\textsuperscript{19, 28}. This creates a pro-coagulant environment causing liver production of acute phase proteins\textsuperscript{19}. Interestingly, cross-sectional studies comparing obese children with age- and sex-matched non-obese children have consistently found significantly higher circulating leptin levels in obese children\textsuperscript{15, 16, 30-32} (Table 1.1a), suggesting leptin resistance occurs. In support of this leptin resistance theory, administration of exogenous leptin in obese patients does not ameliorate obesity\textsuperscript{20}.

1.1.3.2 **Adiponectin**

Adiponectin is a 30kDa bioactive polypeptide produced specifically by differentiated adipocytes that has anti-inflammatory effects upon endothelial cells, macrophages, and leukocytes\textsuperscript{28}. Adiponectin inhibits nuclear factor (NF)-kB, IL-6 and TNF-\(\alpha\) production and up-regulates the production of IL-10 and IL-1 receptor antagonists\textsuperscript{22}.
Table 1.1a: Cross-sectional studies in children that have measured systemic inflammation
(modified from Jensen et al\textsuperscript{33})

<table>
<thead>
<tr>
<th>Inflammatory Marker</th>
<th>Study Design</th>
<th>Participants</th>
<th>Sample size</th>
<th>Difference in inflammatory marker</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leptin</td>
<td>Cross- sectional\textsuperscript{15}</td>
<td>Obese and non-obese children, aged 7-10 years. Obesity defined as BMI SDS &gt;3 (Turkish reference population)</td>
<td>O=60; NO=60</td>
<td>↑ leptin in O vs NO.</td>
</tr>
<tr>
<td></td>
<td>Case-control \textsuperscript{16}</td>
<td>Obese and non-obese children, aged 6-9 years. Obesity defined as &gt;90th BMI percentile for reference population</td>
<td>O=51; NO=51</td>
<td>↑ leptin in O vs NO. Leptin an independent predictor of BMI in O.</td>
</tr>
<tr>
<td></td>
<td>Case-control \textsuperscript{30}</td>
<td>Obese and non-obese children, aged 7-10 years. Obesity defined as 120% mean body weight for Turkish reference population and when BMI for age &gt;99h%</td>
<td>O=63; NO=63</td>
<td>↑ leptin in O vs NO. Leptin correlated with BMI in obese.</td>
</tr>
<tr>
<td></td>
<td>Case-control\textsuperscript{12}</td>
<td>Obese and healthy-weight children with mean age 10 years. Obesity defined as BMI &gt;95th percentile for reference population</td>
<td>O=45; NO=40</td>
<td>↑ leptin in O vs HW.</td>
</tr>
<tr>
<td></td>
<td>Cross-sectional\textsuperscript{31}</td>
<td>Obese and non-obese children, with mean age 10.9 years. Obesity defined as &gt;97th BMI percentile (WHO standards). NO defined as &lt;90th BMI percentile</td>
<td>O=67; NO=62</td>
<td>↑ leptin in O vs NO.</td>
</tr>
<tr>
<td>Adiponectin</td>
<td>Case-control \textsuperscript{16}</td>
<td>Obese and non-obese children aged, 6-9 years. Obesity defined as&gt;90th BMI percentile for reference population</td>
<td>O=51; NO=51</td>
<td>↓ adiponectin in O vs NO. Adiponectin correlated negatively with BMI.</td>
</tr>
<tr>
<td></td>
<td>Case-control \textsuperscript{34}</td>
<td>Obese and non-obese children, aged 8-15 years. Obesity defined as ≥95th percentile (reference population not specified)</td>
<td>O=73; NO=30</td>
<td>↓ adiponectin in O vs NO. Adiponectin negatively correlated with BMI</td>
</tr>
<tr>
<td></td>
<td>Cross-sectional \textsuperscript{35}</td>
<td>Obese and healthy weight children, aged 14-18 years. Obesity defined as BMI &gt;30</td>
<td>HW=6; O=15</td>
<td>↓ adiponectin levels in O vs HW. BMI, %body fat &amp; trunk fat mass negatively correlated with adiponectin levels; %lean mass positively associated with adiponectin.</td>
</tr>
</tbody>
</table>

BMI body mass index; CRP C-Reactive Protein; HW Healthy Weight; O Obese; OW Overweight; NO Non-Obese; SDS standard deviation score.

Adiponectin reduces smooth muscle cell proliferation, macrophage transformation to foam cells, and monocyte adhesion in the vascular wall, and stimulates angiogenesis and nitric oxide production in the endothelium\textsuperscript{20}. Adiponectin also reduces gluconeogenesis, increases fatty acid oxidation and glucose uptake in the liver and skeletal muscle, and directly improves insulin sensitivity\textsuperscript{20, 28, 36}. Cross-sectional studies have found that adiponectin levels are significantly lower in obese children.
compared to non-obese children\textsuperscript{16, 34, 35} and correlate negatively with adiposity indices\textsuperscript{16, 34, 35, 37} (Table 1.1a). Lower adiponectin levels have also been associated with increased CRP levels in obese children and adolescents compared to non-obese participants\textsuperscript{38}.

1.1.3.3 \textit{C-Reactive Protein}

C-Reactive protein (CRP) is a classic general marker of both low-grade and acute inflammation\textsuperscript{39}. Inflammation, infection or tissue damage stimulate hepatocytes to rapidly produce CRP in response to cytokine stimulation, specifically IL-1 and IL-6, and NF-kB\textsuperscript{39-41}. CRP in turn activates NF-kB signalling, complement & tissue factor, and the production of cytokines and chemokines which subsequently produce more CRP, creating a positive feedback loop\textsuperscript{19}. Studies have identified a positive relationship between BMI and CRP with significantly higher CRP levels consistently documented in obese children and adolescents compared to non-obese controls\textsuperscript{16, 31, 34, 40, 42-45} (Table 1.1b). Furthermore, CRP levels reportedly increase in parallel with adiposity and insulin resistance measures, with waist circumference identified as an independent predictor of CRP levels in children\textsuperscript{38}. In fact, an increase in BMI of one standard deviation is reportedly associated with an increase in CRP of approximately 50% in children and adolescents\textsuperscript{46}. Notably, a cross-sectional study in 228 Canadian children aged 10-19 years old, found approximately 16% of the population had CRP levels between 3-10mg/l, which according to their national guidelines indicates a high CVD risk\textsuperscript{38}. Elevations in CRP was associated with increasing obesity, as indicated by BMI and \%body fat\textsuperscript{38}. 
Table 1.1b: Cross-sectional studies in children that have measured systemic inflammation
(modified from Jensen et al\textsuperscript{33})

<table>
<thead>
<tr>
<th>Inflammatory Marker</th>
<th>Study Design</th>
<th>Participants</th>
<th>Sample size</th>
<th>Difference in inflammatory marker</th>
</tr>
</thead>
<tbody>
<tr>
<td>CRP</td>
<td>Cross-sectional \textsuperscript{40}</td>
<td>Obese and non-obese children, aged 2.3–19 years. Obesity defined as &gt;95\textsuperscript{th} BMI percentiles (NCHS 2000 reference values).</td>
<td>O=131; NO=114</td>
<td>↑ CRP in O vs NO. Positive association between CRP and BMI.</td>
</tr>
<tr>
<td></td>
<td>Cross-sectional \textsuperscript{47}</td>
<td>Representative sample of US children (NHANES III), aged 8–16 years. Overweight defined as &gt;85\textsuperscript{th} BMI percentile or sum of 3 skinfolds for reference population (NHANES III)</td>
<td>n=3512</td>
<td>OW more likely to have ↑ CRP (OR 3.17 girls and 3.74 boys based on BMI; OR 2.89 girls and 5.11 boys based on skinfolds)</td>
</tr>
<tr>
<td></td>
<td>Cross-sectional \textsuperscript{42}</td>
<td>Healthy weight and obese children, aged 15–16 years. Obesity defined as BMI &gt;30</td>
<td>HW=6; O=15</td>
<td>↑ CRP in O vs HW. CRP positively correlated with BMI and %body fat.</td>
</tr>
<tr>
<td></td>
<td>Cross-sectional \textsuperscript{43}</td>
<td>Obese and non-obese children, with mean age 11 years. Obesity defined as ≥97\textsuperscript{th} BMI percentile based on population-specific data</td>
<td>NO=14; O=31</td>
<td>↑ CRP in O vs NO. CRP correlated with SDS-BMI.</td>
</tr>
<tr>
<td></td>
<td>Case-control \textsuperscript{16}</td>
<td>Obese and non-obese children aged, 6–9 years. Obesity defined as &gt;90\textsuperscript{th} BMI percentile for reference population</td>
<td>O=51; NO=51</td>
<td>↑ CRP in O vs NO. CRP positively correlated with BMI.</td>
</tr>
<tr>
<td></td>
<td>Case-control \textsuperscript{34}</td>
<td>Obese and non-obese children, aged 8–15 years. Obesity defined as ≥95\textsuperscript{th} percentile (reference population not specified)</td>
<td>O=73; NO=30</td>
<td>↑ CRP in O vs NO. CRP positively correlated with BMI.</td>
</tr>
<tr>
<td></td>
<td>Cross-sectional \textsuperscript{15}</td>
<td>Obese and healthy weight children, aged 12-15 years. Obesity defined according to the International Obesity Task Force.</td>
<td>O=51; HW=30</td>
<td>↑ CRP in O vs HW. CRP positively correlated with BMI z-score.</td>
</tr>
<tr>
<td></td>
<td>Cross-sectional \textsuperscript{44}</td>
<td>Obese and non-obese children, aged 5–17 years. Obesity defined as BMI z-score ≥1.64.</td>
<td>O=38; NO=113</td>
<td>↑ CRP in O vs NO.</td>
</tr>
<tr>
<td></td>
<td>Cross-sectional \textsuperscript{31}</td>
<td>Obese and non-obese children, with mean age 10.9 years. Obesity defined as &gt;97\textsuperscript{th} BMI percentile (WHO standards). NO defined as &lt;90\textsuperscript{th} BMI percentile</td>
<td>O=67; NO=62</td>
<td>↑ CRP in O vs NO.</td>
</tr>
</tbody>
</table>

BMI body mass index; CRP C-Reactive Protein; HW Healthy Weight; O Obese; OW Overweight; NO Non-Obese; SDS standard deviation score.
### Table 1.1c: Cross-sectional studies in children that have measured systemic inflammation (modified from Jensen et al\(^33\))

<table>
<thead>
<tr>
<th>Inflammatory Marker</th>
<th>Study Design</th>
<th>Participants</th>
<th>Sample size</th>
<th>Difference in inflammatory marker</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL-6</td>
<td>Case-control</td>
<td>Obese and non-obese children, aged 6-9 years. Obesity defined as &gt;90(^\text{th}) BMI percentile for reference population</td>
<td>O=51; NO=51</td>
<td>IL-6: No difference between O vs NO.</td>
</tr>
<tr>
<td></td>
<td>Cross-sectional</td>
<td>Healthy weight and obese children, aged 15-16 years. Obesity defined as BMI &gt;30</td>
<td>HW=6; O=15</td>
<td>↑ IL-6 in O vs HW. IL-6 positively correlated with BMI &amp; %body fat.</td>
</tr>
<tr>
<td></td>
<td>Cross-sectional</td>
<td>Healthy weight and obese children, aged 10-13 years. Obesity defined as BMI &gt;85(^\text{th}) percentile for age and sex (NHANES I data)</td>
<td>O=49; HW=69</td>
<td>↑ IL-6 in O vs HW. No correlation between IL-6 and BMI or %body fat.</td>
</tr>
<tr>
<td></td>
<td>Case-control</td>
<td>Obese and non-obese children, aged 7-10 years. Obesity defined as 120% mean body weight for Turkish reference population and when BMI for age &gt;99(^\text{th}) percentile</td>
<td>O=63; NO=63</td>
<td>↑ IL-6 in O vs NO.</td>
</tr>
<tr>
<td></td>
<td>Cross-sectional</td>
<td>Obese and healthy weight children, aged 12-15 years. Obesity defined according to the International Obesity Task Force.</td>
<td>O=51; HW=30</td>
<td>↑ IL-6 in O vs HW.</td>
</tr>
<tr>
<td></td>
<td>Cross-sectional</td>
<td>Obese and non-obese children, with mean age 10.9 years. Obesity defined as &gt;97(^\text{th}) BMI percentile (WHO standards). NO defined as &lt;90(^\text{th}) BMI percentile</td>
<td>O=67; NO=62</td>
<td>IL-6: No difference between O vs NO.</td>
</tr>
<tr>
<td></td>
<td>Cross-sectional</td>
<td>Obese and non-obese children, with mean age 11 years. Obesity defined as ≥97(^\text{th}) BMI percentile based on population-specific data</td>
<td>NO=14; O=31</td>
<td>↑ TNF-(\alpha) in O vs NO. TNF-(\alpha) correlated with SDS-BMI.</td>
</tr>
<tr>
<td></td>
<td>Case-control</td>
<td>Obese and non-obese children, aged 8-15 years. Obesity defined as ≥95(^\text{th}) percentile (reference population not specified)</td>
<td>O=73; NO=30</td>
<td>↑ TNF-(\alpha) in O group vs NO. TNF-(\alpha) positively correlated with BMI.</td>
</tr>
<tr>
<td></td>
<td>Case-control</td>
<td>Obese and non-obese children, aged 7-10 years. Obesity defined as 120% mean body weight for Turkish reference population and when BMI for age &gt;99(^\text{th}) percentile</td>
<td>O=63; NO=63</td>
<td>↑ TNF-(\alpha) in O vs NO</td>
</tr>
<tr>
<td></td>
<td>Case-control</td>
<td>Obese and healthy-weight children with mean age 10 years. Obesity defined as BMI &gt;95(^\text{th}) percentile for reference population</td>
<td>O=45; NO=40</td>
<td>↑ TNF-(\alpha) in O vs HW.</td>
</tr>
<tr>
<td></td>
<td>Cross-sectional</td>
<td>Obese and healthy weight children, aged 12-15 years. Obesity defined according to the International Obesity Task Force.</td>
<td>O=51; HW=30</td>
<td>↑ TNF-(\alpha) in O vs HW.</td>
</tr>
</tbody>
</table>

BMI body mass index; HW Healthy Weight; IL-6 interleukin-6; O Obese; OW Overweight; NO Non-Obese; SDS standard deviation score; TNF-\(\alpha\) tumour necrosis factor-\(\alpha\).
1.1.3.4 Interleukin-6

Interleukin (IL)-6 is a pro-inflammatory cytokine secreted by adipocytes and the adipose tissue matrix and circulates in glycosylated form\(^20\). The IL-6 receptor is identical to the leptin receptor and is expressed by adipocytes and the adipose tissue matrix\(^20\). IL-6 contributes to inflammation by raising CRP levels and suppressing adiponectin production\(^20\). Multiple cross-sectional studies have identified significantly elevated levels of IL-6 in obese compared to non-obese children\(^30, 42, 45, 48\), while two studies failed to identify a significant difference in IL-6 levels between these two groups\(^16, 31\) (Table 1.1c). Interestingly, one paediatric study demonstrated that IL-6 levels are positively associated with adipocyte diameter but not with percentage fat or BMI\(^49\), suggesting that adipocyte hypertrophy may be more important to the metabolic consequences of adipose tissue.

1.1.3.5 Tumour Necrosis Factor-alpha

Tumour necrosis factor (TNF)-α is a transmembrane protein identical to cachexin and produced by stromovascular cells and adipocytes\(^20\). The 17kDa biologically active form of TNF-α stimulates calcium signalling causing increased muscle contraction and causes peripheral muscle catabolism via Type I and II receptors\(^20, 28\). TNF-α receptors are also expressed by adipocytes and enable TNF-α to affect gene expression in adipose tissue as well as other metabolically active tissues\(^20\). It is via this mechanism that TNF-α influences the expression of adiponectin, IL-6 and other adipocyte derived products\(^20\). TNF-α contributes to a proinflammatory environment by stimulating NF-kB signalling and increasing production of acute phase proteins and cytokines\(^19, 28\). Chronically elevated TNF-α levels impair the anorexigenic effects of leptin and insulin\(^50\), perhaps suggesting increased TNF-α levels may contribute to the apparent leptin resistance in the obese population. Levels of TNF-α have been
found to be significantly higher in obese children and adolescents compared to non-obese subjects\textsuperscript{30, 32, 34, 43, 45} (Table 1.1c). Interestingly, a positive correlation between adipocyte diameter and TNF-α levels is documented, although BMI and fat mass have failed to correlate with TNF-α levels\textsuperscript{49}, again suggesting that adipocyte hypertrophy may be more important to the metabolic consequences of adipose tissue.

1.2 Asthma in children and adolescents

1.2.1 Prevalence and manifestation

Asthma is a heterogeneous chronic inflammatory airway disease which involves activation of the immune system by specific stimuli, manifesting with airway hyper-reactivity, variable airflow obstruction (with expiratory airflow limitation), and airway inflammation\textsuperscript{51, 52}. Asthma manifests with clinical features of shortness of breath, chest tightness, sputum production, wheeze and coughing, particularly in the evening and early hours of the morning.

Asthma has become increasingly prevalent worldwide over the past few decades in both children and adults, paralleling atopy, obesity and Westernisation\textsuperscript{53}. Asthma is estimated to affect up to 18% of the world’s population\textsuperscript{54}. While this trend may have reached a plateau or even slightly reversed in Australian children in recent years\textsuperscript{55}, asthma remains the most common chronic condition impacting Australian children, affecting approximately one in ten children under 15 years of age\textsuperscript{56}. Notably, asthma-related emergency department presentations, hospital admission rates and hospital separation rates in Australia, are highest amongst this age group\textsuperscript{56}. Asthma accounts for over $600 million of the Australian health care expenditure, with prescription medications accountable for over half of asthma-related costs\textsuperscript{56}. Furthermore, quality of life and social health has been rated more poorly in asthma sufferers\textsuperscript{56}.
The origins of asthma are complex and not completely elucidated. Genetic predisposition, occupational exposures, viral infections, aero-allergens, pollution, smoking, dietary components and obesity, have been implicated in the development and pathogenesis of asthma, and may contribute to increased symptoms in susceptible individuals\textsuperscript{54, 57}. Fundamental to the pathogenesis of asthma is altered airway inflammation.

1.2.2 Airway inflammation in asthma

Airway inflammation occurs in both acute and chronic states of asthma\textsuperscript{58}. The inflammation is generally localised to the conducting airways but, with increasing severity and chronicity, spreads to the smaller airways\textsuperscript{59}. Increased airway inflammation with eosinophils is associated with a poorer asthma prognosis in children, as indicated by increased asthma severity, nocturnal symptoms, wheezing and airflow obstruction\textsuperscript{51}. Altered airway inflammation has been reported in infants exhibiting wheezy symptoms, with a higher neutrophil count and lower lymphocyte count, compared to infants without wheeze\textsuperscript{60}. Chronic inflammation can lead to localised tissue injury and subsequent structural changes in the airways, termed remodelling\textsuperscript{58}.

Pathological changes in asthmatic airways are evidenced by hyperplasia and hypertrophy of smooth muscle cells, hyperplasia and metaplasia of mucous glands, subepithelial fibrosis, thickening of the airway wall and increased vascularisation\textsuperscript{58}. Airway smooth muscle in asthma has been reported to play a role in cytokine, chemokine and growth factor production\textsuperscript{58}. Mucus hypersecretion, oedema and sensitisation of the conducting airways occurs in conjunction\textsuperscript{59}, resulting in respiratory symptoms of wheeze, shortness of breath, chest tightness, and cough. Changes in airway pathology are suggested to underlie the deterioration in lung
function of subjects with asthma, whom are non-responsive to corticosteroid treatment.\textsuperscript{58}

Airway inflammation in asthma may arise via the acquired immune system, mediated via a T-helper (Th)2 pathway, with the key defining feature being the dominating presence of airway eosinophils.\textsuperscript{59} This sub-type of asthma is associated with allergic sensitisation and is termed atopic asthma or eosinophilic asthma. Asthma may also present via the innate immune system, mediated via a Th1 pathway and characterised by increased levels of airway neutrophils in the absence of eosinophilia.\textsuperscript{61,62} This sub-type of asthma is typically present in the absence of atopy and is termed non-atopic asthma or neutrophilic asthma. The Th1 and Th2 cells involved in asthma pathogenesis differentiate from a common ancestor in response to cues from both environmental triggers and immune regulatory cells.\textsuperscript{58} However, eosinophilic and neutrophilic asthma are activated by different stimuli and function via different pathways (Figure 1.2).

\textbf{Figure 1.2} Eosinophilic and neutrophilic asthma are activated by different stimuli and operate via different inflammatory pathways.

(Figure from Simpson et al\textsuperscript{63})
Eosinophilic asthma is associated with an allergic response to specific environmental allergens which trigger the acquired immune system upon inhalation (predominantly Immunoglobulin (Ig)E mediated)\textsuperscript{59}. The inflammatory pathway is primarily driven by IL-5\textsuperscript{59}. The Th2 cell infiltration leads to the release of Th2 cytokines (IL-4, IL-13, IL-5, IL-9) that are responsible for the activation and localisation of inflammatory cells in the airways (eosinophils, macrophages and basophils); the stimulation of IgE release from B-cells; and the release of chemokines, cell adhesion molecules, metalloproteases, and nitric oxide which leads to the degradation of the airway epithelium\textsuperscript{58-60}. Eosinophils contribute to structural changes in the airways through the production of TGF-β1 and encourage fibroblast proliferation, collagen synthesis and myofibroblast growth\textsuperscript{59}. Generally, this subtype of asthma responds to corticosteroids\textsuperscript{58}.

Although the Th2 immune response typically predominates in mild-moderate asthma\textsuperscript{59} and reportedly underlies the development of asthma in children with wheeze\textsuperscript{60}, increased recruitment of Th1 cells occurs with increasing chronicity and severity\textsuperscript{59}. Indeed, the sputum from children during acute asthma exacerbations has approximately 10 times higher counts of eosinophils, neutrophils and mast cells compared to sputum samples from children with stable asthma\textsuperscript{64}. One third to one half of asthma presentations have been attributed to non-eosinophilic inflammation, associated with raised neutrophils and elevated IL-8 levels\textsuperscript{52, 61, 62}. Neutrophilic asthma is associated with innate immune activation by various stimuli, including viruses, bacteria, pollutants, tobacco smoke and particular dietary components, which bind to specific innate immune receptors (toll-like receptors and nod-like receptors) in the airways and trigger the NF-kB inflammatory pathway\textsuperscript{61, 62, 65-67}. Importantly, this type of asthma has also been associated with obesity\textsuperscript{57}. The inflammatory response in
neutrophilic asthma is predominantly driven by IL-8. A subsequent influx of neutrophils causes a further increase in Th1 cytokines, including IL-1, IL-6, IL-8, and TNF-α. Notably, neutrophilic asthma appears unresponsive to corticosteroid therapy.

1.3 Obese Asthma in Children & Adolescents

Asthma and obesity are highly prevalent in children and adolescents, being the most common chronic morbidities in children from economically developed regions. The prevalence of overweight and obesity is higher in children with asthma compared to children without asthma, potentially affecting more than one third of asthmatic children, with this trend being reported in America, Australia and Denmark. Likewise, the prevalence of asthma has also been reported to be significantly greater in obese and morbidly obese children compared to those of a healthy weight. In fact, obesity has been indicated as a significant risk factor for asthma in adults and children.

Evidence from studies published over the past two decades support an association between asthma and obesity in children, adolescents and adults. Importantly, most longitudinal studies demonstrate that excess weight gain precedes the development of asthma and onset of respiratory symptoms in children. A meta-analysis identified a respective 20% and 50% increased risk for asthma in infants with a higher birth weight and in school aged children with a higher BMI. In contrast, data from the Longitudinal Study of Australian Children suggests that asthma precedes the development of obesity. This study reported children aged 4-5 years suffering from wheeze or asthma, regardless of their baseline weight status, to be more likely to become overweight or obese at age 6-7 years, compared to non-asthmatic children.

Gender differences in asthma risk have been noted with some studies only finding an
increased risk or stronger association for females\textsuperscript{80-82}, while others have reported stronger associations between being overweight and risk of asthma onset in male children\textsuperscript{83}. A cross-sectional study of 20,000 6-7 year old Italian children found that children in the highest BMI quintile compared to children in the lowest BMI quintile had a significantly increased risk of new asthma diagnosis and wheeze\textsuperscript{82}. This association was stronger for females than for males\textsuperscript{82}. The likelihood of 11-13 year old females developing asthma symptoms and bronchial hyper-responsiveness is reportedly 5.5-7 fold higher in females who became overweight between the ages of 6-11 years\textsuperscript{80}. Likewise, non-asthmatic female children with a higher initial BMI and non-asthmatic female children who had the greatest increases in annual BMI z-score during a 5 year study, had a higher risk of asthma with persistent wheeze\textsuperscript{81}. On the other hand, males that exhibited the smallest and greatest increases in BMI z-score both had an increased risk of asthma\textsuperscript{81}, possibly highlighting the inadequacy of BMI/BMI z-score as an indicator of adiposity. Likewise, longitudinal analysis of annual data from The Children’s Health Study (1993-98) identified an increased relative risk of new asthma diagnosis in children who were overweight and obese, with this being stronger in males than females\textsuperscript{83}.

Evidence in the adult population suggests that obesity is associated specifically with non-atopic asthma, with two studies reporting that the association between asthma and obesity only occurs in non-atopic disease\textsuperscript{84, 85}. Likewise, large epidemiological studies have shown no relationship between obesity and atopy\textsuperscript{86, 87}. In the European Respiratory Community Health Survey, no association was found between BMI and total or specific IgE levels\textsuperscript{87}. This data supports the hypothesis that obesity promotes the development of a Th1-mediated non-atopic form of asthma in adults. A recent report in children found no association between BMI and atopy\textsuperscript{77}, while a second
study reported overweight children were more likely to be atopic compared to their healthy weight peers\textsuperscript{88}. Although the association between obesity and asthma may exist in both atopic and non-atopic children, evidence suggests it is stronger in the absence of atopy\textsuperscript{78, 83}. In the National Health and Nutrition Examination Study (NHANES III), an independent relationship between BMI and atopy or systemic eosinophil counts in children was not identified, with authors surmising that adiposity may negatively impact clinical asthma outcomes via non-atopic inflammatory pathways\textsuperscript{86}.

The high prevalence of the obese subject with asthma has triggered the study of the ‘obese phenotype’ in asthma. Given the ongoing rise in global childhood obesity rates, the presentation of the obese asthmatic child will likely become more common. Therefore, it is essential that we begin to understand the mechanisms and manifestations of asthma in this subgroup of children to appropriately inform clinical management.

### 1.4 Manifestations of the Obese Phenotype in Asthma

A recent cluster analysis was used to identify distinct asthma phenotypes in adults, which exhibit different clinical responses to treatment\textsuperscript{89}. One of the four key clusters identified was obese, non-eosinophilic asthma, characterised by symptoms in the absence of airway eosinophils\textsuperscript{89}. A recent study also conducted an unsupervised cluster analysis of mild-moderate asthmatics and revealed the ‘persistently non-eosinophilic’ group to be characterised by higher BMI, the absence of atopy, and unresponsive to corticosteroid therapy\textsuperscript{90}.

Although study of the obese phenotype in asthma has gained momentum in recent years and has been characterised in adults\textsuperscript{89-91}, its clinical presentation in childhood
has not been thoroughly investigated and the pathogenesis of ‘obese asthma’ in children is unknown. The manifestations, mechanisms and management of both obesity and asthma differ through the life stages. Importantly, the pathogenesis and presentation of obese asthma in children is likely to differ from that of adults (Table 1.2). The clinical, physiological and pathological manifestations of asthma in the obese population are discussed in this section.

Table 1.2: Manifestations of obese asthma in children and adults
(modified from Jensen et al⁹²)

<table>
<thead>
<tr>
<th>Manifestations</th>
<th>Child</th>
<th>Adult</th>
</tr>
</thead>
<tbody>
<tr>
<td>Airway Inflammation:⁸⁹, ⁹¹, ⁹³-⁹⁵</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>- Exhaled nitric oxide</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>- Sputum cell counts</td>
<td>?</td>
<td>↑ (neutrophilic)</td>
</tr>
<tr>
<td>Systemic Inflammation:⁹¹, ⁹⁴, ⁹⁶-¹⁰¹</td>
<td>—</td>
<td>↑</td>
</tr>
<tr>
<td>- Leptin</td>
<td>—</td>
<td>↑</td>
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<tr>
<td>- Adiponectin</td>
<td>↓</td>
<td>↓</td>
</tr>
<tr>
<td>- Interleukin (IL)-6</td>
<td>—</td>
<td>↑</td>
</tr>
<tr>
<td>- C- Reactive Protein (CRP)</td>
<td>—</td>
<td>↑</td>
</tr>
<tr>
<td>- Tumour Necrosis Factor (TNF)-α</td>
<td>—</td>
<td>↑</td>
</tr>
<tr>
<td>Steroid response¹⁰²-¹⁰⁵</td>
<td>↓</td>
<td>↓</td>
</tr>
<tr>
<td>Bronchial hyperresponsiveness:¹⁸, ⁹³, ⁹⁴, ⁹⁶, ¹⁰⁶, ¹⁰⁷</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>- Prevalence</td>
<td>— / ↑</td>
<td>— / ↑</td>
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<tr>
<td>- Severity</td>
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<td>Severity:</td>
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<tr>
<td>- Symptoms</td>
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<tr>
<td>- Health care usage</td>
<td>— / ↑</td>
<td>↑</td>
</tr>
<tr>
<td>- Medication use</td>
<td>↑</td>
<td>↑</td>
</tr>
<tr>
<td>Lung function:¹⁶, ⁹⁶, ¹¹²-¹¹⁵</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>- Obstruction</td>
<td>— / ↑</td>
<td>↑</td>
</tr>
<tr>
<td>- Restriction</td>
<td>↑</td>
<td>↑</td>
</tr>
<tr>
<td>Quality of life¹¹⁸, ⁹⁶</td>
<td>— / ↓</td>
<td>↓</td>
</tr>
</tbody>
</table>

— No difference, ↑ increased in obese asthma, ↓ decreased in obese asthma; ? data not yet measured
1.4.1 Symptoms, exacerbations and Quality of Life

The burden of obesity in childhood asthma has been highlighted in previous studies which have reported an increased frequency of bronchial hyper-responsiveness, an increased number of prescribed medications and inhaled corticosteroid (ICS) use, an increased number of school days missed, and a significantly reduced quality of life associated with overweight and obesity in asthmatic children\textsuperscript{18, 92, 110, 111}. Analysis of The National Study of Health and Growth data identified a higher prevalence of asthma attacks in obese children compared to children of normal weight from as early as 1982-94\textsuperscript{116}. A higher number of hospital and emergency department admissions, a significantly longer length of stay in hospital, including in an intensive care unit, and extended use of steroid medications, albuterol and oxygen supplementation following an acute asthma exacerbation, has also been reported in obese compared to non-obese children with asthma\textsuperscript{111, 117}. Two recent analyses of large cohorts reported that increased BMI and/or adiposity in children was associated with increased asthma symptoms, asthma exacerbations and emergency department presentations\textsuperscript{78, 108}, while a contrasting report found no significant difference in baseline quality of life, asthma severity classification, or prospective health care usage between healthy weight, overweight, or obese children\textsuperscript{68}. Clinically and statistically significant reductions in quality of life, have also been reported in overweight asthmatic children compared to healthy weight asthmatic and non-asthmatic children, and compared to overweight non-asthmatic children\textsuperscript{18}. Interestingly, this study demonstrated that obesity did not affect quality of life in children without asthma but significantly reduced the quality of life in children with asthma.

Although it has been suggested that obese children have an increased perception of respiratory symptoms\textsuperscript{117} and that the diagnosis of ‘pseudo-asthma’ may be more
prevalent in the obese population, objective measures indicate that clinical asthma outcomes are worsened in children who are obese\textsuperscript{118}. A large study in children and adolescents has provided evidence that asthma is highly prevalent in obese children, as diagnosed by both general physicians, respiratory specialists and objective criterion\textsuperscript{118}. In fact, the diagnosis of ‘pseudo-asthma’ was lower in the obese children.

1.4.2 Lung function and airway hyper-responsiveness

Obesity adversely affects dynamic and static lung function in adults, both with and without asthma. The greatest reductions in adults are seen in expiratory reserve volume (ERV) and functional residual capacity (FRC)\textsuperscript{96, 112}. However, in the obese child, ventilatory function appears to be largely unaffected. A recent large prospective trial which enrolled children and adults with asthma, reported significantly decreased baseline forced vital capacity (FVC) in obese adults which was not present in obese children\textsuperscript{68}. In fact, multiple studies have reported no difference in baseline forced expiratory volume in 1 second (FEV\textsubscript{1}) or FVC between healthy weight, overweight and obese asthmatic children\textsuperscript{68, 88, 93, 94, 102}. However, reduced peak expiratory flow (PEF) in overweight and obese asthmatic children compared to non-overweight asthmatic children has been reported\textsuperscript{18, 110}. More recently, a post-hoc analysis of data from the Childhood Asthma Management Program (CAMP) found a significantly reduced FEV\textsubscript{1}/ FVC, a marker of airway obstruction, in children \(\geq 85^{th}\) BMI percentile compared to healthy weight children\textsuperscript{102}. This was supported by similar findings in Korean adolescent males\textsuperscript{88}. Likewise, in a large sample of predominantly non-Caucasian urban American youths, an inverse relationship between FEV\textsubscript{1}/ FVC and both percentage body fat and BMI was reported\textsuperscript{108}. Conversely, Peters et al recently reported the proportion of asthmatic children with severe obstruction, as indicated by a FEV\textsubscript{1}/ FVC <70%, to be similar between normal weight, overweight and obese
children\textsuperscript{68}. Given that the greatest deficits in lung function with adult obesity are seen in ERV, it would indicate that lung volume assessment is needed in future studies of childhood obesity. Lung volume measurements have only recently been reported in two studies which found that RV and FRC, expressed as a function of %predicted or TLC, is significantly lower in overweight and obese children compared to non-overweight children with asthma, while ERV was not reported\textsuperscript{114,115}.

It is unclear whether the prevalence or severity of bronchial hyperresponsiveness is greater in obese children. Two recent studies did not detect a significant difference in the prevalence of airway hyper-responsiveness (AHR) between overweight/obese and normal weight asthmatic children\textsuperscript{93,94}. Although, another study did find AHR to be significantly higher in overweight asthmatic females\textsuperscript{88}. In a cross-sectional study, obese asthmatic children had the greatest FEV\textsubscript{1} reduction post-exercise and a greater AUC\textsubscript{20} (area under the FEV\textsubscript{1} time curve over a 20 mins post-exercise time period, expressed as a percentage change from baseline x minutes) compared to healthy weight asthmatic children, indicating that although bronchoconstriction in asthmatic children may not be more prevalent, it may be more severe, in those who are obese\textsuperscript{94}. A similar study found obese children, both with and without asthma, had significantly lower post-exercise MEF\textsubscript{75} compared to healthy controls\textsuperscript{93}. However, there was no difference between obese and healthy weight asthmatics. Furthermore, the healthy weight asthmatics experienced a significant reduction in FEV\textsubscript{1}, FVC and FEF\textsubscript{25-75} post-exercise, while the obese asthmatics had a significant reduction in only FEV\textsubscript{1}/FVC post-exercise\textsuperscript{93}.

\subsection*{1.4.3 Airway and systemic inflammation}

Studies examining airway differential cell counts from sputum have suggested that obese asthma in adults leads to a non-eosinophilic pattern of airway inflammation
with significant neutrophilia, particularly predominant in females. However, there are minimal sputum studies in children, and to our knowledge none have investigated airway inflammation in obese children. In adults with asthma, BMI has been positively associated with exhaled 8-isoprostane levels, a marker of airway oxidative stress, but negatively associated with exhaled nitric oxide (eNO) levels, a marker of eosinophilic airway inflammation. These associations did not exist in the group of adults without asthma. Levels of eNO are increased in asthmatic children compared to healthy controls. However, no clinically significant difference in eNO has been found between obese and healthy weight asthmatic children. In fact, excess weight as determined by adjusted BMI or total percentage body fat is not associated with eNO in asthmatic children. However, differential cell count studies may demonstrate differences in airway inflammatory cells between obese and non-obese children and further investigation is required.

Increased systemic inflammation is evident in obese adults compared to non-obese adults with asthma, particularly increased plasma leptin, CRP and IL-6 levels, and reduced adiponectin levels. Significantly elevated TNF-α levels have also been documented in obese female adults with asthma when compared to obese females without asthma, and non-obese females, with and without asthma. This study also found IL-6 levels to be significantly higher in obese asthmatics compared to non-obese asthmatics and healthy controls. Another study evaluated the association between leptin, asthma and BMI across 3 age groups and found no association between leptin and asthma in age groups 3-18 years and 9-24 years but found higher leptin levels in obese asthmatic adults aged 24-39 years, compared to non-obese asthmatic adults. In a contrasting 12 year follow-up study of children, leptin levels were significantly higher in overweight children with asthma, compared to
overweight children without asthma. Interestingly, there was no difference in leptin levels between the non-overweight children, with and without asthma. No difference in TNF-α levels was detected between overweight and healthy weight children, with or without asthma, in this same study. Likewise, increased leptin and reduced adiponectin levels have recently been reported in obese children as compared to non-obese children with asthma, while there are reportedly no differences in IL-6 or TNF-α levels. However, another study of a small sample of obese and non-obese children, with and without asthma, failed to find a significant difference in CRP levels between the groups.

### 1.4.4 Medication use

Characteristic of non-eosinophilic asthma, obese asthmatic adults are relatively resistant to steroid treatment, and thus this group require alternate treatment approaches. Data from double-blind, placebo-controlled randomised trials in adults with asthma, have found that obesity is associated with resistance to inhaled corticosteroids (ICS), with beclomethasone response inversely associated with BMI. Indeed, there is evidence suggesting that routine corticosteroid treatment is less effective in obese asthmatic children, attributable to more frequent and more severe exacerbations, and increased use of rescue short acting β2-agonists and steroid based medications, compared to non-obese children with asthma. Given that anti-inflammatory asthma medications primarily target eosinophil-based inflammation, this may suggest that airway inflammation in obese children with asthma follows a non-eosinophilic pattern. The effect of inhaled budesonide in overweight and obese children enrolled in the 4 year prospective CAMP trial, was reportedly reduced in magnitude and significance, suggesting reduced steroid efficacy in children with a higher BMI. Compared to subjects receiving placebo/
nedocromil, healthy weight asthmatics had initial and continued improvements in FEV$_1$, FEV$_1$/FVC and bronchodilator response with budesonide treatment, whereas overweight and obese children had only minor initial improvements in FEV$_1$ and bronchodilator response, with no further improvements in any lung function variables over time$^{102}$. Furthermore, although there was a significant reduction in the number of prednisone courses required by overweight and obese children receiving budesonide, compared to those on placebo/ nedocromil, the magnitude of effect was not as great as that reported for healthy weight asthmatics$^{102}$. Unlike the healthy weight asthmatics, there was no change in emergency department presentations or hospital admissions for obese children treated with the same ICS dose$^{102}$. These results may suggest that ICS efficacy in the obese asthmatic child is reduced. This is an important finding given the predominate use of ICS to control childhood asthma, and is relevant to the clinical management of the obese child and requires further investigation.

1.5 Mechanisms of the Obese Phenotype in Asthma

Multiple mechanisms pertaining to obesity physiology have been proposed including the physiological restriction of the thoracic cage by excess weight and the hypersecretion of pro-inflammatory mediators as the link between obesity and asthma$^{33, 106}$. Obesity may also contribute to respiratory symptoms through cardio-respiratory deconditioning or, conversely, asthma could potentially increase the risk of weight gain via disease specific and medication related effects on metabolism and energy control, including activity limitation. Obesity and asthma may be linked by a common aetiology such as genetic predisposition, factors influencing in-utero development, and associated co-morbidities, including gastro-oesophageal reflux and sleep disordered breathing$^{28, 117}$. However, studies which controlled for improvements in these co-morbidities following surgically-induced weight loss found the relationship
between obesity and asthma remained significant\textsuperscript{28}. Given that a rise in prevalence of both conditions has paralleled Westernisation, it is plausible that common environmental factors underlie the rise of both obesity and asthma in childhood. Characteristic of Westernisation is the ‘obesogenic environment’, which encourages a chronic excess intake of energy and an increased ratio of physical inactivity: activity. However, the nature of the relationship between obesity and asthma in children remains to be elucidated.

\textbf{Figure 1.3.} Mechanisms of Paediatric Obese Asthma.

Multiple factors influence body composition, which may be an important factor when considering the effects of excess mass upon respiratory status. Lean mass may attenuate detriments in lung function caused by excess adiposity and may have neutral effects on other clinical asthma markers. (Figure from Jensen et al\textsuperscript{92})

More specifically, hypothesised mechanisms for the deleterious effect of obesity upon respiratory status have focused on the effects of excess fat mass, either as a function of its endocrine capabilities or by mechanical restriction of the thoracic cage and diaphragm (\textbf{Figure 1.3}). However, most studies define obesity by measures of BMI, a crude translation of adiposity. In fact, one study identified that 32\% and 42\% of
adolescent females and males are incorrectly classified as overweight or obese using BMI criteria, despite not having a high adiposity level\textsuperscript{121}. Obesity defined by BMI and BMI $z$-score describes excess collective mass, i.e. both lean and fat mass. This is an important consideration particularly in paediatrics, as the changes in body composition that occur with growth and obesity may have differential effects upon lung function.

1.5.1 Inflammation in Obese Asthma

The chronic inflammatory process created by excess adiposity has been implicated in the pathophysiology of numerous obesity-related long-term complications including cardiovascular disease and Type II Diabetes mellitus, and inflammatory-based conditions such as rheumatoid arthritis, inflammatory bowel disease and multiple sclerosis\textsuperscript{22}. Obesity-related inflammation has been implicated as an underlying factor contributing to asthma development and pathogenesis, under the hypothesis that an increase in systemic inflammatory mediators potentially exacerbates pulmonary inflammation, a direct component of asthma pathophysiology\textsuperscript{33} (Figure 1.4). Specifically, this low-grade inflammation has been implicated as an underlying factor in non-eosinophilic asthma. However, this hypothesis has not been thoroughly investigated in children largely due to a lack of descriptive airway inflammation studies.

Leptin has been reported to stimulate Th1 cytokine production\textsuperscript{22} while decreasing Th2 cytokine production, indicating that leptin may be involved in non-atopic asthma\textsuperscript{28}. Cell studies and animal models have suggested that leptin potentially contributes to asthma pathogenesis via contributing to structural changes in the airways and/or airway hyper-reactivity. In animal models, exogenous leptin has been shown to
enhance AHR to ovalbumin challenge\textsuperscript{122}; augment the airway inflammatory response to ozone, as evidenced by increases in broncho-alveolar levels of IL-6, eotaxin, macrophage inflammatory protein (MIP)-2, keratinocyte chemoattractant (KC), sTNF receptor 1 and sTNF receptor 2\textsuperscript{123}; and increase bronchoalveolar lavage (BAL) levels of neutrophils, IL-6 and leukotriene B\textsubscript{4}, and macrophage phagocytosis\textsuperscript{124}. In human airway smooth muscle (ASM) cells, leptin stimulates the release of vascular endothelial growth factor, which consequentially induces airway remodelling and angiogenesis\textsuperscript{125}. However, a contrasting study reported that leptin inhibited the migration and proliferation of ASM cells in vitro and did not affect cytokine production or contraction of ASM\textsuperscript{126}. Conversely, adiponectin has been shown to inhibit vascular smooth muscle proliferation\textsuperscript{28}. If this function is exerted in the airways, ASM mass may increase in the presence of reduced adiponectin levels, contributing to asthma. However, in vitro studies have not been able to establish this link\textsuperscript{125}.

**Figure 1.4.** Hypothesised link between obesity and asthma.

Excess adiposity increases systemic inflammation, subsequently augmenting airway inflammation and contributing to asthma pathogenesis. AHR airway hyper-responsiveness; ASM airway smooth muscle; TNF, tumour necrosis factor; IL, interleukin. (Figure from Jensen et al\textsuperscript{33})
The increasing ratio of leptin to adiponectin is reportedly associated with reduced eNO levels in asthmatic adults\textsuperscript{119}. Interestingly, this association is not present in non-asthmatic adults\textsuperscript{119}. This may suggest that adipokine levels propagate non-eosinophilic airway inflammation in the adult obese asthmatic population. Cross-sectional studies have found significantly higher leptin levels in healthy weight asthmatic children versus controls\textsuperscript{127, 128}, with increased leptin levels identified as a predictive factor in asthma onset\textsuperscript{128}. Although the prevalence of atopic and non-atopic asthma in children reportedly increases in parallel with leptin levels\textsuperscript{129}, it is unclear whether a leptin-asthma association is stronger in the presence of atopy. Some studies report the association is stronger for children with non-atopic asthma\textsuperscript{78, 83, 86, 129}, while one study has reported children with atopic asthma have significantly higher leptin levels compared to children with non-atopic asthma\textsuperscript{128}, and a recent study has reported a stronger association between overweight status and atopy\textsuperscript{88}. Conversely, a cohort study found no association between adiponectin and asthma during childhood, adolescence or adulthood\textsuperscript{99}. In addition, cross-sectional analysis of non-atopic asthma, atopic asthma, and controls found no difference in adiponectin levels between the groups of children\textsuperscript{37}.

Recent studies have indicated that adipokines may be related to asthma outcomes in children\textsuperscript{94, 108}. Adiponectin is reportedly positively associated with asthma control, FEF\textsubscript{25-75}\textsuperscript{37} and FEV\textsubscript{1}/FVC; and negatively correlated with asthma symptoms, exacerbations, and markers of exercise-induced bronchoconstriction severity, even after controlling for BMI\textsuperscript{94, 108}. Serum leptin has been shown to increase with clinical asthma severity in children aged 2-14 years\textsuperscript{130}. A recent study found baseline leptin was a significant negative predictor, and adiponectin was a significant positive predictor, of baseline FVC\% and PEF\% in a group of obese adolescents with asthma.
However, this association was not true for adolescents without asthma\textsuperscript{131}. The odds of an asthmatic child experiencing exercise-induced bronchoconstriction, and exercise-induced bronchoconstriction of a greater severity, is reportedly higher in the presence of raised serum leptin levels, independent of BMI\textsuperscript{94}. These results may indicate that the mechanism is not the excess weight \textit{per se}, but the associated increase in the leptin/ adiponectin ratio. In a cross-sectional study, obese asthmatic children experienced more severe post-exercise bronchoconstriction compared to healthy weight asthmatic children\textsuperscript{94}. However, these differences were no longer evident after adjusting for serum leptin and adiponectin levels\textsuperscript{94}, suggesting that the mechanism of action in bronchoconstriction may be via increased systemic inflammation created by excess adiposity, rather than the mechanical effects of excess weight. Whether this mechanism involves increased airway inflammation is not known. Airway inflammation, as measured by eNO, has not been found to correlate with systemic inflammatory markers\textsuperscript{94}. This is not surprising given there are no reported differences in eNO between obese and normal weight asthmatic children\textsuperscript{94} and it is therefore unlikely that obese asthma operates via increased eosinophilic inflammation. Given the distinct differences in airway inflammation in obese asthmatic adults, and the implication for alternate asthma treatment, there is a need for more descriptive airway inflammation studies in paediatric obese asthma.

IL-6 is a non-specific stimulant of B and T cells and is one of the interleukin molecules found to directly generate inflammation in asthma\textsuperscript{58, 132}. It is involved in the acute-phase and late-phase asthma response\textsuperscript{19, 81} and correlates with asthma disease activity\textsuperscript{98}. Cell studies have demonstrated a significantly increased release of IL-6 from the epithelial cells of children with asthma, compared to children without asthma\textsuperscript{133}. Likewise, peripheral whole blood cells from asthmatic adults were found to
produce significantly higher IL-6 levels compared to healthy non-atopic controls found that IL-6 levels were significantly higher in asthmatic subjects, but there was no difference between subjects with atopic and non-atopic asthma. Increased levels of plasma IL-6 were recently reported to be associated with worsened clinical outcomes in obese asthmatic adults, and significantly correlated with airway neutrophils. However, a recent study found no significant difference in IL-6 levels between obese and non-obese children, with and without asthma.

A weak association between airway neutrophils and CRP was also documented and CRP levels have been related to asthma symptoms of wheeze, nocturnal cough and breathlessness following exertion in adults. In non-obese asthmatic children, serum CRP levels are elevated during periods of exacerbation and negatively correlate with FEV₁. Interestingly, the largest CRP reduction following resolution of exacerbation was measured in those in the lowest BMI percentile suggesting the leanest children have low levels of background CRP. In a second study of non-obese children, serum CRP levels were found to be significantly higher in asthmatics compared to controls. Furthermore, CRP levels in steroid-naive uncontrolled asthmatics were approximately double the CRP levels of ICS-responsive controlled asthmatics. In contrast, a study of obese and non-obese children, with and without asthma, found no significant difference in CRP levels across the four groups. However, CRP tended to be highest in the obese asthmatic children. In adults, elevated serum CRP levels have been reported in non-atopic asthmatics, but not atopic asthmatics, compared to controls. Authors also reported a significant positive relationship between CRP and respiratory symptoms of wheeze, breathlessness on exertion and nocturnal cough, concluding that systemic inflammation may also contribute to the pathology of non-atopic asthma.
TNF-α is a key cytokine in asthma pathophysiology via the activation of transcription factor, thus extending eosinophil life, and amplifying the inflammatory response\textsuperscript{58}. TNF-α receptors are located on ASM cells and human in vivo studies have demonstrated AHR with TNF-α stimulation\textsuperscript{28}. TNF-α levels are reported to be 5.6 times higher in the alveolar macrophages of infants with wheeze compared to infants without wheeze\textsuperscript{60}. Furthermore, elevated levels of TNF-α and TNF receptor-1 and receptor-2, have been documented in children and adults with AHR, compared to those without hyper-reactivity\textsuperscript{135}. In addition, these TNF markers negatively correlated with FEV\textsubscript{1}\textsuperscript{135}. However, a recent study of obese and non-obese children, with and without asthma, did not detect a significant difference in serum TNF-α levels across the groups\textsuperscript{94}.

Indeed, there is evidence of increased systemic inflammation in children with asthma which may be exacerbated by the systemic inflammatory process caused by obesity\textsuperscript{94, 100, 101, 127, 128}. However, whether this alters asthma outcomes in children via augmenting airway inflammation is unknown.

1.5.2 Growth, body composition and the mechanical effects of obesity

Obesity may produce asthma symptoms without altering the airway inflammatory environment of asthma. Adipose tissue within the android and thoracic regions are hypothesised to impart a compressive effect on the chest wall and cause downward movement of the diaphragm, leading to increased breathlessness\textsuperscript{136}. Alterations in breathing patterns, such as reduced lung expansion associated with decreased tidal breathing in obese subjects, may lead to increased contraction of ASM resulting in subsequent narrowing of the airways and increased AHR\textsuperscript{28, 86}. One study attributed the abnormal alveolar diffusion capacity, observed in one third of obese non-asthmatic children, to structural changes in the airways following lipid deposits or reduced
surface area on the alveoli\textsuperscript{137}. Body composition may therefore be important, and particularly in children, growth is a factor to consider as part of body composition changes.

Children follow a non-linear growth trajectory characterised by periods of growth acceleration. Characteristic of growth is the primary deposition of lean mass, followed by fat mass. However, in adults the deposition of fat predominates with increasing weight. The rate of weight gain and change in body composition are rapid in children and adolescents during these periods of growth. There are three critical periods of high adiposity risk that are thought to determine persistent childhood obesity: the prenatal period, the period of adiposity rebound, and adolescence\textsuperscript{138}. Periods of growth outside these ‘critical’ windows for increased adiposity risk may not be linked to asthma outcomes. One study noted that the development of obesity at age 5 years, corresponding approximately to the period of adiposity rebound, was associated with increased risk of asthma diagnosis and poorer lung function at age 6 and 8 years\textsuperscript{139}. However, excess weight gained in the period prior to this was not related to any future asthma outcomes. In fact, a higher BMI in infancy was associated with a reduced risk of asthma and greater lung function as a child\textsuperscript{139}. The first 12 months of life is a rapid growth stage and a higher BMI may reflect the optimal deposition of lean mass and/or a state of adequate nutrition, which may influence lung development in early life.

Changes in body composition are also gender specific, leading to differences in the amount and distribution of lean and fat mass. During puberty, males accumulate a higher amount of lean mass at a much quicker rate than females and over a longer period, while their fat mass remains relatively stable\textsuperscript{140, 141}. On average, healthy weight males finish puberty with an additional 20kg of lean mass compared to females, a 13\% body fat value and an android body shape\textsuperscript{141}. Females, on the other
hand, accumulate significantly more adipose tissue at a higher rate than males throughout puberty, with an associated increase in hip size\textsuperscript{140,141}. On average, healthy weight females finish puberty with an extra 5-6kg fat mass compared to males, a body fat value of 25\% and a gynoid body shape\textsuperscript{141}. These pubertal changes are attributable to sex-specific hormone levels and distinguish males from females on many levels, but specifically in terms of body composition. Importantly, body composition at the time of puberty predicts body composition and health in adulthood\textsuperscript{141}. Indeed, the importance of body composition has been demonstrated in adults with and without asthma, with direct and indirect measurement of total and regional adiposity associated with respiratory outcomes\textsuperscript{142-144}. Interestingly, body composition in females has been found to be an important predictor of the presence of self-reported asthma in a large cohort of adults\textsuperscript{145}. A recent study of overweight and obese adults with asthma, reported an inverse association between lung function and both lean mass and fat mass in females\textsuperscript{144}. Conversely, in males, lung function was positively associated with lean mass, while there was no association with fat mass\textsuperscript{144}. In children without asthma, DEXA-measured adiposity is also reportedly inversely associated with lung function\textsuperscript{137}.

Further well designed studies to investigate associations between body composition and respiratory outcomes in children are required, as use of BMI z-score can be misleading. One study found no difference in weight or BMI z-score between asthmatic children and healthy controls\textsuperscript{146}. Despite this, the percentage of adiposity was significantly greater in asthmatic versus non-asthmatic children\textsuperscript{146}. Recent data have suggested that an increased childhood BMI is associated with increased adult FEV\textsubscript{1} and FVC\textsuperscript{147}. However, these seemingly positive effects disappeared after adjusting for indirect measures of lean body mass (handgrip strength and skin-fold
test derivations). This highlights the importance of differentiating between lean and adipose tissue, and also suggests that obesity and body composition in childhood may have implications for adult respiratory health.

Differential effects of obesity on lung function may also reflect the duration of obesity i.e. the longer the period of obesity, the more detrimental the effect upon respiratory status. It was recently reported that reduced lung function among overweight and obese female adults was evident only in those who were overweight or obese as a child, and not in those overweight and obese female adults who were classed as a healthy weight child. Furthermore, an interaction effect between age and BMI was reported in this group of women, suggesting an accelerated decline in lung function with persisting obesity as they aged. In another recent study, obese paediatric and adult subjects were categorised by the duration of their obesity (≤5 years, >5-10 years, >10-15 years, >15 years). After controlling for age and the presence of co-morbidities, including asthma, decrements in ventilatory and static lung function were significantly greater in those with the longest duration of obesity. This suggests that the onset and duration of obesity, as well as the composition of the excess weight, may be an important consideration when assessing effects upon lung function and should be considered in future studies.

### 1.6 Obesity Risk Factors in Children with Asthma

The rise in asthma and obesity prevalence has paralleled the development of Western society. Characteristic of Westernisation is the ‘obesogenic’ environment, which encompasses widespread changes to the physical, social, cultural and familial environment. This has greatly impacted dietary practices and activity patterns. In addition, a gradual decline in total sleep time and reduction in the quality of sleep has occurred worldwide. In effect, the obesogenic environment supports and encourages
poor sleeping behaviours and energy imbalance, whereby there is excessive caloric intake, unbalanced micro- and macro-nutrient intake, and a decrease in energy expenditure through activities of daily living. Potentially the obesogenic environment may have a greater impact on children with asthma, with data from the Longitudinal Study of Australian Children suggesting that the presence of asthma may increase the risk of overweight and obesity in children, regardless of their initial weight status. However, the question of whether the risk for obesity is greater in children with asthma, due to a higher prevalence of specific lifestyle factors, has not been addressed. Indeed, sleep disturbance, a significant risk factor for overweight and obesity in children and adolescents, is reportedly greater in the asthmatic population. Early intervention is key to reduce the onset of overweight and obesity, and therefore it is essential to understand the prevalence of modifiable risk factors in this population. The following sections examine the literature surrounding key modifiable lifestyle risk factors for obesity in children, both with and without asthma.

1.6.1 Sleep as a Risk Factor for Obesity in Asthma

Sleep deprivation is a significant problem worldwide in children and adolescents. The difference in sleep requirement and actual self-reported sleep time may be as large as 2 hours. Daytime sleepiness is also a significant concern worldwide, with up to 40% of adolescents reporting some aspect of daytime sleepiness. Interestingly, the increase in obesity rates has paralleled the decrease in sleep duration in children and adolescents over the past few decades and lack of sleep has been described as a risk factor for weight gain in both children and adults. Indeed, sleep duration is notably reduced in children who are obese, and an inverse association between sleep and BMI z-score, and more specifically adiposity, has been noted. A
meta-analysis concluded that for every one hour increase in sleep duration the pooled risk for overweight and obesity in children decreases by about 9%\textsuperscript{14}.

In children with asthma, disturbed sleep may be a potential risk factor for overweight and obesity. Children with asthma have reported more disturbed sleep patterns than children without asthma\textsuperscript{79}, with an estimated one in five children regularly woken by their asthma during the night\textsuperscript{56} or reporting difficulty falling asleep\textsuperscript{154}. In adults, poor sleep quality is a significant predictor of poor asthma control in both severe and non-severe asthmatics\textsuperscript{155}. A study investigating the prevalence of obstructive sleep apnoea (OSA) in adults, found that sleep disruption measured by home polysomnography (PSG), was significantly higher in the severe asthmatic group, as evidenced by a poorer sleep efficiency and a higher arousal index (AI), compared to moderate asthmatics and healthy controls\textsuperscript{156}. Notably, the asthmatic participants, regardless of asthma severity, experienced a significantly shorter period of slow-wave sleep, compared to non-asthmatic participants\textsuperscript{156}. In children, the risk of nocturnal awakenings is reportedly higher for those with more severe asthma, a lower FEV\textsubscript{1}/FVC, and greater atopy\textsuperscript{157}. Indeed, sleep quality as measured by validated questionnaire, has been found to worsen with increasing nocturnal asthma symptoms in a group of predominantly non-Caucasian children\textsuperscript{158}. Morning and evening PEF measurements have also been inversely associated with frequent night awakenings in children\textsuperscript{159}. Poorer self-rated sleep quality, but not self-reported sleep duration, has also been associated with asthma symptom severity in children\textsuperscript{160}. In fact, reduced sleep quality predicted more severe symptoms the next day\textsuperscript{160}. Yet another study found that children with asthma reported more disturbed sleep, compared to healthy controls, even when their asthma is clinically stable\textsuperscript{161}. Furthermore, difficulty awakening, increased daytime sleepiness, self-reported depression, and significantly
poorer parental reported mood and behavioural functioning, memory recall and learning difficulty, is evident in children suffering nocturnal asthma compared to healthy controls.\textsuperscript{159, 162}

Sleep disturbance has not been well described using objective measures in children with asthma. The majority of studies reporting sleep habits in children have used parental or self-report\textsuperscript{157, 160, 161, 163, 164}, while others have used actigraphy\textsuperscript{159, 163} and one has used electroencephalographic studies\textsuperscript{165}. Only three studies have used the gold-standard technique of PSG to describe sleep patterns in children, with and without asthma\textsuperscript{162, 166, 167}. These studies were limited by their sample size, the use of self-reported asthma, failure to adequately characterise asthma status and medication use, and the presence of confounding factors, particularly obesity and OSA. However, despite these limitations, previous studies using PSG data suggest that sleep disturbance is greater in children with asthma compared to controls, as supported by an increased number of awakenings\textsuperscript{162}, an increased arousal index\textsuperscript{166}, a poorer sleep efficiency\textsuperscript{162, 167}, an extended sleep onset latency\textsuperscript{167} and a reduced total sleep duration\textsuperscript{167}. Given that sleep is a significant risk factor for overweight and obesity in children, one may hypothesise that children with asthma are at an increased risk of obesity via increased sleep disturbance.

Sleep deprivation and obesity are increasingly prevalent in Western society and it is possible that sleep may be linked to obesity via alterations in lifestyle practices key to maintaining the balance between energy input and output. However, the mechanism by which poor sleep increases the risk for excess weight gain in childhood is unclear, largely due to a lack of exploratory studies. Sleep may be negatively associated with overweight and obesity\textsuperscript{151, 168} through adversely impacting physical activity and/ or dietary practices. This may take effect via metabolic disturbances e.g. alterations in
appetite signalling and/ or energy utilisation and storage, or via adversely impacting daytime functioning causing reductions in physical activity, increases in sedentary activity, poorer nutritional choices and increased caloric intake\textsuperscript{150, 151}. In addition, increased waking hours presents an increased opportunity for excess energy intake in the context of reduced energy expenditure. These mechanisms may contribute to the increased risk of obesity that is observed with sleep deprivation\textsuperscript{150} (Figure 1.5).

\textbf{Figure 1.5.} Mechanisms by which sleep disturbance in asthma may lead to increased risk of weight gain

\textbf{1.6.2 Altered Appetite Control & Dietary Intake with Sleep Disturbance}

Chronically sustained sleep deprivation can adversely impact multiple systems that are influenced by the circadian rhythm and sleep-wake cycle, including neuro-hormonal and metabolic function\textsuperscript{169}, and are thought to contribute to an increased risk of lifestyle disease, notably cardiovascular disease, Type-II diabetes mellitus and obesity\textsuperscript{149, 169-171}. Multiple regulatory systems within the body are influenced by the circadian rhythm and sleep-wake cycle and therefore are potentially vulnerable to reduced sleep quantity and poor sleep quality. Sleep restriction studies in adults have reported clinically important metabolic and endocrine alterations, most notably...
decreased glucose tolerance and insulin sensitivity, and disturbances in growth hormone and cortisol secretion\textsuperscript{150}. Chronically sustained alterations in these biomarkers may contribute to changes in energy storage, utilisation and mobilisation, and body composition and growth changes, and significantly alter disease risk. Suboptimal sleep duration in adults has been linked to alterations in various adipokines, specifically an decrease in the satiety hormone leptin and an increase in the appetite-stimulating hormone ghrelin\textsuperscript{151}. Unlike leptin, which is produced by adipose tissue, ghrelin is produced in the stomach\textsuperscript{172}. Both the acyl and des-acyl forms of ghrelin are able to cross the blood-brain barrier where it influences appetite control centres in the central nervous system\textsuperscript{172}.

Changes in appetite and hunger scores, assessed using a visual analogue scale, have been documented following sleep debt in adults, with appetite for high-carbohydrate and high-fat foods most significantly enhanced\textsuperscript{151}. In addition, a study in adults comparing sleep restriction by 1.5 hours versus sleep extension by 1.5 hours, found an increased energy intake from snacks was associated with sleep-restriction compared to sleep extension\textsuperscript{150}. These studies suggest that sleep–deprived people are more likely to consume excess energy throughout the day, particularly carbohydrate and fat rich foods. In contrast, another adult study compared two nights of 4 hour sleep duration with two nights of 8 hour sleep duration, and did not detect a difference in serum leptin or plasma ghrelin levels; appetite, hunger or satiety ratings; or energy intake from snacks and/ or meals\textsuperscript{173}. However, fat consumption was approximately 90grams higher during the sleep deprived state\textsuperscript{173}. A recent study found a single night of sleep fragmentation in adult males – i.e. a reduction in rapid eye movement (REM) sleep but no change in total sleep time (TST), was associated with adverse changes in insulin, glucagon like protein-1, cortisol levels, fatigue ratings and fullness ratings the
next day\textsuperscript{174}, indicating that sleep quality is also associated with adverse changes in metabolic and appetite measures.

Whether alterations in appetite hormones are associated with acute or chronic sleep disturbance in children, with or without asthma, has not been investigated. Only two studies have assessed self-reported sleep and measured levels of endocrine hormones and metabolic markers in children\textsuperscript{152, 175}. Serum leptin levels have been inversely associated with self-reported sleep duration in female children without asthma\textsuperscript{175}. Adiponectin levels are also reportedly lower in male non-asthmatic children with self-reported sleep of 8 hours versus 10 hours\textsuperscript{175}. Sleep duration, as measured by actigraphy, has been inversely associated with metabolic disturbances in children in a more recent study, specifically CRP and low density lipoprotein (LDL)-cholesterol\textsuperscript{152}. However, this was not found in a second study, where no differences were detected in plasma lipids, glucose or insulin levels between children classified as short or long sleepers\textsuperscript{175}.

Dietary-related risk factors for weight gain have been inversely associated with sleep duration in non-asthmatic children. Interestingly, these risk factors are highly prevalent in children with self-reported sleep duration up to only 1-2 hours below the recommended 9-10 hours\textsuperscript{175}, indicating that only mild reductions in sleep time may be associated with poor lifestyle habits. A difference in sleep duration of 2 hours has been associated with an almost 20\% difference in the prevalence of frequent soft drink consumption among male children\textsuperscript{175}. However, a difference in the total nutrition score (derived from a validated FFQ), was not detected between short and long sleepers for either boys or girls in the same study\textsuperscript{175}. Notably, sleep duration was inversely associated with BMI z-score and waist circumference in this group of non-asthmatic children\textsuperscript{175}. One study provided evidence to suggest that delayed sleep
initiation may lead to anxiety and consequential emotional eating, thereby increasing risk of excess energy intake and weight gain\textsuperscript{176}. In a group of 9-11 year old Finnish children without asthma, self-reported sleep duration was inversely associated with consumption of energy-rich foods (e.g. fast foods and confectionery), with this association stronger in males\textsuperscript{177}. In the same study, consumption of nutrient-dense foods was positively associated with self-reported sleep duration in girls only\textsuperscript{177}. Interestingly, these associations for both boys and girls were attributable to sleep duration during school nights, not weekend nights\textsuperscript{177}. Amongst a group of non-asthmatic adolescents, those attaining self-reported ‘adequate’ sleep (defined by the study as 6-8 hours on more than four days per week) were approximately three times more likely to achieve a higher frequency score for positive nutritional practices (e.g. eating 3 meals per day, including breakfast, selection of low fat foods, drinking $\geq 1500$ml), compared to those with inadequate sleep duration throughout the week\textsuperscript{178}. In fact, adequate sleep was a significant predictor of a higher frequency score for positive nutrition behaviours in this group of adolescents\textsuperscript{178}. Daytime sleepiness has also been found to be a positive predictor of energy-rich food consumption in male non-asthmatic children, whereby self-reported daytime tiredness occurring more than four days per week increased the risk of energy-rich food consumption more than eight-fold\textsuperscript{177}. On the other hand, difficulty awakening in the morning was a negative predictor of nutrient-dense food intake in this group of male children\textsuperscript{177}. Unfortunately, these studies are limited by the use of non-validated subjective sleep and dietary assessment tools, and did not adequately describe the dietary patterns/behaviours measured. It remains that the association between sleep, appetite control and dietary intake requires further exploration using objective, validated and reliable measurements in children, with and without asthma.
1.6.3 Altered Physical & Sedentary Behaviour with Sleep Disturbance

The change in the modern society with regards to technology, transportation and practices within the home, school and work environment, has supported a rise in sedentary activity. In combination with reductions in active play and active transport, there is a considerable reduction in the energy expended by an individual in their daily activities. Daytime fatigue, as a consequence of poor sleep, may contribute to a concomitant reduction in physical activity levels and increase in sedentary behaviours. Indeed, multiple aspects of daytime functioning appear susceptible to sleep problems, including social, school and work performance, concentration and attention, emotional and mood stability. There is also the potential increased risk of exercise limitations in children with asthma, due to disease-specific restrictions, whether perceived or actual.

Cross-sectional measures of sleep quantity and quality have been associated with adverse changes in physical and sedentary activity behaviours in non-asthmatic children. Calamaro et al calculated a multi-tasking index from reported small-screen usage in a sample of predominantly Caucasian adolescents. A significantly lower multi-tasking index (i.e. lower small-screen use) was detected in adolescents who reported 8-10 hours sleep, compared to children reporting 6-8 hours or 3-5 hours sleep, per weeknight. In addition, the multi-tasking index was a significant positive predictor of difficulty initiating sleep and falling asleep during school. Conversely, associations between self-reported sleep and media usage were not observed in another group of adolescents. ‘Adequate’ sleep (defined as 6-8 hours on more than four days per week) was a significant predictor of positive exercise behaviours in a study of Taiwanese adolescents. In fact, adolescents reporting ‘adequate’ sleep duration were just over twice as likely to achieve a higher frequency score for positive
exercise behaviour, compared to adolescents reporting inadequate weekly sleep\textsuperscript{178}. Notably, even mild reductions in self-reported sleep time, of up to 1-2 hours below the recommended 9-10 hours\textsuperscript{175}, may be associated with adverse sedentary and physical activity behaviours in non-asthmatic children. Amongst children with a self-reported mean sleep duration of 8 hours, media usage is reportedly 1.6 times higher in females and 2 times higher in males, compared to children sleeping up to 2 hours longer\textsuperscript{175}. Physical activity was also found to be less prevalent in female short sleepers compared to female long sleepers\textsuperscript{175}. Sleep restriction studies in adults have demonstrated that low-intensity activity increases while high-intensity activity decreases, the day following acute sleep deprivation, with activity counts significantly lower following 4 hours versus 8 hours sleep\textsuperscript{173}. In addition, this weakly corresponded with reported tiredness which was significantly enhanced with sleep deprivation\textsuperscript{173}, which may suggest that daytime tiredness, as a consequence of poor sleep, may lead to a reduction in energy expenditure through daily activities.

On the other hand, sleep disturbance time, but not sleep duration, has been inversely associated with physical activity measured using 24 hour actigraphy in a sample of 13 year old non-asthmatic children\textsuperscript{180}. This result remained significant for females only. In fact, for every one hour increase in sleep disturbance, there was a 3% decrease in physical activity\textsuperscript{180}. Likewise, increased sleep latency in another group of non-asthmatic children has been linked with decreased physical activity and increased sedentary behaviour\textsuperscript{181}. Sleep latency reportedly increased by 3 minutes and decreased by 5.7 minutes, for every one hour of sedentary and vigorous activity, respectively\textsuperscript{181}. In a group of Chinese adolescents, participation in regular physical activity less than three times per week was identified as the greatest risk factor for insomnia symptoms, increasing the risk by more than 2-3 times that of adolescents.
reporting habitual physical activity\textsuperscript{182}. Unfortunately previous studies are limited by the use of non-validated subjective sleep and activity assessment tools, and the poor description of the physical and sedentary behaviours. In addition, whether alterations in the sedentary: physical activity ratio is associated with sleep alterations in children with asthma is unknown. Furthermore, longitudinal studies are needed to investigate the direction of these associations. It remains that the association between sleep, sedentary activity and physical activity requires further exploration using objective, validated and reliable measurements in children, with and without asthma.

\textbf{1.7 Weight loss in Asthma}

Given the high rates of obese asthma in children and adults, there is a definite need for weight loss intervention. However, few weight loss intervention studies have been conducted in the asthmatic population. Previous weight loss interventions have been conducted in adults, with the exception of one recent uncontrolled study that investigated the impact of weight loss in adolescents with asthma\textsuperscript{131}. The majority of studies have reported the effect of surgically-induced weight loss\textsuperscript{183-185}, while few have investigated the effects of dietary intervention, either alone\textsuperscript{33} or in combination with other lifestyle interventions\textsuperscript{131, 186}. Weight loss achieved in asthmatic adults ranges from 35-45\% within 1-3 year follow-up\textsuperscript{183, 184} and 8-19\% within 8-26 weeks\textsuperscript{33, 186}, for surgical and dietary interventions, respectively. Only one adequately designed RCT, conducted in adults, met the criteria for a 2003 Cochrane review on dietary-induced weight loss interventions in the asthmatic population, with the majority of other diet-induced weight loss studies either non-randomised or uncontrolled\textsuperscript{187}. However, a randomised trial was recently published investigating the effects of a 10 week very-low calorie diet and/ or exercise regime on asthma outcomes in obese adults, including a 10 week follow-up period\textsuperscript{186}. Only one study has been conducted
in adolescents, aged 15-19 years, with and without asthma. Recently published, this uncontrolled study achieved up to 13.5% loss of body weight using a 1 year combined therapy of diet, exercise, psychological and medical management. Intervention studies involving a dietary component have typically employed a very-low to low calorie diet, an alternate day energy restriction and ad libitum diet, or national nutritional guidelines for the general population. Importantly, these studies have achieved improvements in one or more asthma-related outcomes following weight loss (Figure 1.6).

Figure 1.6. Weight loss in asthma.

Improvements in asthma measures following weight loss in the asthmatic population. ASM, airway smooth muscle; FEV₁, forced expiratory volume in 1 second; FVC, forced vital capacity; PEFR, peak expiratory flow rate; TLC, total lung capacity; ERV, expiratory reserve volume; FRC, functional residual capacity.

(Figure from Jensen et al)

1.7.1 Symptoms, Quality of Life and medication usage with weight loss

Obese asthmatic adults randomised to a 14 week weight reduction program that involved an 8 week very-low calorie diet, achieved a mean 14.5% weight loss with a subsequent improvement in reported dyspnoea and rescue medication use, compared
to the control group\textsuperscript{188}. Similar improvements in dyspnoea score were reported with a mean weight loss of 13.5kg in an uncontrolled study of an 8 week very-low calorie diet in asthmatic adults\textsuperscript{189}. A recent randomised trial of a 10 week very-low calorie diet and/or exercise intervention in 38 obese adults with asthma, reported a significant weight loss of approximately 8.5\% in the dietary and combination groups\textsuperscript{186}. Importantly, a clinically significant improvement in asthma control and quality of life was detected in these groups. Although the effects of weight loss on asthma control has not been reported in the paediatric population, a recent uncontrolled study of 75 asthmatic adolescents found self-rated symptoms of cough and wheeze reduced following a 12 month interdisciplinary weight loss intervention\textsuperscript{131}. Importantly, the severity of asthma in this group of adolescents reduced significantly at 6 and 12 months, with the proportion of adolescents classified with mild-moderate persistent asthma reducing from 74\% at baseline to 19\% by study finalisation\textsuperscript{131}. Improvements in self-rated symptoms, asthma control and severity, quality of life and medication use have also been reported in adults following surgically-induced weight loss\textsuperscript{183-185}. While medication use and asthma control remained stable in the control group, there was a significant reduction in ICS and SABA medication use, and a clinically significant improvement in asthma control, in adults post-bariatric surgery\textsuperscript{184}. Likewise, Dixon et al reported a clinically significant improvement in asthma control and asthma-related quality of life, and a reduction in SABA use in adults 12 months following surgically-induced weight loss\textsuperscript{185}.

1.7.2 Lung function and airway hyper-responsiveness with weight loss

Significant improvements in ventilatory function (FEV\textsubscript{1}, FVC) following weight loss of almost 15\% have been demonstrated in asthmatic adults randomised to a 14 week dietary intervention, as compared to the control group\textsuperscript{188}. Smaller uncontrolled and
non-randomised studies have also reported significant improvements in FEV$_1$, FVC, morning and evening PEF, and post-salbutamol FEV$_1$, with weight loss following 8 weeks energy restriction$^{189, 191}$. A longer non-randomised uncontrolled trial of 6 months, prescribed 58 obese females a energy restricted diet, which included a 6 week or 12 week period of liquid meal replacements, depending on the level of obesity$^{190}$. This study reported a dose-dependent effect, with greater weight loss associated with significantly greater improvements in FEV$_1$, FVC and total lung capacity (TLC)$^{190}$. Likewise, a pooled analysis of overall weight loss in a recent randomised trial with three intervention arms, demonstrated that those asthmatic adults achieving more than 10% weight loss in 10 weeks had significant improvements in FRC and ERV of almost 0.5L$^{186}$. Improvements in spirometry values up to 20% of the predicted value, have also been reported following weight loss in obese adolescents, both with and without asthma$^{131}$. In fact, obstruction reduced significantly in the asthmatic adolescents, as indicated by a mean change in FEV$_1$/FVC% from 71% at baseline to 93% at 12 months$^{131}$. However, the effect of weight loss upon static lung function and airway responsiveness has not been reported in the paediatric population. Surgically-induced weight loss is also associated with large improvements in lung function, notably static lung function$^{183-185}$. Boulet et al recently reported significant improvements in %predicted values for FEV$_1$, FVC, FRC, FRC/TLC, VC and ERV following surgically-induced weight loss in asthmatic adults, while these values were unchanged in the control group$^{184}$. In fact, by 12 months post-surgery, there was an approximate doubling in ERV %predicted with weight loss. Improvements in airway reactivity (provocation concentration (PC)$_{20}$ to metacholine) have been demonstrated in adults following bariatric surgery$^{184, 185}$, but interestingly this was reported to occur in non-atopic individuals only$^{185}$. 69
1.7.3 Airway and systemic inflammation with weight loss

Systemic inflammation has been found to reduce following weight loss in adolescents and adults with asthma. Scott et al recently reported a reduction in leptin levels of up to 38% in asthmatic adults following a 10 week very-low calorie diet, either alone or in combination with an exercise program\textsuperscript{186}. In addition, IL-6 levels reduced by approximately 13% with combined dietary and exercise intervention\textsuperscript{186}. Adiponectin and CRP, however, did not change significantly in either treatment arm\textsuperscript{186}. In adolescents with and without asthma, leptin and CRP levels were found to reduce following a 12 month interdisciplinary intervention, while adiponectin levels increased, compared to baseline\textsuperscript{131}. Notably, these changes in inflammatory mediators were not detected at 6 months, perhaps indicating that greater weight loss is needed for changes in inflammatory biomarkers. Furthermore, change in adiponectin was found to be a significant positive predictor of change in FVC %predicted in asthmatic adolescents at 12 months, while change in BMI, leptin, CRP and maximum oxygen uptake (VO2max) were not significant predictors of the change in lung function\textsuperscript{131}. CRP has been shown to reduce following substantial weight loss in asthmatic adults, with reductions of 50% by 6 months, and 80% by 12 months, post-bariatric surgery\textsuperscript{184}. Another recent bariatric surgery intervention reported a non-statistically significant decrease of almost 50% in serum leptin and a significant increase in serum adiponectin of almost 83% 12 months post-surgery\textsuperscript{185}.

Two studies have reported the effect of weight loss on airway inflammatory mediators in adults with asthma. A randomised trial using a 10 week dietary and/or exercise intervention detected a reduction in eosinophils in the exercise group only\textsuperscript{186}. Although this study did not detect changes in sputum inflammatory cells following overall weight loss for the three treatment arms, a significant positive association
between %neutrophils and weight change, specifically DEXA-measured fat mass, was reported in females\textsuperscript{186}. Another study measured broncho-alveolar lavage (BAL) inflammatory markers and found a significant increase in %lymphocytes in adults 12 months following surgically-induced weight loss\textsuperscript{185}. In addition, the function of lymphocytes was reportedly enhanced, as evidenced by an associated increase in peripheral cytokine production. The same study also detected an increase in BAL adiponectin levels, while BAL leptin levels remained unchanged\textsuperscript{185}. The effect of weight loss upon airway inflammation is yet to be investigated in children and adolescents.

1.8 Hypotheses and Aims of Thesis

1.8.1 Key Points of Background to Thesis

- Obesity is a highly prevalent problem and is associated with poorer outcomes in children with asthma. However, the obese asthma phenotype in children is yet to be adequately defined.

- Identifying whether the presence of modifiable risk factors for obesity is common in children with asthma is important information for early intervention strategies to prevent weight gain in this population.

- Weight loss studies in obese children with asthma are limited but previous studies do indicate that a reduction in weight may provide clinically important improvements for the asthmatic child.
1.8.2 Hypotheses of Thesis

Study 1 & 2: Obesity causes systemic inflammation, augmenting airway inflammation and worsening asthma outcomes in children with asthma.

Study 3: a) Children with asthma experience more sleep disruption than children without asthma i.e. (i) sleep duration is shorter; and/ or (ii) sleep quality is poorer in children with asthma. b) Sleep disturbance impacts energy balance by altering appetite control (subsequently increasing energy intake) and reducing physical activity.

Study 4: Weight reduction, specifically fat mass, will reduce systemic and airway inflammation and improve lung function and clinical outcomes in obese children with asthma.

1.8.3 Aims of Thesis

Study 1 & 2: To describe systemic and airway inflammation, lung function, and clinical outcomes in obese children with asthma.

Study 3: a) To investigate whether the presence of asthma increases sleep disturbance in children i.e. (i) sleep duration is reduced; and/ or (ii) sleep quality is poorer. b) To investigate whether sleep disturbance is associated with a higher prevalence of obesity risk factors in children with asthma i.e. (i) altered levels of systemic metabolic and appetite markers; (ii) poorer dietary intake; (iii) reduced physical and increased sedentary activity behaviours.

Study 4: To examine the effect of weight loss on systemic and airway inflammation, lung function and clinical outcomes in obese children with asthma.
2. Chapter II: General Methods
2.1 Clinical Assessment

2.1.1 Anthropometry

Height was measured to nearest 0.1cm using a 2m scale suspended from a wall (Surgical and Medical Supplies Pty Ltd, SA Australia). Weight was measured to nearest 100g using max capacity 150kg scales (EB8271 NuWeigh, Newcastle Weighing Services, NSW Australia). Waist circumference (WC) was measured to nearest 0.1cm at the midpoint between the lower costal edge and the iliac crest, using a non-extensible steel tape (Lufkin W606PM, Cooper Tools, Apex NC). Waist to height ratio was calculated (waist circumference (cm) / height (cm)).

BMI was calculated using height and weight (weight (kg) / height (m)\(^2\)). The Centre for Disease Control and Prevention (CDC) BMI-for-age percentile charts\(^{192}\) were used to screen for overweight and obesity in the paediatric population. Overweight was defined as ≥85\(^{th}\) BMI-for-age percentile and <95\(^{th}\) BMI-for-age percentile while obesity was defined as ≥95\(^{th}\) BMI-for-age percentile. BMI z-score was calculated with reference to the CDC 2000 Growth Charts using SAS Software (SAS Institute Inc., USA)\(^{193}\). BMI z-score was reported in all studies with obesity defined as a BMI z-score ≥1.64SDS, which equates to the CDC obesity definition of ≥95\(^{th}\) BMI-for-age percentile.

2.1.2 Body composition

Total body scans were carried out using a Dual Energy X-ray Absorptiometry (DEXA) machine and associated software (GE Lunar Prodigy, Medtel; GE Healthcare encore 2007 software Version 11.40.004, Madison USA). Participants were scanned on the appropriate setting (thin / standard / thick mode). Total and regional absolute fat mass, muscle mass and bone mineral density were calculated (kg). Percentage total
and regional body fat were also calculated (%). A urine pregnancy test (Pregnow, Fertility Solutions, Australia) was carried out before the scan to screen female participants. If there was a chance the participant was pregnant, the scan was not performed.

2.1.3 Pulmonary Function Testing

Dynamic lung function was measured using a Koko spirometer (Windows KoKo PFT System Version 4.9 2005, PDS Inc Louisville USA). Predicted values for forced expiratory volume in one second (FEV$_1$) and forced vital capacity (FVC) was calculated using NHANES III data, which accounts for gender, age, and height$^{194}$. FEV$_1$ and FVC values were then expressed as a percentage of the predicted values (FEV$_1$ %predicted and FVC %predicted, respectively).

Lung volumes (total lung capacity (TLC), functional residual capacity (FRC), residual volume (RV) and expiratory reserve volume (ERV)) were measured using a plethysmograph and associated software (MedGraphics Elite Series Plethysmograph, USA; Breeze Suite 6.4.1.14 Version 510 2008, MedGraphics Corp., USA). The predicted values for the lung volume measurements were calculated using prediction equations by Polgar & Promadhat$^{195}$. The lung volume values were then expressed as a percentage of the predicted values (TLC %predicted, FRC %predicted, RV %predicted, ERV %predicted) and in the original scale (L).

2.1.4 Sputum Induction/ Challenge

Airway hyper-responsiveness (AHR) was determined, and a sputum sample was collected, by exposing the patient to a mist of 4.5% saline created by a nebuliser (ULTRA-NEB™ ultrasonic nebuliser, DeVilbiss, Model 2000). The saline aerosol was inhaled through a mouthpiece for incremental time periods to a maximum of
15.5mins. Before commencing the saline inhalation, the participant underwent spirometry to determine their best FEV$_1$ from 3 attempts. FEV$_1$ was measured at regular intervals between periods of saline inhalation and compared to this baseline value.

AHR was determined if the participant’s FEV$_1$ dropped ≥15% from their initial baseline value. AHR could only be determined if the participant had withheld their medications for the appropriate period of time. Participants who experienced AHR were administered 600mcg short-acting beta$_2$-agonist (SABA) through a volumatic spacer and their lung function assessed after a 10 minute period. Lung function was to return to within 10% of their baseline FEV$_1$ before continuing the test. The saline test was ceased if the participant’s FEV$_1$ dropped ≥15% twice during the test or if the participant elected not to continue.

Participants were encouraged to cough and clear the throat to help dislodge sputum off their chest wall. Any contents in the mouth were emptied into a specimen jar. Sputum samples were collected pre-prandial.

### 2.1.5 Exhaled Nitric Oxide

Exhaled Nitric Oxide (eNO) was measured using NiOX chemiluminescent Detector unit (Aerocrine, Australian Supplier Zynergy Medical). eNO was measured following a ≥12hr period of fasting and was reported in the unit measure, parts per billion (ppb). NiOX measures fractional eNO from exhaled breath of humans. Increased eNO indicates the presence of airway inflammation. Participants were instructed to maintain a constant flow exhalation. To aid the process, visual image of a hot-air balloon was displayed on the screen and participants were to continue exhaling until
the balloon had landed on the other side. The average of 3 technically acceptable attempts was recorded.

2.1.6 Allergy Testing

An allergy skin prick test was carried out to determine whether the participant was atopic. Participants were to withhold anti-histamine medication prior to the procedure. Common allergens (Aspergillus fumigatus, Alternaria tenius, Dust mite (Dermatophagoides Pteronyssinus), Cockroach mix, Grass mix) were applied to the skin (Hollister-Stier, Link Group). A positive histamine control and negative saline control were also applied to the skin to validate the test. A skin prick lancet was then used to gently break the skin surface beneath the allergen without causing visible damage or drawing blood. The weal for the positive histamine control was measured after 10 minutes and the weals of the remaining allergens were measured after 15 minutes. The timing for measurement was consistent for all participants. The size of the allergen weal determined whether the participant was atopic. If one or more allergen weals were greater than 3mm$^2$, then the participant was deemed atopic.

2.1.7 Asthma Classification

Clinical asthma pattern was classified according to the Global Initiative for Asthma (GINA) paediatric guidelines$^{196}$. These guidelines classify asthma as intermittent, mild persistent, moderate persistent or severe persistent. Current asthma control status was assessed using the Juniper Asthma Control Questionnaire. This tool is validated for use with children 6 years and older$^{197}$ and was administered by the research officer. The tool consists of 7 questions, each with seven responses, scored from 0 through to 6. The total score is calculated as an average of the seven scores. A higher number indicates poorer asthma control. The clinical definition of poorly controlled
asthma is a score of $\geq 1.5$. A clinically significant difference or change in ACQ is defined as a difference or change in score of $\geq 0.5$.

### 2.1.8 Quality of Life: Asthma

The Juniper Paediatric Asthma Quality of Life Questionnaire (standardised) (PAQLQ(s)) is a validated tool used to assess quality of life (QoL) in children with asthma\textsuperscript{198, 199}. The tool was self-administered, requiring approximately 10mins to complete. The tool consists of 23 questions, each with seven responses, scored from 1 through to 7. A total QoL score is calculated as an average of the 23 scores. In addition, the questionnaire assesses 3 QoL domains by averaging the scores for specific questions according to a code (symptoms, activity limitation, and emotional function). A higher score indicates a greater QoL. A clinically significant difference or change in QoL is defined as a difference or change in the PAQLQ(s) score of $\geq 0.5$.

### 2.1.9 Urinary Cotinine Levels

Participants were asked to provide a urine sample. A dipstick (NicAlert, Nymox Pharmaceutical Corp, USA NJ) was submerged into the sample for 10 seconds. After a period of approximately 10 mins, or until the blue line disappeared, the level of cotinine in the sample was indicated by the darkest line on the stick. The level indicated on the stick corresponded to the level of exposure to smoking and was categorised as 0 (nil exposure), 1 (minimum exposure, non-smoker) or 2 (current smoker).
2.2 Sleep assessment

2.2.1 Polysomnography (PSG)

Sleep architecture was determined using the gold-standard measurement, PSG, which records electrical signalling, respiration and muscle movement while asleep. Overnight PSG was performed using the modified 10-20 application system (E-Series, Compumedics Ltd, Victoria Australia). Monitoring included electrocardiogram (ECG); electroencephalogram (EEG); pulse rate; oximetry (SpO₂); airflow, including transcutaneous CO₂ (tcCO₂), via nasal thermistor and nasal prong pressure transducer where tolerated; and diaphragmatic and respiratory effort via diaphragmatic electromyography (EMG) and Peizo respiratory bands located on the chest and abdomen. Scoring of polysomnographic recordings was carried out by a sleep technician in the Paediatric Sleep Unit, John Hunter Hospital (Newcastle, Australia), using Profusion PSG 2 software (Compumedics Ltd, Victoria Australia 2001-2007). Sleep-wake state was scored using R & K Rules criteria for children >6mths of age. EEG arousals with and without a respiratory event were scored using modified American Thoracic Society criteria²⁰⁰,²⁰¹. Use of these scoring guidelines was under the direction of the paediatric respiratory sleep physicians. The Paediatric Sleep Unit meets accreditation by the Thoracic Society of Australia and New Zealand, and the Australasian Sleep Association.

2.2.2 Pediatric Daytime Sleepiness Scale (PDSS)

The Pediatric Daytime Sleepiness scale (PDSS) is a validated 8-item questionnaire designed to measure excessive daytime sleepiness in school aged children²⁰². Each question is given a score from one to four, with a total score ranging from zero to a maximum of 32. A greater score indicates greater daytime sleepiness. A score >26 for signifies clinically abnormal daytime sleepiness.
2.3 Biochemical Assessment

Blood samples were collected from patients following a ≥12hr period of fasting. Participants were offered food immediately following the blood test and sputum induction. All blood was collected by a trained paediatric phlebotomist from the Hunter Medical Research Institute or alternatively from the Hunter Area Pathology Service (HAPS), John Hunter Hospital.

2.3.1 Cholesterol and triglycerides

Blood collection: Blood was collected into a 4ml lithium heparin coated tube. Preparation: Samples were centrifuged at 3800 rpm, 4°C for 10 minutes and plasma separated from whole blood within 2hrs of collection.

2.3.1.1 Plasma Triglycerides

Determination: Plasma triglycerides were determined following a series of enzymatic reactions with a bichromatic (510, 700nm) endpoint (TRIG Flex reagent cartridge, Dimension Vista System, Siemans Healthcare Diagnostics Inc. 2008, Newark USA).

2.3.1.2 Plasma Total Cholesterol

Determination: Plasma total cholesterol was determined in an enzymatic process with a polychromatic (540, 452, 700nm) endpoint, whereby cholesterol esters are hydrolysed and resulting free cholesterol is oxidised (CHOL Flex reagent cartridge, Dimension Vista System, Siemans Healthcare Diagnostics Inc. 2008, Newark USA).

2.3.1.3 Plasma High Density Lipoprotein Cholesterol (HDL-C)

Determination: Plasma high density lipoprotein cholesterol (HDL-C) levels were determined using a HDLC assay (HDLC Flex reagent cartridge, Dimension Vista System, Siemans Healthcare Diagnostics Inc. 2008, Newark USA). This assay uses a
2 reagent format with a bichromatic (600, 700nm) endpoint: the first reaction separates HDL-C from other lipoproteins using dextran sulphate in the presence of magnesium; the second reaction oxidises HDL-C and subsequently produces a coloured dye, the intensity of which is directly proportional to the level of serum HDL-C.

### 2.3.1.4 Low Density Lipoprotein Cholesterol (LDL-C)

**Determination:** Low density lipoprotein cholesterol (LDL-C) was determined using the calculation: \( \text{LDL-C} = \text{Total cholesterol} - (\text{HDL-C}) - (0.4545 \times \text{Triglycerides}) \).

### 2.3.2 Insulin and Glucose

**Blood collection:** Blood was collected into a 4ml lithium heparin tube.

**Preparation:** Samples were centrifuged at 3800 rpm, 4°C for 10 minutes and plasma separated from whole blood within 2hrs of collection.

**Determination:** Plasma glucose levels were determined using an enzymatic method involving hexokinase and oxidation, with a bichromatic (340, 383nm) endpoint (GLU Flex reagent cartridge, Dimension Vista System, Siemans Healthcare Diagnostics Inc. 2008, Newark USA). Plasma insulin levels were determined using an immunoassay (Access Ultrasensitive Insulin assay, Beckman Coulter Inc. 2008, CA USA).

### 2.3.3 Systemic Inflammatory Markers

#### 2.3.3.1 High sensitivity C-reactive protein (hsCRP)

**Blood collection:** Blood was collected into a 4ml lithium heparin coated tube.

**Preparation:** Samples were centrifuged at 3800 rpm, 4°C for 10 minutes and plasma separated from whole blood within 2hrs of collection.
**Determination**: Samples were mixed with monoclonal antibody-coated polystyrene particles, specific for human CRP (CRP Flex reagent cartridge, Dimension Vista System, Siemens Healthcare Diagnostics Inc. 2008, Newark USA). These formed aggregates, which scatter light as it passes through the sample. The intensity of scattered light was proportional to the protein content of the sample. The result was then compared with a standard of known hsCRP concentration.

### 2.3.3.2 Interleukin (IL)-6

**Blood collection**: Blood was collected into a 4ml EDTA tube.

**Preparation**: Samples were processed within 1hr of collection. Samples were centrifuged at 3000 rpm, 4°C for 10 minutes. Plasma and red blood cell aliquots were stored at -80°C until bulk analysis on all samples could be completed.

**Determination**: Plasma IL-6 was measured using a high-sensitivity commercial ELISA assay (R&D Systems, Minneapolis MN USA). The sensitivity for this assay was 0.039pg/ml. All samples were tested in duplicate.

### 2.3.4 Hormones

#### 2.3.4.1 Leptin and Adiponectin

**Blood collection**: Blood was collected into a 4ml serum tube and a 4ml EDTA tube.

**Preparation**: Samples were centrifuged at 3000rpm, 4°C for 10 minutes and transferred to a clean tube. Serum, plasma and red blood aliquots were stored at -80°C until bulk analysis on all samples could be completed. Centrifugation was repeated on thawing to clear sample of precipitate.

**Determination**: Serum/ plasma leptin was measured using a commercial ELISA assay (Bio-Rad, Hercules CA USA) at a 1:4 dilution of sample. Serum adiponectin was measured using a commercial ELISA assay (Bio-Rad, Hercules CA USA) at a 1:400
dilution of sample. Assay sensitivity was 3.1pg/ml and 32.7pg/ml, respectively. All samples were tested in duplicate.

2.3.4.2 Ghrelin

**Blood collection:** Blood was collected into a 4ml EDTA tube.

**Preparation:** Samples were processed within 1hr of collection. Samples were centrifuged at 3000 rpm, 4°C for 10 minutes. Plasma and red blood cell aliquots were immediately stored at -80°C until bulk analysis on all samples could be completed.

**Determination:** Total plasma ghrelin was measured using a commercial ELISA assay (Bio-Rad, Hercules CA USA). The sensitivity for this assay was 100pg/ml. All samples were tested in duplicate.

2.3.4.3 Cortisol

**Blood collection:** Blood was collected into a 4ml lithium heparin coated tube.

**Preparation:** Samples were centrifuged at 3800 rpm, 4°C for 10 minutes and plasma separated from whole blood within 2hrs of collection.

**Determination:** Plasma cortisol was measured using a commercial immunoenzymatic assay (Access Immunoassay Systems, Beckman Coulter, Fullerton CA USA). The sensitivity for this assay was 11nmol/L.

2.3.4.4 Insulin-like Growth Factor (IGF)-1

**Blood collection:** Blood was collected into a 4ml lithium heparin coated tube.

**Preparation:** Samples were centrifuged at 3800 rpm, 4°C for 10 minutes and plasma separated from whole blood within 2hrs of collection.
Determination: Plasma IGF-1 was measured using a double antibody radioimmunoassay kit (Bioclone, Marrickville NSW AUS). The sensitivity for this assay was 0.018ng/ml. All samples were tested in duplicate.

2.4 Airway inflammatory cells

Sputum analysis: Sputum was collected and chilled. All samples were processed within 30mins. Opaque mucocellular sputum portions were selected from saliva and processed using dithiothreitol (DTT)\textsuperscript{203, 204}. Cytospins were prepared, stained (May-Grunwald Geimsa) and a differential cell count obtained from 400 non-squamous cells. Sputum cells were stored in RLT buffer at -80°C. Supernatant were stored in aliquots at -80°C.

2.5 Dietary Assessment

2.5.1 Australian Child & Adolescent Eating Survey (ACAES)

Dietary intake was assessed using the Australian Child & Adolescent Eating Survey (ACAES), a 120-item semi-quantitative food-frequency questionnaire (FFQ), completed by either the parent or child. The ACAES has been tested for reliability and relative validity and demonstrates acceptable accuracy for ranking nutrient intakes in Australian youth 9 to 16 years\textsuperscript{205}.

The ACAES FFQ is designed to collect information about usual frequency of intake of 120 food items over the previous 6 months. A standard portion size is provided for each food item, determined using 'natural' serving size (eg. slice of bread) or portion sizes derived from the 1995 National Nutrition Survey (unpublished data purchased from the Australian Bureau of Statistics). An individual response is required for each food item, with frequency options for most items ranging from ‘Never’ to ‘4 or more times per day’. Food items are organised according to their food group including
drinks, breads and cereals, dairy food, main meals, sweets and snacks, fruit and vegetables.

The ACAES includes 15 additional questions about age, use of vitamin supplements, food-related behaviours and sedentary activity, including frequency of takeaway food consumption, breakfast consumption, and eating while watching television, as well as time watching television and playing computer games.

The questionnaire was analysed by computer scan technology through the School of Health Science, University of Newcastle, Australia and the results returned to the research officer for statistical analysis. Individual mean daily macro- and micronutrient intakes are calculated using FFQ-specific programs utilising the Australian food composition database, AusNut 1999 (All Foods) Revision 14 (Australian Government Publishing Service, Canberra).

A subsample of questions from the ACAES (eight food group components – 70 questions), consistent with the eating patterns recommended in the Australian Dietary Guidelines for Children and Adolescents, were utilised to calculate the Australian Child and Adolescent Recommended Food Score (ACARFS). The ACARF score ranges from zero to 73, with a higher score indicating consumption patterns more closely in line with Australian Dietary Guidelines.

2.5.2 Food Craving Inventory (FCI)-II

The Food Craving Inventory (FCI)-II is a reliable and validated measure of food cravings, with four conceptual subscales: high fats; sweet foods; carbohydrates and starches; and fast-food fats. The FCI-II is a 28-item questionnaire designed to collect information about cravings for 28 food items over the past month. The FCI-II is scored on a Likert scale from 1-5, with 1 being ‘never’ and 5 being ‘always / almost
every day’. The total score is obtained by averaging all 28 items, while the score for each sub-scale is obtained by averaging the responses for each item listed for that scale. A higher score indicates a higher frequency of a food craving in the past month.

2.6 Physical Activity Assessment

2.6.1 Adolescent Physical Activity Recall Questionnaire (APARQ)

The Adolescent Physical Activity Recall Questionnaire (APARQ) has documented reliability and validity for children 13yo-15yo\textsuperscript{207}. Authors were contacted and advised that, although there are no validation studies, the use of this tool would be appropriate in younger age groups. Participants were prompted to think of organised and non-organised activities in which they participate during a normal school week in summer and winter. Responses were divided into 4 subscales: organised summer activities, organised winter activities, non-organised summer activities, and non-organised winter activities. Activities undertaken for <10 minutes or activities with an estimated metabolic equivalent (MET) value <3.0 were excluded from analyses. A total average MET value and total weekly activity time was calculated from the responses. The average MET value and the weekly activity time were also calculated for each subscale.

2.6.2 Adolescent Sedentary Activity Questionnaire (ASAQ)

The Adolescent Sedentary Activity Questionnaire (ASAQ) has been validated for children 11-15yrs\textsuperscript{208}. Authors were contacted and advised that, although there are no validation studies, the use of this tool would be appropriate in younger age groups. Participants were instructed to record the usual amount of time spent each day in a number of sedentary activities during a typical school week. Sedentary activities included passive travel, music practice / reading, small-screen recreation and
homework. The total time spent in sedentary activities for the 7-day week was then calculated. The time spent in sedentary activities was also divided into weekdays (Monday-Friday) and weekend (Saturday-Sunday).

2.7 Statistical Analysis

All data were de-identified, entered and stored in a Microsoft Access 2007 database (Microsoft Corporation, USA). Statistical analysis was carried out using Intercooled Stata Version 11 for Windows (StataCorp., College Station, Texas, USA 1984-2005). Continuous data variables were tested for normality. Where appropriate, log transformation was used to normalise data. Continuous data are presented as mean (standard deviation, SD) or median [interquartile range, IQR]. Categorical data are presented as proportion (n, (%)). Where applicable, outcome data are reported as change (Δ) from baseline.

Results were considered statistically significant if p≤0.05. Continuous data were assessed using a paired mean-comparison t-test or Wilcoxon sign-rank test for within-group comparisons. Where there were comparisons between two groups only, an independent two-group mean-comparison t-test or Wilcoxon rank-sum test was used for continuous data. Comparisons between multiple groups (i.e. ≥3 groups) for continuous data were carried out using oneway analysis of variance (ANOVA) with post-hoc two-sample unpaired t-testing, or Kruskal Wallis with post-hoc Wilcoxon rank-sum testing. The p-value for post-hoc tests were adjusted by using the following calculation: p-value=0.05 / (number of groups-1).

Categorical data were assessed using Pearson’s Chi-squared (X²) test. Associations between variables were investigated using Pearson’s correlation coefficient,
Spearman’s Rank correlation coefficient, or multivariable linear regression analysis, where appropriate.

2.8 Ethics

Prior to participation in all studies described in Chapter 3, 4, 5 and 6, written informed consent from a parent / guardian and written informed assent from the child / adolescent was obtained. Each study described in Chapters 3, 4, 5 and 6 were undertaken at Hunter Medical Research Institute, John Hunter Hospital & John Hunter Children’s Hospital, in partnership with the University of Newcastle, NSW Australia. Approval for all studies described in Chapters 3, 4, 5 and 6 was obtained from the appropriate Institutional Review Board (IRB): the Hunter New England Human Research Ethics Committee (HNEHREC) and the University of Newcastle Human Research Ethics Committee (UNHREC). The clinical trial described in Chapter 5 is registered with the Australian New Zealand Clinical Trials Registry (ACTRN12610000955011).
3. Chapter III: Characterising Paediatric Obese Asthma: Airway and systemic inflammation in obese children with asthma
3.1 Introduction

Overweight and obesity is highly prevalent among children with asthma, and exceeds the general population rate in Australia, Denmark and America\textsuperscript{68-71}, with a recent report that up to 45\% of asthmatic children are carrying excess weight\textsuperscript{68}. Obesity appears to complicate the management of childhood asthma, with previous studies indicating that obesity is associated with an increased risk of exacerbations, poorer asthma control and increased medication use, including steroids\textsuperscript{92, 209}. Recently, significant reductions in lung volume indices have also been associated with overweight and obesity in asthmatic children\textsuperscript{114, 115}.

While the alarmingly high rates of obesity among asthmatic children warrant the development of specific interventions for this population, the first step is to better understand the presentation of obese asthma in order to inform clinical management. The mechanisms linking obesity and asthma remain poorly understood and multiple hypotheses have been proposed. Obesity may be associated with respiratory symptoms via cardio-respiratory de-conditioning, physiological restriction of the chest wall by excess mass, or co-morbidities, including gastro-oesophageal reflux and sleep disordered breathing\textsuperscript{106}. Alternatively, chronic systemic inflammation, characteristic of obesity, has been hypothesised as an underlying factor in obese asthma that may contribute to altered airway inflammation and poorer clinical outcomes\textsuperscript{33}.

As a chronic inflammatory disease of the airways, asthma is traditionally characterised by eosinophilic airway inflammation. However, in adults, obese asthma has been described as a distinct clinical phenotype, involving non-eosinophilic airway inflammation and being unresponsive to current pharmacological treatment\textsuperscript{89, 91, 103}.  

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Raised systemic inflammation is also characteristic of obese asthma in adults and has been linked to airway inflammation and poorer clinical outcomes\(^9\). However, airway inflammation in obese asthmatic children has not been adequately described. Therefore, the aim of this study is to characterise obese asthma in children by describing airway inflammation, systemic inflammation and clinical asthma outcomes in obese and non-obese children, with and without asthma.

### 3.2 Methods

#### 3.2.1 Subjects

Obese and non-obese children, with and without asthma, aged 8-17 years, were recruited from the general community and John Hunter Hospital (JHH) outpatient clinics, Newcastle NSW, Australia, from July 2004-2011. Asthma was defined by physician diagnosis and current respiratory symptoms. Obesity was defined as a body mass index (BMI) \(z\)-score \(\geq 1.64\) standard deviation score (SDS). Participants were assigned to one of four groups: obese asthma (OA, \(n=74\)); non-obese asthma (NOA, \(n=249\)); obese control (OC, \(n=9\)); or non-obese control (NOC, \(n=29\)). Exclusion criteria included unexplained weight change during the past three months, inflammatory/ endocrine disease, or respiratory disorder other than asthma. The Hunter New England and University of Newcastle Human Research Ethics Committees approved this study (09/05/20/5.08). Informed participant assent (Appendix 1) and guardian consent (Appendix 2) were obtained.

#### 3.2.2 Clinical assessment

Participants attended JHH for clinical testing after an overnight fast and withholding antihistamines and asthma medications (Appendix 3). Clinical asthma pattern, current asthma status, and quality of life was assessed using the Global Initiative for Asthma
(GINA) guidelines\textsuperscript{196}, Juniper Asthma Control Questionnaire (ACQ)\textsuperscript{210}, and Paediatric Asthma Quality of Life Questionnaire (standardised) (PAQLQ(s))\textsuperscript{199}, respectively. Asthma stability was confirmed, defined as no exacerbation, respiratory tract infection or oral corticosteroid use in the past 4 weeks. Exhaled Nitric Oxide (eNO) was measured (NiOX chemiluminescent detector unit, Aerocrine, Zynergy Medical). This measurement always occurred prior to lung function testing and the combined bronchial provocation testing and sputum induction (described below). Atopy was determined by positive skin prick test to common allergen(s) (Aspergillus fumigatus, Alternaria tenius, Dust mite (Dermatophagoides Pteronyssinus), Cockroach mix, Grass mix). Tobacco exposure was measured by urinary cotinine (NicAlert, Nymox Pharmaceutical Corp, USA NJ). Weight and height were measured using 150 kg max scales (EB8271 NuWeigh, Newcastle Weighing Services NSW, Australia) and 2 m wall-suspended measuring tape with wall stop (Surgical and Medical Supplies Pty Ltd SA, Australia). BMI was calculated (weight (kg) / height (m)\textsuperscript{2}) and converted to BMI \textit{z}-scores\textsuperscript{193}. All participants performed spirometry (Windows KoKo PFT System Version 4.9 2005, PDS Inc Louisville USA) and a subset of participants performed lung plethysmography (MedGraphics Elite Series Plethysmograph, USA; Breeze Suite 6.4.1.14 Version 510 2008, MedGraphics Corp., USA). Lung function variables are presented as a percentage of their predicted value\textsuperscript{194, 195}.

3.2.3 Sputum inflammatory cells

Participants underwent combined bronchial provocation testing and sputum induction with hypertonic saline (4.5%) (ULTRA-NEB\textsuperscript{TM} ultrasonic nebuliser, DeVilbiss, Model 2000), as previously described\textsuperscript{64}. Airway hyperresponsiveness (AHR) was defined as a fall in FEV\textsubscript{1} \textgeq 15\% from baseline. The dose response slope (DRS) and
log-transformed provocation dose (log-\(PD_{15}\)) were calculated. Sputum was selected, dispersed with dithiothreitol, and total cell counts and viability determined. Cytospins were prepared, stained (May-Grunwald Geimsa) and a differential cell count obtained. Eosinophilic asthma was defined as sputum eosinophilia \(\geq 2.0\%\).

### 3.2.4 Systemic inflammatory mediators

Blood samples collected from a participant subset were centrifuged at 3000 rpm, 4°C for 10 minutes. High sensitivity C-Reactive Protein (CRP) was measured from serum mixed with monoclonal antibody-coated polystyrene particles, specific for human CRP (CRP Flex reagent cartridge, Dimension Vista System, Siemans Healthcare Diagnostics Inc. 2008, Newark USA). Commercial ELISAs were used to measure plasma interleukin (IL)-6 (R&D Systems, Minneapolis MN USA), and serum leptin and adiponectin (Bio-Rad, Hercules CA USA). Assay sensitivity was 0.039pg/ml, 3.1pg/ml and 32.7pg/ml, respectively. All samples were tested in duplicate.

### 3.2.5 Statistical analysis

Data are presented as mean±SD, median[IQR], or proportion \((n, \%)\). Data variables were assessed for normality prior to analysis using oneway-ANOVA with post-hoc two-sample unpaired t-testing or Kruskal Wallis with post-hoc Wilcoxon rank sum testing for continuous data, and Pearson’s Chi-squared test for categorical data. Alpha was set at \(\leq 0.05\) for all tests. Statistical analysis was performed using Intercooled Stata Version 11.0 for Windows (StataCorp, College Station, Texas, USA 1984-2005).

### 3.3 Results

Subject characteristics are presented in Table 3.1. The prevalence of atopy was lower in OC compared to both asthma groups, and lower in NOC compared to NOA. FEV\(_1\)
%predicted, FVC %predicted, and TLC %predicted did not differ by statistical significance across the four groups. However, FEV$_1$/TLC% was significantly lower in both asthma groups compared to NOC. FRC %predicted (p=0.046), ERV %predicted (p=0.004), RV %predicted (p=0.003) and RV/TLC% (p=0.005) differed significantly across groups (Figure 3.1). While there was no significant difference in FRC %predicted in post-hoc analysis, differences between OA and NOC for ERV %predicted, and between OC and OA for RV %predicted, were detected. Likewise, RV/TLC% was significantly different between OC and both NOA and OA (Figure 3.1).

Table 3.1: Subject characteristics & lung function summarised by obesity and asthma status

<table>
<thead>
<tr>
<th>Subject Characteristics</th>
<th>Asthma</th>
<th>Controls</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Obese (n=74)</td>
<td>Non-Obese (n=249)</td>
<td></td>
</tr>
<tr>
<td>Age (years); mean(SD)</td>
<td>11.1(2.8)</td>
<td>11.1(3.0)</td>
<td>0.344</td>
</tr>
<tr>
<td>Gender (% females); %</td>
<td>47.3</td>
<td>38.6$^b$</td>
<td></td>
</tr>
<tr>
<td>Height (cm); mean(SD)</td>
<td>150.5(15.3)$^a$</td>
<td>143.8(16.1)</td>
<td>0.014</td>
</tr>
<tr>
<td>Weight (kg); mean(SD)</td>
<td>65.4(24.7)$^{ab}$</td>
<td>39.1(13.4)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>BMI z-score (SDS); mean(SD)</td>
<td>2.1(0.3)$^{ab}$</td>
<td>0.2(0.9)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Atopic (Y/N); n (%Y)$^\dagger$</td>
<td>27/12$^c$ (69.2)</td>
<td>42/8$^{bc}$ (84.0)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>FEV$_1$ (%$^\ddagger$); mean(SD)</td>
<td>96.1(14.1)</td>
<td>96.3(14.0)</td>
<td>0.908</td>
</tr>
<tr>
<td>FVC (%$^\ddagger$); mean(SD)</td>
<td>100.5(11.2)</td>
<td>97.4(11.9)</td>
<td>0.581</td>
</tr>
<tr>
<td>FEV1/FVC (%)$^\ddagger$; mean(SD)</td>
<td>79.2(6.6)$^b$</td>
<td>80.7(8.2)$^b$</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>TLC (%)$^\ddagger$; mean(SD)$^\dagger$</td>
<td>106.8(13.3)</td>
<td>100.3(10.7)</td>
<td>0.568</td>
</tr>
<tr>
<td>FEV$_1$/TLC (%)$^\ddagger$; mean(SD)</td>
<td>88.5(12.9)$^b$</td>
<td>86.5(9.4)$^b$</td>
<td>0.003</td>
</tr>
</tbody>
</table>

BMI, body mass index; FEV$_1$, forced expiratory volume in 1 second; FVC, forced vital capacity; SDS, standard deviation score; TLC, total lung capacity; $^a$ vs non-obese asthma; $^b$ vs non-obese controls; $^c$ vs obese controls; p<0.017 for post-hoc analyses; $^\dagger$data available on a subset of participants only; $^\ddagger$ expressed as a percentage of predicted values.

The prevalence of AHR was similar in OA and NOA (42.5 vs 35.3%, p=0.346), as was the DRS (1.02[0.53, 2.9] vs 1.25[0.38, 3.22]%fall/ml, p=0.798) and log-PD$_{15}$ (1.26(1.47) vs 1.24(1.06)ml, p=0.928). The OA had a significantly lower PAQLQ
score (5.9[4.7, 6.4] vs 6.4[6.0, 6.6], p=0.035) and a smaller proportion used inhaled corticosteroids (ICS) (52.0 vs 75.8%, p=0.004), compared to NOA. However, there was no significant difference in the median ACQ score (0.6[0.3, 1.3] vs 0.7[0.4, 1.0], p=0.933) or steroid dose (400[400, 800] vs 400[200, 675] beclomethasone equivalents, p=0.721), between OA and NOA. A statistically significant difference (p=0.034) in the proportion of OA versus NOA classified as intermittent (35.7 vs 24.1%), mild (19.1 vs 44.8%), moderate (33.3 vs 27.6%) and severe (11.9 vs 3.5%) asthma was detected.

![](image)

**Figure. 3.1** Lung volumes indices as measured by lung plethysmography.

a) functional residual capacity (FRC) %predicted, b) expiratory reserve volume (ERV) %predicted, c) residual volume (RV) %predicted, d) RV as a percentage of total lung capacity (TLC). OA, obese asthma (n=31); NOA, non-obese asthma (n=12); OC, obese control (n=9); NOC, non-obese control (n=15).

Compared to all groups, eNO was significantly lower in OC (Table 3.2). However, there was no difference between NOA and OA. The number and proportion of eosinophils did not differ by statistical significance between the four groups.
Conversely, %neutrophils were significantly different across the four groups, with a trend towards a higher proportion in the asthmatic groups versus the control groups. However, post-hoc analysis did not detect a statistically significant difference. Significantly lower %macrophages were detected in the NOA compared to both control groups, while absolute macrophage numbers were significantly lower compared to all groups.

Among OA, %eosinophils were significantly higher in males versus females (Table 3.3), and a significantly higher proportion of females compared to males had non-eosinophilic asthma (Figure 3.2). This was not true for NOA. However, no statistically significant gender differences were evident in airway neutrophils (Table 3.3). Given the difference in %eosinophils in the OA, we compared medication usage between males and females. There was no difference in ICS or SABA usage, with a similar median steroid dose between male and female OA (400[200, 400] vs 400[400, 800] beclomethasone equivalents, p=0.199).

![Figure 3.2](image)

**Figure 3.2** The proportion of eosinophilic asthma (≥2% sputum eosinophils) and non-eosinophilic asthma among obese and non-obese, male and female children.
OA, obese asthma; NOA, non-obese asthma.
Table 3.2: Exhaled nitric oxide and sputum inflammatory cell counts by obesity and asthma status

<table>
<thead>
<tr>
<th>Airway Inflammatory Markers</th>
<th>Asthma</th>
<th>Controls</th>
<th>$p$-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Obese (n=55)</td>
<td>Non-Obese (n=199)</td>
<td>Obese (n=9)</td>
</tr>
<tr>
<td>Exhaled nitric oxide (ppb); median[IQR]</td>
<td>27.9[10.5, 46.7]$^a$</td>
<td>27.5[16.8, 55.7]$^a$</td>
<td>6.1[2.4, 10.4]</td>
</tr>
<tr>
<td>Interleukin-8 (pg/ml); median[IQR]</td>
<td>1.7[0.4, 6.4]</td>
<td>1.2[0.3, 3.1]</td>
<td>n/a</td>
</tr>
<tr>
<td>Total cell count (x 10$^6$/ml); median[IQR]</td>
<td>2.5[1.3, 4.6]</td>
<td>1.7[0.7, 3.7]$^b$</td>
<td>3.7[2.9, 6.5]</td>
</tr>
<tr>
<td>Eosinophils (%)</td>
<td>0.8[0.5, 5.9]</td>
<td>1.1[0, 7.0]</td>
<td>0.0[0.0, 0.3]</td>
</tr>
<tr>
<td>Eosinophils (x 10$^6$/ml); median[IQR]</td>
<td>0.02[0, 0.1]</td>
<td>0.02[0, 0.1]</td>
<td>0.00[0.0, 0.02]</td>
</tr>
<tr>
<td>Neutrophils (x 10$^6$/ml); median[IQR]</td>
<td>0.5[0.2, 1.5]</td>
<td>0.4[0.2, 1.2]</td>
<td>0.5[0.1, 1.2]</td>
</tr>
<tr>
<td>Macrophages (%)</td>
<td>61.8(22.6)</td>
<td>51.9(25.9)$^{bc}$</td>
<td>79.6(20.6)</td>
</tr>
<tr>
<td>Macrophages (x 10$^6$/ml); median[IQR]</td>
<td>1.8[1.1, 2.5]$^a$</td>
<td>1.0[0.5, 1.6]$^{bc}$</td>
<td>2.7[1.8, 5.4]</td>
</tr>
<tr>
<td>Lymphocytes (%)</td>
<td>0.9[0.2, 2.4]</td>
<td>0.5[0.1, 1.5]</td>
<td>1.1[0.0, 1.3]</td>
</tr>
<tr>
<td>Lymphocytes (x 10$^6$/ml); median[IQR]</td>
<td>0.02[0, 0.1]</td>
<td>0.01[0, 0.04]$^b$</td>
<td>0.04[0.0, 0.1]</td>
</tr>
<tr>
<td>Epithelial (%)</td>
<td>2.3[0.5, 5.5]$^b$</td>
<td>3.0[0.5, 7.0]$^b$</td>
<td>0.3[0.0, 2.3]</td>
</tr>
</tbody>
</table>

$^a$ vs non-obese asthma; $^b$ vs non-obese controls; $^c$ vs obese controls; $p$<0.017 for post-hoc analyses
<table>
<thead>
<tr>
<th>Airway inflammatory markers</th>
<th>Obese asthma</th>
<th>Non-obese asthma</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Males n=26</td>
<td>Females n=26</td>
<td>Males n=120</td>
</tr>
<tr>
<td>Exhaled nitric oxide (ppb); median[IQR]</td>
<td>33.5[12.8, 49.8]</td>
<td>11.2[8.6, 28.2]</td>
<td>28.0[20.3, 73.7]</td>
</tr>
<tr>
<td>Interleukin-8 (pg/ml); median[IQR]</td>
<td>2.27[0.5, 5.56]</td>
<td>1.67[0.03, 6.84]</td>
<td>1.19[0.15, 3.29]</td>
</tr>
<tr>
<td>Eosinophils (%) ; median[IQR]</td>
<td>3.8[0.5, 9.8]a</td>
<td>0.0[0.0, 1.0]</td>
<td>1.4[0.0, 7.5]</td>
</tr>
<tr>
<td>Eosinophils (x 10^6/ml); median[IQR]</td>
<td>0.11[0.01, 0.12]</td>
<td>0.0[0.0, 0.04]</td>
<td>0.03[0.0, 0.14]</td>
</tr>
<tr>
<td>Neutrophils (%) ; median[IQR]</td>
<td>21.6[8.5, 29.8]</td>
<td>19.6[9.5, 42.0]</td>
<td>28.0[13.0, 56.5]</td>
</tr>
<tr>
<td>Neutrophils (x 10^6/ml); median[IQR]</td>
<td>0.5[0.2, 2.4]</td>
<td>0.5[0.1, 1.3]</td>
<td>0.4[0.2, 1.2]</td>
</tr>
<tr>
<td>Macrophages (%) ; mean(SD)</td>
<td>60.0(22.4)</td>
<td>63.7(23.1)</td>
<td>51.9(25.6)</td>
</tr>
<tr>
<td>Macrophages (x 10^6/ml); median[IQR]</td>
<td>1.8[1.1, 3.0]b,e</td>
<td>1.7[1.1, 2.5]b,e</td>
<td>1.0[0.5, 1.8]</td>
</tr>
</tbody>
</table>

*a vs obese asthma females; b vs non-obese asthma males; c vs non-obese asthma females; p<0.0167 for post-hoc analyses
Leptin (p=0.032), adiponectin (p=0.010) and IL-6 (p=0.025) levels differed significantly across the four groups, while CRP levels did not (p=0.197). In post-hoc analysis, leptin levels were significantly higher in OC compared to NOA and NOC, and adiponectin levels were lower in OA compared to NOC, while no statistically significant difference was detected for IL-6 (Figure 3.3). Gender differences were not evident in systemic inflammatory biomarkers.

Figure. 3.3 Systemic inflammatory biomarkers in obese & non-obese children, with & without asthma. a) serum leptin (ng/ml), b) serum adiponectin (μg/ml), c) plasma interleukin (IL)-6 (pg/ml), d) serum C-Reactive Protein (mg/ml). OA, obese asthma (n=32); NOA, non-obese asthma (n=14); OC, obese control (n=9); NOC, non-obese control (n=16).

3.4 Discussion

Obese asthma was not associated with elevated airway or systemic inflammation in this group of children. However, a higher proportion of obese females had non-eosinophilic asthma compared to obese males, suggesting gender differences in
airway inflammation exist. Obesity was associated with lung restriction, which manifested as reduced ERV in asthma and reduced RV in controls. Furthermore, obesity was associated with a clinically significant reduction in quality of life in asthmatic children.

To our knowledge this is the first study to report airway inflammatory cell counts in obese children. In adults, obese asthma follows a non-eosinophilic steroid-resistant pattern of airway inflammation, characterised by neutrophilia and predominant among females. An association between %neutrophils and BMI has been reported in female, but not male, adults with asthma. Our data did not detect altered airway neutrophils in obese female children with asthma. However, among obese asthmatics, %eosinophils were higher in males, and a significantly higher proportion of obese females had non-eosinophilic asthma. The obese female children also had a lower eNO, consistent with a non-eosinophilic pattern of airway inflammation. This requires further examination in a larger cohort.

The current study suggests children with asthma have a lower number and proportion of sputum macrophages, with a significant reduction in NOA compared to controls. Previous studies have observed a lower proportion and number of macrophages in non-obese children and adults with asthma, compared to controls. However, macrophage functional capacity, rather than infiltration, may be more relevant to the initiation and propagation of airway inflammation. Indeed, the presence of mature macrophages is greater in asthmatics, with airway macrophages from wheezy infants found to release greater amounts of pro-inflammatory mediators compared to non-wheezy infants. Macrophages were not statistically significantly reduced in the obese asthmatics compared to controls. An obesity-induced rise in tissue-resident macrophage numbers may translate into the airways and account for the preserved
number and proportion of macrophages seen in the obese asthmatic group in our study.

Systemic inflammation occurs in adult obese asthma, with IL-6 almost 2-3 times higher and CRP approximately 7-8 times higher in obese versus non-obese adults. Previous studies have also reported adiponectin to be 50% lower in obese children and adults, compared to non-obese counterparts. In our study, serum adiponectin was reduced in asthmatic and non-asthmatic obese children, compared to non-obese controls. However, this reduction was statistically significant in obese asthmatics only, and may reflect the small sample of obese controls. IL-6 and CRP were not significantly raised in the asthmatic or non-asthmatic obese children in our study population, which agrees with previous paediatric studies that did not detect elevated CRP, IL-6 or tumour necrosis factor (TNF)-α levels in obese children. The type and distribution of adipose tissue, duration of obesity, gender and age are important factors predicting the metabolic effects of obesity, including the up-regulated secretion of inflammatory mediators and impaired endocrine cross-talk with other organs involved in the inflammatory process, such as the liver. In the early stages of obesity, and in youth, adipose tissue expands largely in subcutaneous depots, which are less metabolically active than visceral adipose tissue. The point at which adipose tissue becomes pathological may be an important distinction between children and adults when considering the effects of obesity on inflammatory and clinical outcomes.

Leptin has been suggested as a mediator between asthma and obesity, with leptin levels reportedly 2-5 times higher in obese adults with asthma. Studies in asthmatic and non-asthmatic children have found leptin levels to be 2-3 times higher in the obese versus non-obese. In contrast, leptin levels in the current study were
not elevated in obese asthmatics compared to non-obese asthmatics or controls, but were only significantly raised in obese children without asthma. This is a novel finding and may suggest that Th2 cytokines, which drive immune responses in childhood asthma, are influencing tissue-resident macrophage function. In obesity, there is a shift from alternatively activated macrophages (M2), which are induced by Th2 cytokines and can modulate and down-regulate proinflammatory mediators, to classically activated macrophages (M1), which are induced primarily by Th1 cytokines and responsible for the chronic secretion of proinflammatory mediators, including leptin and IL-6. Therefore, obesity-induced metabolic abnormalities may depend on the balance in Th1/Th2 signalling which influence macrophage function.

Recently, eosinophils have been shown to direct macrophages in adipose tissue to a M2 phenotype through release of Th2 cytokines. Given that childhood asthma is predominantly driven by a Th2 immune response, the modulation of macrophage function by Th2 cytokines may explain the low leptin levels observed in the obese asthmatic children. However, this hypothesis requires further investigation. It should be noted that there was variability in leptin levels in the OA, and a number of individuals had elevated leptin levels. Thus, this area warrants further investigation in a larger sample size.

Obesity exerts its greatest detriment on lung volume measurements, with reductions observed in ERV and FRC in adults, with and without asthma. Similarly, reductions in ERV were detected in obese asthmatic children, compared to non-obese children without asthma. However, in contrast to recent reports in children, the obese controls had a significantly reduced RV %predicted and RV/ TLC%, which was not observed in the obese asthmatic children. Rastogi et al recently reported RV %predicted and RV/ TLC% to be significantly lower in obese asthmatic children,
compared to non-obese children without asthma\textsuperscript{115}. Likewise, Mahut et al reported RV/ TLC to be lower in overweight and obese children, compared to normal-weight children with asthma, identifying a negative relationship between BMI z-score and FRC and RV, expressed as both %predicted and relative to TLC\textsuperscript{114}. Preserved RV in the asthmatic children in the presented study may be attributable to the obstructive effect of asthma, causing ‘air-trapping’ in the distal airways and an inflated RV\textsuperscript{216}. Importantly, reductions in lung function associated with obesity were not detected through routine spirometry, and our data identifies lung volume assessment as an important clinical measure in this group of children.

Among asthmatic children, quality of life was both clinically and statistically significantly lower in the obese versus non-obese. Although some studies have found obesity is associated with poorer quality of life in asthmatic children, other studies have found no difference between obese and non-obese children, and it has been suggested that obesity may interfere with the perception of respiratory symptoms in children\textsuperscript{92, 217}. Alternatively, obese asthmatic children may perceive worse symptoms related to a clinically significant reduction in ERV. However, obesity was not associated with poorer asthma control, as measured by ACQ. Furthermore, airway reactivity to hypertonic saline did not differ between obese and non-obese asthmatics in our study. Previous studies examining obesity and AHR in children and adults provide conflicting results, with some reporting a higher prevalence or severity of AHR in obese compared to non-obese asthmatics, while others report no difference\textsuperscript{92}. Previous studies also report increased medication use in obese asthmatic children and adults\textsuperscript{92, 209}. Our data didn’t detect a difference in steroid dose between obese and non-obese children with asthma. In fact, a smaller proportion of obese children were
reportedly taking ICS medication. However, our data is limited by patient/guardian report of medication use, which may not reflect actual practice by this group.

Obesity appears to influence asthma in childhood via mechanical restriction, with significant reductions in static lung function and quality of life. Obesity was not associated with altered airway or systemic inflammation in this cross-sectional analysis of children with stable asthma. However, a gender difference in sputum eosinophilia among obese children with asthma was noted and warrants further investigation. Obese asthma in adults is associated with distinct airway and systemic inflammatory alterations which may become apparent in this group of children as they transition through adolescence into adulthood. Therefore, longitudinal studies, including repeated measures of sputum and systemic inflammatory markers, are needed to improve our understanding of obese asthma.
4. Chapter IV: Characterising Paediatric Obese Asthma: Relationships between body composition and lung function in children with and without asthma
4.1 Introduction

Obesity is a highly and increasingly prevalent condition in children\(^2\), with approximately one in four Australian children and one in three American children now above the 85\(^{th}\) body mass index (BMI) percentile\(^7,218\). Importantly, obesity is a clinically significant risk factor for asthma in children and adults\(^33,73\). Evidence suggests that obesity alters respiratory function in adults, with and without asthma. Reductions in forced expiratory volume in one second (FEV\(_1\)) and forced vital capacity (FVC) have been reported in obese adults compared to healthy controls\(^219\). However, obesity in adults appears to have a more clinically important detriment on static lung function\(^136\), with a strong inverse relationship between BMI and both functional residual capacity (FRC) and expiratory reserve volume (ERV) reported\(^112,136,220\). In children, the data are inconsistent. Higher values for FEV\(_1\) and FVC have been reported to parallel body weight and BMI in cross-sectional studies for children with and without asthma\(^219,221\). However, a negative association between childhood weight and both dynamic and static lung function has also been reported\(^107,114,115\).

Body weight and BMI, although important and useful tools clinically, do not reveal information regarding body composition\(^222\). It is likely that different types of tissue have different physiological effects and therefore the composition of body weight may have clinical relevance to lung function\(^92,223\). In adults, total and regional fat mass have been negatively associated with lung function, with strong correlations reported for FRC and ERV\(^142\). Differentiating fat versus lean mass may therefore be an important factor in examining the relationship between lung function and obesity. The relationship between body composition and lung function in children with and without asthma has not been adequately described. Only one previous study in
morbidly obese non-asthmatic children has reported reductions in lung volumes with increased DEXA-measured percentage body fat.

Recent literature has also indicated that the regional distribution of body composition may be important to respiratory function in adults. Increases in DEXA-measured thoracic adiposity in non-asthmatic adults has been associated with poorer lung function and lower lung volume values, indicating that this region may be an important anthropometric measure. However, it’s use and reproducibility has not been reported in children.

We hypothesise that a) body weight negatively predicts FRC and ERV in school-aged children, with and without asthma; and b) any observed detrimental effect of obesity on FRC and ERV is associated with increased adiposity. Therefore, the primary aim of this study was to investigate whether body weight and body composition are predictive of FRC and ERV in school-aged children with and without asthma, using multi-variable linear regression analysis. A secondary aim was to assess the reproducibility of DEXA-measured total and regional body composition in children, including the thoracic region, by examining agreement between dual total body scans using intraclass correlation coefficients (ICC).

4.2 Materials and Methods

4.2.1 Study participants
Obese and non-obese participants aged 8-17 years, with and without asthma, were recruited from the general community in Newcastle, Australia using media advertisement, medical centres, John Hunter Hospital outpatient clinics and school newsletters, during the period June 2009 to March 2011. Participants were given nutritional consult(s) with an Accredited Practising Dietitian and feedback on results.
Asthma was defined by physician diagnosis and episodic respiratory symptoms in the past year. Exclusion criteria included metabolic or cardiac co-morbidities, inflammatory disease, respiratory disorder other than asthma, or unexplained weight change during the past three months. The study was approved by Hunter New England and University of Newcastle Human Research Ethics Committee (Approval number: 09/05/20/5.08). Participant assent and guardian consent was obtained.

4.2.2 Clinic Visits

Participants attended the John Hunter Hospital for testing and were instructed to withhold antihistamines and asthma medications for 6-24hrs prior to the visit if applicable. Clinical asthma pattern was classified according to GINA guidelines. Current asthma status was assessed using the Juniper Asthma Control Questionnaire. Asthma stability was confirmed and defined as no exacerbation, respiratory tract infection or oral corticosteroid use in the past 4 weeks. Airway hyperresponsiveness to hypertonic saline (4.5%) was defined by a ≥15% drop in baseline FEV\textsubscript{1}. Atopy was evaluated by a positive skin prick test to common allergen(s) (Aspergillus fumigatus, Alternaria tenius, Dust mite (Dermatophagoides Pteronyssinus), Cockroach mix, Grass mix). Tobacco smoke exposure was measured via urinary cotinine assay (NicAlert, Nymox Pharmaceutical Corp, USA NJ).

4.2.3 Anthropometry

4.2.3.1 Non-DEXA measurements

Weight and height were measured to nearest 100g using 150kg maximum scales (EB8271 NuWeigh, Newcastle Weighing Services NSW, Australia) and 2m wall-suspended measuring tape to nearest 0.1cm with wall stop (Surgical and Medical
Supplies Pty Ltd SA, Australia) with the children barefoot and wearing light clothing. BMI was calculated \( \frac{\text{weight (kg)}}{\text{height (m)}^2} \) and converted to BMI \( z \)-scores\(^{193}\).

### 4.2.3.2 DEXA measurements

Dual total body scans were conducted on all participants using DEXA (GE Lunar Prodigy, Medtel; GE Healthcare encore 2007 software Version 11.40.004, Madison USA). Participants were scanned on the appropriate setting as per the machine selection (thin / standard / thick mode). The participants arose from the table after the first scan, walked briefly around the room, and were then repositioned immediately by the same technician for the second scan. The scanner provided percentage total and regional body fat, and absolute total and regional bone mineral density, fat mass and lean mass. Manual adjustments were made to each scan to ensure the regions of the body were contained within the set parameters. The height of the android region of interest was equal to 20% of the distance between the pelvis and the neck of femur, with the inferior border defined by the upper part of the pelvis. The height of the gynoid region was 1.5 times the height of the android region, with the upper border at the superior part of the trochanter major (Figure 4.1). The thoracic region was framed using the ‘scanner region of interest’ function of the software. A box was first overlaid and then adjusted to ensure dissection through the acromioclavicular joint by two vertical lines bordering the rib cage, a horizontal line beneath the chin, and a horizontal line at the

![Figure 4.1. DEXA scan depicting key regions of interest. Image prior to manual adjustment.](image-url)
infero-lateral limit of the rib cage. This region of interest has previously been described\textsuperscript{142}.

### 4.2.4 Respiratory Function Assessment

Forced expiratory volume in one second (FEV\textsubscript{1}) and forced vital capacity (FVC) were measured using spirometry (KoKo PD Instrumentation Louisville CO USA). Lung volumes were measured using a plethysmograph (MedGraphics Elite Series Plethysmograph, USA). The instruments were calibrated prior to patient assessment. The best of three technically acceptable values was accepted for use in the primary statistical analysis. For descriptive tabulation, these values were expressed as a percentage of the predicted value\textsuperscript{194}.

### 4.2.5 Statistical Analysis

Subject characteristics are cross-classified by sex and gender and summarised in terms of counts (n), means (with standard deviations (SD)), and medians (with interquartile range (IQR)). All tests were of size alpha = 0.05 and no adjustments were made for multiple comparisons.

Given that both fat and lean regional mass variables were highly correlated with the respective total fat and lean mass, only the total fat and total lean mass variables were included in the regression models. The average values for total fat and total lean mass (kg) were calculated from the dual DEXA scans. To reduce any potential error from the assumption that the chosen reference values are applicable to our study group\textsuperscript{224}, lung function variables remained in their original scale (L) for inclusion in the models. Multivariable regression models were used, controlling for age, gender, height and asthma, in which a lung function variable was the dependent variable of interest and the independent variable of interest was (i) body weight, (ii) total fat...
mass, or (iii) total lean mass. The equation used was of the form: E[lung function variable] = A_1 + A_2*MALE + A_3*ASTHMA + A_4*AGE + A_5*HEIGHT + B*MEASURE; in which MALE is a binary indicator of male gender, ASTHMA is a binary indicator of asthma diagnosis, and MEASURE is the anthropometric measure.

The adjusted-R^2 (R^2) and p-value for the final model, and the β-coefficient and respective 95% confidence interval and p-value for the independent variables are reported. The beta-coefficient is a statistical estimate of the change ('B') in the population mean response for each 1 unit increase in the independent variable. The null hypothesis that 'B' is zero was tested using an F-test procedure of size alpha=0.05. For each model fitted, auxiliary analyses were performed to evaluate goodness of fit and to evaluate the model's assumption that the residuals follow a Gaussian distribution.

Reproducibility of the DEXA scans was assessed using intraclass correlations and Bland Altman plots. The intraclass correlation coefficient (ICC) (-95%CI, +95%CI) for each manually-adjusted DEXA region of interest are reported. The data being pairs of measures, the estimator of the ICC was the Pearson correlation coefficient (r). An r > 0.80 indicates strong correlation between the two measurements. Intercooled Stata Version 11.0 for Windows (StataCorp, College Station, Texas, USA 1984-2005) was used for all statistical computations.

### 4.3 Results

Subject characteristics are summarised by sex and asthma status (Table 4.1). The majority of children with asthma were classified as intermittent or mild persistent according to the GINA classification system. Urinary cotinine levels were negligible in all participants and therefore tobacco exposure was not given further consideration.
### Table 4.1: Subject characteristics by gender and asthma status

<table>
<thead>
<tr>
<th>Subject Characteristics</th>
<th>Non-asthmatic children</th>
<th>Asthmatic children</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Females (n=14)</td>
<td>Males (n=11)</td>
</tr>
<tr>
<td><strong>Age (years); mean(SD)</strong></td>
<td>13.1(1.7)</td>
<td>12.0(2.1)</td>
</tr>
<tr>
<td><strong>Weight (kg); mean(SD)</strong></td>
<td>56.5(16.4)</td>
<td>57.8(24.0)</td>
</tr>
<tr>
<td><strong>Height (cm); mean(SD)</strong></td>
<td>157.5(12.4)</td>
<td>153.6(15.6)</td>
</tr>
<tr>
<td><strong>BMI z-score (SDS); median[IQR]</strong></td>
<td>0.7[-0.4, 1.3]</td>
<td>0.7[0.1, 1.4]</td>
</tr>
<tr>
<td><strong>Total Body Fat (%); mean(SD)</strong></td>
<td>34.2(11.4)</td>
<td>31.8(14.5)</td>
</tr>
<tr>
<td><strong>FEV₁ %predicted (%); mean(SD)</strong></td>
<td>94.7(9.9)</td>
<td>97.0(13.9)</td>
</tr>
<tr>
<td><strong>FVC %predicted (%); mean(SD)</strong></td>
<td>96.1(9.1)</td>
<td>98.8(11.5)</td>
</tr>
<tr>
<td><strong>FEV₁/FVC (%)</strong></td>
<td>86.5(6.2)</td>
<td>84.8(6.6)</td>
</tr>
<tr>
<td><strong>Atopic (Y/N); n (%Y)</strong></td>
<td>5/9(35.7)</td>
<td>5/5(50.0)</td>
</tr>
<tr>
<td><strong>AHR (Y/N); n (%Y)</strong></td>
<td>N/A</td>
<td>N/A</td>
</tr>
<tr>
<td><strong>DRS (%fall/ml); median[IQR]</strong></td>
<td>N/A</td>
<td>N/A</td>
</tr>
<tr>
<td><strong>PDₑ₁₂ (ml); median[IQR]</strong></td>
<td>N/A</td>
<td>N/A</td>
</tr>
<tr>
<td><strong>ACQ; median[IQR]</strong></td>
<td>N/A</td>
<td>N/A</td>
</tr>
<tr>
<td><strong>GINA classification; n (%)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Intermittent</td>
<td>N/A</td>
<td>N/A</td>
</tr>
<tr>
<td>Mild persistent</td>
<td>N/A</td>
<td>N/A</td>
</tr>
<tr>
<td>Moderate persistent</td>
<td>N/A</td>
<td>N/A</td>
</tr>
<tr>
<td>Severe persistent</td>
<td>N/A</td>
<td>N/A</td>
</tr>
<tr>
<td><strong>ICS (Y/N); n (%Y)</strong></td>
<td>N/A</td>
<td>N/A</td>
</tr>
<tr>
<td><strong>SABA (Y/N); n (%Y)</strong></td>
<td>N/A</td>
<td>N/A</td>
</tr>
<tr>
<td><strong>Beqs; median[IQR]</strong></td>
<td>N/A</td>
<td>N/A</td>
</tr>
</tbody>
</table>

ACQ, asthma control questionnaire; AHR, airway hyperresponsiveness; Beqs, beclomethasone equivalents; BMI, body mass index; DRS, dose response slope; FEV₁, forced expiratory volume in 1 second; FVC, forced vital capacity; GINA, Global Initiative for Asthma; ICS, inhaled corticosteroid; PD, provocation dose; SABA, short-acting beta-agonist; SDS, standard deviation score.

In a multivariable regression model (Table 4.2) body weight was a statistically significant negative predictor of both FRC and ERV while height was a positive predictor. The presence of asthma was a negative predictor of ERV. Total fat mass negatively predicted FRC and ERV in separate models but this reached statistical significance for FRC only (Table 4.3). Asthma negatively predicted ERV in this model also. However, total lean mass and the presence of asthma were statistically significant negative predictors of ERV but not FRC (Table 4.4).
Table 4.2: Body weight as a predictor of FRC and ERV

<table>
<thead>
<tr>
<th>Final model</th>
<th>FRC(L)</th>
<th>ERV(L)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$R^2=0.521$, p&lt;0.001</td>
<td>$R^2=0.418$, p&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>$\beta$-coefficient (95% CI)</td>
<td>p-value</td>
</tr>
<tr>
<td>Age (yrs)</td>
<td>0.080 (-0.004, 0.164)</td>
<td>0.060</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>0.034 (0.020, 0.048)</td>
<td>0.000</td>
</tr>
<tr>
<td>Gender</td>
<td>0.093 (-0.127, 0.313)</td>
<td>0.401</td>
</tr>
<tr>
<td>Asthma</td>
<td>0.109 (-0.109, 0.328)</td>
<td>0.321</td>
</tr>
<tr>
<td>Body weight (kg)</td>
<td>-0.008 (-0.015, -0.002)</td>
<td>0.017</td>
</tr>
</tbody>
</table>

FRC, functional residual capacity; ERV, expiratory reserve volume

Table 4.3: Total body fat as a predictor of FRC and ERV

<table>
<thead>
<tr>
<th>Final model</th>
<th>FRC(L)</th>
<th>ERV(L)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$R^2=0.526$, p&lt;0.0001</td>
<td>$R^2=0.403$, p&lt;0.0001</td>
</tr>
<tr>
<td></td>
<td>$\beta$-coefficient (95% CI)</td>
<td>p-value</td>
</tr>
<tr>
<td>Age (yrs)</td>
<td>0.080 (-0.003, 0.163)</td>
<td>0.058</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>0.030 (0.016, 0.043)</td>
<td>0.000</td>
</tr>
<tr>
<td>Gender</td>
<td>0.060 (-0.162, 0.283)</td>
<td>0.590</td>
</tr>
<tr>
<td>Asthma</td>
<td>0.126 (-0.098, -0.349)</td>
<td>0.267</td>
</tr>
<tr>
<td>Fat mass (kg)</td>
<td>-0.012 (-0.021, -0.003)</td>
<td>0.010</td>
</tr>
</tbody>
</table>

FRC, functional residual capacity; ERV, expiratory reserve volume

Table 4.4: Total body lean mass as a predictor of FRC and ERV

<table>
<thead>
<tr>
<th>Final model</th>
<th>FRC(L)</th>
<th>ERV(L)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$R^2=0.467$, p&lt;0.0001</td>
<td>$R^2=0.438$, p&lt;0.0001</td>
</tr>
<tr>
<td></td>
<td>$\beta$-coefficient (95% CI)</td>
<td>p-value</td>
</tr>
<tr>
<td>Age (yrs)</td>
<td>0.050 (-0.038, 0.139)</td>
<td>0.262</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>0.029 (0.008, 0.051)</td>
<td>0.009</td>
</tr>
<tr>
<td>Gender</td>
<td>0.072 (-0.186, 0.330)</td>
<td>0.581</td>
</tr>
<tr>
<td>Asthma</td>
<td>0.024 (-0.201, 0.248)</td>
<td>0.834</td>
</tr>
<tr>
<td>Lean mass (kg)</td>
<td>-0.002 (-0.030, 0.026)</td>
<td>0.902</td>
</tr>
</tbody>
</table>

FRC, functional residual capacity; ERV, expiratory reserve volume

We further explored the association between lung function and body composition, by examining additional components of TLC, FEV$_1$ and FVC. In a multivariable linear regression model (Table 4.5), controlled for age, gender, height and asthma, in which the dependant variable was FEV$_1$ and the independent variable was total lean mass, both total lean mass, age and height were statistically significant predictors of an
increase in FEV\textsubscript{1}. Similarly, total lean mass was predictive of FVC and TLC. Body weight (Table 4.6) and total fat mass (Table 4.7) were not statistically significant predictors of FEV\textsubscript{1}, FVC or TLC in multivariable regression models.

**Table 4.5: Total body lean mass as a predictor of FEV\textsubscript{1}, FVC and TLC**

<table>
<thead>
<tr>
<th>Final model</th>
<th>FEV\textsubscript{1}(L)</th>
<th>FVC(L)</th>
<th>TLC(L)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$R^2=0.795$, p&lt;0.0001</td>
<td>$R^2=0.845$, p&lt;0.0001</td>
<td>$R^2=0.816$, p&lt;0.0001</td>
</tr>
<tr>
<td>B-coefficient (95% CI)</td>
<td>p-value</td>
<td>B-coefficient (95% CI)</td>
<td>p-value</td>
</tr>
<tr>
<td>Age (yrs)</td>
<td>0.084 (0.020, 0.148)</td>
<td>0.010</td>
<td>0.060 (-0.008, 0.128)</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>0.016 (0.000, 0.032)</td>
<td>0.049</td>
<td>0.031 (0.014, 0.048)</td>
</tr>
<tr>
<td>Gender</td>
<td>0.104 (-0.084, 0.291)</td>
<td>0.275</td>
<td>0.195 (-0.004, 0.394)</td>
</tr>
<tr>
<td>Asthma</td>
<td>-0.162 (-0.327, 0.003)</td>
<td>0.054</td>
<td>0.030 (-0.145, 0.205)</td>
</tr>
<tr>
<td>Lean mass (kg)</td>
<td>0.033 (0.013, 0.054)</td>
<td>0.002</td>
<td>0.037 (0.016, 0.059)</td>
</tr>
</tbody>
</table>

FEV\textsubscript{1}, forced expiratory volume in 1 second; FVC, forced vital capacity; TLC, total lung capacity

**Table 4.6: Total body weight as a predictor of FEV\textsubscript{1}, FVC and TLC**

<table>
<thead>
<tr>
<th>Final model</th>
<th>FEV\textsubscript{1}(L)</th>
<th>FVC(L)</th>
<th>TLC(L)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$R^2=0.773$, p&lt;0.0001</td>
<td>$R^2=0.838$, p&lt;0.0001</td>
<td>$R^2=0.784$, p&lt;0.0001</td>
</tr>
<tr>
<td>B-coefficient (95% CI)</td>
<td>p-value</td>
<td>B-coefficient (95% CI)</td>
<td>p-value</td>
</tr>
<tr>
<td>Age (yrs)</td>
<td>0.106 (0.040, 0.172)</td>
<td>0.002</td>
<td>0.074 (0.003, 0.144)</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>0.035 (0.023, 0.047)</td>
<td>0.000</td>
<td>0.050 (0.037, 0.063)</td>
</tr>
<tr>
<td>Gender</td>
<td>0.251 (0.070, 0.433)</td>
<td>0.007</td>
<td>0.357 (0.162, 0.551)</td>
</tr>
<tr>
<td>Asthma</td>
<td>-0.158 (-0.338, 0.021)</td>
<td>0.082</td>
<td>0.019 (-0.173, 0.211)</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>0.002 (-0.004, 0.007)</td>
<td>0.568</td>
<td>0.006 (-0.000, 0.011)</td>
</tr>
</tbody>
</table>

FEV\textsubscript{1}, forced expiratory volume in 1 second; FVC, forced vital capacity; TLC, total lung capacity

**Table 4.7: Total body fat mass as a predictor of FEV\textsubscript{1}, FVC and TLC**

<table>
<thead>
<tr>
<th>Final model</th>
<th>FEV\textsubscript{1}(L)</th>
<th>FVC(L)</th>
<th>TLC(L)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$R^2=0.765$, p&lt;0.0001</td>
<td>$R^2=0.817$, p&lt;0.0001</td>
<td>$R^2=0.751$, p&lt;0.0001</td>
</tr>
<tr>
<td>B-coefficient (95% CI)</td>
<td>p-value</td>
<td>B-coefficient (95% CI)</td>
<td>p-value</td>
</tr>
<tr>
<td>Age (yrs)</td>
<td>0.117 (0.050, 0.185)</td>
<td>0.001</td>
<td>0.088 (0.016, 0.161)</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>0.036 (0.025, 0.048)</td>
<td>0.000</td>
<td>0.053 (0.040, 0.065)</td>
</tr>
<tr>
<td>Gender</td>
<td>0.217 (0.032, 0.403)</td>
<td>0.022</td>
<td>0.331 (0.131, 0.531)</td>
</tr>
<tr>
<td>Asthma</td>
<td>-0.102 (-0.287, 0.083)</td>
<td>0.273</td>
<td>0.059 (-0.141, 0.258)</td>
</tr>
<tr>
<td>Fat mass (kg)</td>
<td>-0.004 (-0.011, 0.003)</td>
<td>0.288</td>
<td>0.000 (-0.007, 0.008)</td>
</tr>
</tbody>
</table>

FEV\textsubscript{1}, forced expiratory volume in 1 second; FVC, forced vital capacity; TLC, total lung capacity
For each total and regional measure of lean mass or fat mass, strong correlations were observed between the first and second DEXA scan (Table 4.8). Bland Altman plots demonstrated good agreement between dual scans for both thoracic fat mass (Figure 4.2a) and thoracic lean mass (Figure 4.2b) in the sampled population of children.

Figure 4.2. Bland-Altman plot of dual DEXA scans of a) thoracic fat mass, and b) thoracic lean mass.
Table 4.8: Intra class correlations between dual DEXA scans

<table>
<thead>
<tr>
<th>DEXA Manually-Adjusted Region</th>
<th>ICC</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Fat Mass</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total Body Fat mass (kg)</td>
<td>0.998</td>
<td>(0.997, 0.999)</td>
</tr>
<tr>
<td>Gynoid Fat mass (kg)</td>
<td>0.998</td>
<td>(0.997, 0.999)</td>
</tr>
<tr>
<td>Android Fat mass (kg)</td>
<td>0.996</td>
<td>(0.994, 0.998)</td>
</tr>
<tr>
<td>Trunk Fat mass (kg)</td>
<td>0.994</td>
<td>(0.991, 0.997)</td>
</tr>
<tr>
<td>Leg Fat mass (kg)</td>
<td>0.998</td>
<td>(0.997, 0.999)</td>
</tr>
<tr>
<td>Arm Fat mass (kg)</td>
<td>0.998</td>
<td>(0.997, 0.999)</td>
</tr>
<tr>
<td>Thoracic Fat mass (kg)</td>
<td>0.983</td>
<td>(0.975, 0.992)</td>
</tr>
<tr>
<td><strong>Lean Mass</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total Body Lean mass (kg)</td>
<td>0.994</td>
<td>(0.990, 0.997)</td>
</tr>
<tr>
<td>Gynoid Lean mass (kg)</td>
<td>0.993</td>
<td>(0.989, 0.997)</td>
</tr>
<tr>
<td>Android Lean mass (kg)</td>
<td>0.985</td>
<td>(0.976, 0.993)</td>
</tr>
<tr>
<td>Trunk Lean mass (kg)</td>
<td>0.978</td>
<td>(0.967, 0.990)</td>
</tr>
<tr>
<td>Leg Lean mass (kg)</td>
<td>0.995</td>
<td>(0.993, 0.998)</td>
</tr>
<tr>
<td>Arm Lean mass (kg)</td>
<td>0.996</td>
<td>(0.994, 0.998)</td>
</tr>
<tr>
<td>Thoracic Lean mass (kg)</td>
<td>0.963</td>
<td>(0.943, 0.982)</td>
</tr>
</tbody>
</table>

DEXA, Dual Energy X-ray Absorptiometry; ICC, intraclass correlation coefficient; CI, confidence interval.

4.4 Discussion

This study investigated whether body weight, and specifically body composition, is associated with differences in FRC and ERV in school-aged children, with and without asthma. Body weight and total body lean mass were statistically significant negative predictors of ERV, while body weight and total fat mass were statistically significant negative predictors of FRC. Conversely, total body lean mass was a statistically significant positive predictor of FEV₁, FVC and TLC.

Previous studies have consistently documented clinically significant reductions in FRC and ERV associated with increasing body weight, BMI, and both total and regional fat mass in adults. Although recent studies have reported reductions in lung volumes indices in overweight and obese children with asthma, only one
study has investigated associations between body composition and lung volumes in children \(^\text{137}\). In morbidly obese children without asthma there were statistically significant negative associations between percentage body fat and TLC %predicted, FRC %predicted and RV %predicted \(^\text{137}\). However, ERV was not reported. In our study, increasing body weight was predictive of a reduced FRC and ERV and an association between FRC and total body fat mass was also detected. Notably, total lean mass but not total body fat, was a strong and statistically significant predictor of ERV. The observed association between lean mass and ERV, and fat mass and FRC, in this group of children, may support the theory that increased weight reduces lung function via mechanical restriction of the chest wall and diaphragm. Although this association between fat mass and FRC has been previously observed in adults, this negative association between ERV and lean mass has not been observed in adults. However, an obesity simulation study in lean, non-asthmatic adults found that compression loading around the chest and abdomen significantly reduced lung volumes, with greatest reductions in ERV \(^\text{225}\). Of interest, a recent study reported that asthma risk was increased by both fat and lean mass in female adults, with lean mass, specifically central lean mass, being a stronger determinant of asthma than fat mass \(^\text{145}\). Authors surmised that the inflammatory effects of intramuscular fat may be responsible for this association \(^\text{145}\). However, it may be that increased absolute mass, regardless of composition, increases the risk of asthma in adults, due to mechanical restriction of the thoracic cage. Indeed, our results are consistent with the conjecture that obesity reduces FRC and ERV in children, and that the presence of excess mass, regardless of the composition, may be detrimental to static lung function.

Further exploration of the association between lung function and body composition was conducted in this group of children. FEV\(_1\) and FVC are easily measured using
spirometry and are routinely used to monitor and assess respiratory status in the clinical setting. Therefore, these are important lung function indices to assess. Our data are consistent with the conjecture that dynamic lung function in children is not impaired by body weight and, in fact, is positively influenced by increases in lean mass. In this group of children, total lean mass was positively associated with \( \text{FEV}_1 \), FVC and TLC. Likewise, total lean mass is reportedly a statistically significant predictor of FVC in non-obese healthy adults\(^{226}\). However, this has not been previously reported in children using an objective measure of body composition. One large cross-sectional study in school-aged children reported positive association of fat-free mass (albeit estimated by triceps skinfold test and arm circumference) with \( \text{FEV}_1 \) for boys and with \( \text{FEV}_1 \), FVC and PEF for girls\(^{227}\). An increase in lean mass may represent increased strength of the diaphragm and chest wall to expand and contract with ventilation producing a greater \( \text{FEV}_1 \), FVC, and subsequently TLC. In addition, this may suggest that morphological changes associated with growth and muscle development in this population may be more important than absolute changes in body weight with regards to lung function.

Considering that lean mass was a negative predictor of ERV, a component of FVC, one may hypothesise that the contrasting positive association between lean mass and FVC is due to a larger inspiratory capacity (IC) (a larger tidal volume and /or inspiratory reserve volume (IRV)). That is, the effects of excess weight may manifest as a reduction in FRC and ERV, but may be compensated by a greater tidal volume and /or maximal inspiration (IRV), which may subsequently preserve and even increase FVC values. Hence, it appears that the restrictive properties of childhood obesity are not detectable through routine spirometry, and measurement of lung volumes may be indicated in this group of children.
Our data demonstrate that body composition measurement can reveal important and unexpected information about the relationship between obesity and lung function in children, which is relevant to future research. However, the clinical significance of the presented data is unclear and requires further investigation. In theory, our results suggest that in an obese school-aged child, who can typically carry an additional 20-30kg body weight compared to a healthy weight child\textsuperscript{228}, there is a potential reduction in FRC and ERV of up to 240ml. The size of this reduction could have a clinically significant impact on the patient population. Of relevance to the clinical setting, the data are consistent with the conjecture that excess weight, regardless of composition, is detrimental to FRC and ERV. Therefore, body weight, an easily accessible, measurable, and reliable indicator collected in routine clinical practice, may be used to assess and monitor this group of children.

Although presenting a novel piece of information regarding lung function and weight in children with and without asthma, the over-representation of obese asthmatics and relatively small sample size have influenced the magnitude and statistical significance of the results obtained. Although the increased prevalence of obesity in the asthmatic groups is reflective of previous findings\textsuperscript{69-71}, this is likely exaggerated due to potential selection bias. The offer of a nutritional consult is likely more attractive to the obese, and a respiratory health assessment is likely more appealing to asthmatics than controls. It is acknowledged that the capabilities of this study were limited by sample size; this study was not designed to investigate all aspects of the relationships in question. Although the reproducibility of DEXA measured total and regional body composition, including the thoracic region, was demonstrated in this group of children, we believe the study was not powered to investigate associations between regional body composition and lung function. We have indicated that thoracic body
composition measurement is a reliable tool and it’s clinical relevance warrants further evaluation. Assessment of this region could be beneficial to future research studies powered to investigate such relationships.

This study investigated whether increased body weight and body composition are associated with FRC and ERV in children, with and without asthma. Body weight and total fat mass were negatively associated with FRC, while body weight and total lean mass were negatively associated with ERV, in this group of children. Further investigation revealed a positive relationship between total lean mass and FEV$_1$, FVC, and TLC. The practical implications and the clinical relevance of these findings require further exploration. The results are consistent with the conjecture that improvements in lean mass yield improvements in dynamic lung function, while excess mass, regardless of composition, is detrimental to static lung function in children.

In summary, similar to adults, the cross-sectional associations observed are consistent with the conjecture that obesity in children reduces FRC and ERV. Routine spirometry may not adequately detect this reduction in lung function in obese children, therefore additional lung function tests e.g. plethysmography, may be indicated when assessing overweight and obese children in both the clinical and research setting. While monitoring body weight is a simple and practical way of tracking weight change in the clinical setting, the measurement of body composition can reveal important information when investigating the effects of obesity upon childhood lung function, and should be considered in future research studies.
5. Chapter V: Risk factors for Obesity in Asthmatic Children
5.1 Introduction

Obese asthma is a highly prevalent problem, with a greater number of asthmatic children overweight\textsuperscript{70, 71} and obese\textsuperscript{68, 69, 79}, compared to children without asthma. In Australia, approximately 30\% to 40\% of children with asthma are overweight or obese\textsuperscript{69}, compared to the general Australian population rate of 23\%\textsuperscript{218}. Early intervention is essential to reduce the prevalence of overweight and obesity in children and avoid the tracking of weight that has been demonstrated to continue through to adulthood\textsuperscript{9}.

Indeed, the risk for weight gain may be greater in children with asthma, with data from the Longitudinal Study of Australian Children suggesting that the presence of asthma or wheeze may increase the risk of overweight and obesity in children and adolescents by 28-40\%, regardless of initial weight status\textsuperscript{79}. Modifiable lifestyle factors are a good target for prevention of weight gain, including sleeping habits, dietary consumption patterns, and activity behaviours. However, whether lifestyle risk factors for weight gain are more prevalent in children with asthma is not known.

Sleep disturbance is greater in children with asthma, affecting approximately one third of asthmatic children\textsuperscript{157}. Furthermore, one in five asthmatic children report problems falling asleep, and approximately one in ten report daytime sleepiness\textsuperscript{154}. Sleep is recognised as a significant risk factor for weight gain in children and adults\textsuperscript{14, 151}. Previous studies in adults have demonstrated that acute reductions in sleep duration are associated with significant physiological changes\textsuperscript{169}, while results from epidemiological studies have indicated that chronic sleep deprivation is associated with increased risk for chronic disease, including cardiovascular disease\textsuperscript{170, 171}. Sleep disturbance in non-asthmatic adults has been linked to alterations in metabolic
markers and appetite hormones\textsuperscript{174, 229, 230}; increased appetite for foods high in fat and sugar\textsuperscript{229, 230}; daytime fatigue\textsuperscript{173, 174} and reduced physical activity\textsuperscript{173}, which may increase the risk for weight gain. Few previous studies have objectively assessed sleep disturbance in children using polysomnography (PSG), and to our knowledge, the association between sleep disturbance and risk factors for obesity have not been investigated in asthmatic and non-asthmatic children.

We hypothesise that children with asthma experience more sleep disruption than children without asthma, which increases the prevalence of obesity risk factors, above that of children without asthma. The aims of this study were to compare a) sleep quantity and sleep quality in children with and without asthma, referred for an overnight PSG for a suspected sleep disorder; and b) risk factors for weight gain in children with and without asthma, including appetite and dietary intake, and physical and sedentary activity, and investigate if these risk factors are related to sleep disturbance.

5.2 Methods

5.2.1 Study participants

Participants were recruited from the population referred to the Paediatric Sleep Unit (PSU), John Hunter Children’s Hospital, Newcastle, Australia for an overnight polysomnography (PSG) for a suspected sleep disorder. Non-obese children and adolescents (BMI $z$-score $<1.64$) aged 7-17 years, with and without asthma, were recruited. Asthma was identified by physician diagnosis. Participants were assigned to the asthmatic group (n=17) or non-asthmatic group (n=17). Exclusion criteria included obesity, defined as a BMI $z$-score $\geq1.64$; medications known to interfere with sleep e.g. antihistamines, methylphenidate; medical conditions, physical deformities, or genetic conditions that may interfere with sleep or negate participation.
in the study e.g. cranio-facial abnormalities, Trisomy 21; diagnosed sleep disorders e.g. narcolepsy, obstructive sleep apnoea (OSA); patients on continuous positive airway pressure (CPAP). This study was approved by the Hunter New England and University of Newcastle Ethics Committees (10/08/18/5.04). Informed participant assent (Appendix 4; Appendix 5) and guardian consent (Appendix 6) were attained.

5.2.2 Clinical assessment

Subjects arrived at the PSU for their overnight polysomnography (PSG) between 1600-1800 hours and departed between 0600-0730 hours the following morning. Participants underwent clinical assessment as part of the study during this time. Medical history and medication usage was collected. Asthma stability was confirmed, defined as the absence of an exacerbation, respiratory tract infection or use of oral corticosteroids in the preceding 4 weeks. Asthma control and severity were assessed using the Juniper Asthma Control Questionnaire (ACQ)\textsuperscript{197, 210}, and Global Initiative for Asthma (GINA) guidelines\textsuperscript{196}, respectively. All participants completed the Pediatric Daytime Sleepiness Questionnaire (PDSS) (Iowa Sleep Disorders Clinic)\textsuperscript{231}. Lung function was assessed using a hand-held EasyOne Spirometer (Model 2001 SN 67548/2008, Medizintechnik AG, Zurich Switzerland). The average FEV\textsubscript{1} and FVC was calculated from three technically acceptable manoeuvres according to ATS guidelines, and expressed as a percentage of their predicted values\textsuperscript{194}. Participants performed spirometry on the eve of their PSG and the following morning.

5.2.2.1 Anthropometry

Height was measured using a wall-suspended electronic stadiometer (Harpenden Stadiometers, Holtain Ltd, Britain). Weight was measured using a max, 200kg electronic, seated chair scale (Sca, Vogel & Hake, Germany). BMI was calculated
(weight (kg) / height (m)²) and converted to BMI z-scores. Waist circumference (WC) was measured to nearest 0.1cm at the midpoint between the lower costal edge and the iliac crest, using a non-extensible steel tape (Lufkin W606PM, Cooper Tools, Apex NC). Waist to height ratio was calculated (waist circumference (cm) / height (cm)).

5.2.2.2 Polysomnography

Overnight PSG was performed using the modified 10-20 EEG application system and computerised sleep system (E-Series, Compumedics Ltd, Victoria Australia). Monitoring included electrocardiogram, EEG (central and occipital), EOG (right and left), EMG (chin), pulse rate, transcutaneous CO₂ (tcCO₂), oximetry (SpO₂) and airflow, via nasal thermistor and nasal prong pressure transducer where tolerated. Respiratory and abdominal effort was monitored via diaphragmatic EMG and Peizo respiratory bands located on the chest and abdomen. Polysomnographic (PSG) recordings were scored by PSU staff using Profusion PSG 2 software (Compumedics Ltd, Victoria Australia 2001-2007). Sleep-wake state and respiratory events were scored using American Academy of Sleep Medicine (AASM) Manual for the Scoring of Sleep and Associated Events (2007). EEG arousals with (RDI, RDI in REM) and without (AI) a respiratory event were scored using modified American Thoracic Society criteria. Use of these scoring guidelines was under the direction of the paediatric respiratory sleep physicians. The PSU meets accreditation by the Thoracic Society of Australia and New Zealand (TSANZ), and the Australasian Sleep Association. Outcomes of interest were sleep quantity (Total sleep time (TST), Total time awake (TTA)) and sleep quality (%sleep efficiency, %REM sleep, sleep latency, Arousal Index (AI) and Respiratory Disturbance Index (RDI)).
5.2.3 Dietary & activity assessment

Food cravings and dietary intake were assessed using the Food Cravings Inventory-II (FCI-II)\textsuperscript{206} and the Australian Child & Adolescent Eating Questionnaire\textsuperscript{232}, respectively. Physical activity levels and sedentary activity levels were assessed using the Adolescent Physical Activity Recall Questionnaire\textsuperscript{207} and the Adolescent Sedentary Activity Questionnaire\textsuperscript{208}, respectively.

5.2.4 Systemic biomarkers

A fasting blood sample was collected by a paediatric phlebotomist in the morning following the PSG and centrifuged at 3000 rpm, 4°C for 10 minutes. All samples underwent duplicate testing. Total plasma ghrelin and plasma leptin were measured using commercial ELISAs (Bio-Rad, Hercules CA USA), with sensitivity of 100pg/ml and 3.1pg/ml, respectively. Inflammation was measured by serum high sensitivity C-Reactive Protein (CRP) (Dimension Vista System, Siemens Healthcare Diagnostics, Newark USA). Plasma cortisol (Access Immunoassay Systems, Beckman Coulter, Fullerton CA USA), IGF-1 (Biocline, Marrickville NSW AUS), insulin (Access Ultrasensitive Insulin assay, Beckman Coulter, Fullerton CA USA), and glucose (Dimension Vista System, Siemens Healthcare Diagnostics, Newark USA) were measured using their respective commercial assays, and homeostasis model assessment of insulin resistance (HOMA-IR) \((\text{glucose (mmol/L)} \times \text{insulin (mU/L)})/ 22.5\) calculated. Plasma cholesterol, high-density lipoprotein cholesterol (HDL-C), and triglycerides were measured using commercial assays (Dimension Vista System, Siemens Healthcare Diagnostics, Newark USA).
5.2.5 Statistical Analysis

Continuous data are presented as mean (standard, deviation, SD) or median [interquartile range, IQR]. Continuous data were compared between groups using the Student’s $t$-test or Wilcoxon rank-sum test. Tabular data are presented as proportions ($n(\%)$) and assessed using Fischer’s exact test. Associations were assessed using spearman- rank correlation coefficients. Statistical significance was set at a $p$-value of $\leq 0.05$. Analysis was conducted using Intercooled Stata Version 11.0 for Windows (StataCorp, College Station, Texas, USA 1984-2005).

5.3 Results

Subject characteristics are presented in (Table 5.1). Participants were similar in age. However, there were significantly more females in the non-asthmatic group, compared to the asthmatic group. Lung function was similar between the asthmatic and non-asthmatic children for both evening and morning spirometry values. However, morning $\text{FEV}_1\%$ and $\text{FEV}_1/\text{FVC}\%$ tended to be lower in the asthmatic group (Table 5.1).

Participants with asthma were well-controlled with a median ACQ score of 0.6[0.1, 1.0], and the majority being classed with intermittent to mild severity asthma (86.7%). Approximately 64.7% were currently prescribed a short-acting beta-agonist (SABA). Just under half of the asthmatic group (47.1%) were currently taking a preventer medication, with a minority (17.6%) taking ICS medication. The median inhaled steroid dose for the asthmatic group was 216.5[200.0, 400] beclomethasone equivalents.
Table 5.1: Subject characteristics of children with and without asthma, referred for an overnight polysomnography

<table>
<thead>
<tr>
<th>Subject Characteristics</th>
<th>Non-asthma (n=17)</th>
<th>Asthma (n=17)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Age (years); mean(SD)</strong></td>
<td>10.8(2.3)</td>
<td>10.7(2.4)</td>
<td>0.928</td>
</tr>
<tr>
<td><strong>Gender (female); n(%)</strong></td>
<td>12(70.6)</td>
<td>5(29.4)</td>
<td><strong>0.016</strong></td>
</tr>
<tr>
<td><strong>Height (cm); median[IQR]</strong></td>
<td>146.0[136.7, 153.0]</td>
<td>139.8[133.0, 147.7]</td>
<td>0.380</td>
</tr>
<tr>
<td><strong>Weight (kg); median[IQR]</strong></td>
<td>37.0[30.5, 46.1]</td>
<td>36.0[28.1, 41.6]</td>
<td>0.502</td>
</tr>
<tr>
<td><strong>BMI z-score (SDS); median[IQR]</strong></td>
<td>0.4[-1.2, 1.0]</td>
<td>0.0[-0.4, 0.6]</td>
<td>0.986</td>
</tr>
<tr>
<td><strong>Waist: height (cm/cm); mean(SD)</strong></td>
<td>0.44(0.06)</td>
<td>0.46(0.05)</td>
<td>0.228</td>
</tr>
</tbody>
</table>

**Evening lung function**
- **FEV\textsubscript{1} % predicted (%)**; mean(SD) | 94.0(12.7) | 93.8(17.3) | 0.974 |
- **FVC %predicted (%)**; mean(SD) | 90.8(11.7) | 93.3(11.9) | 0.552 |
- **FEV\textsubscript{1}/FVC (%)**; mean(SD) | 87.2[86.4, 91.5] | 86.0[77.8, 91.1] | 0.207 |

**Morning lung function**
- **FEV\textsubscript{1} % predicted (%)**; mean(SD) | 93.8(12.3) | 88.1(14.0) | 0.258 |
- **FVC %predicted (%)**; mean(SD) | 90.7(7.9) | 90.3(10.0) | 0.914 |
- **FEV\textsubscript{1}/FVC (%)**; mean(SD) | 88.0[84.5, 89.8] | 79.2[75.7, 88.7] | 0.125 |

*Significantly different between asthma and non-asthma group (p≤0.05); BMI, body mass index; FEV\textsubscript{1}, forced expiratory volume in 1 second; FVC, forced vital capacity; SDS, standard deviation score

Figure 5.1. Sleep latency and total time awake in children referred for an overnight polysomnography

*Significantly different between asthma and non-asthma group, p<0.05
Sleep latency was statistically significantly longer in the asthmatic group compared to the non-asthmatic children (Figure 5.1; Table 5.2). TTA, TST and sleep efficiency (Figure 5.1 & 5.2) were similar between asthmatic and non-asthmatic children (Table 5.2). The PDSS score, AI and RDI were also similar (Table 5.2).

Figure 5.2. Sleep duration and sleep efficiency in children referred for an overnight polysomnography

Table 5.2: Sleep quantity and quality in children with and without asthma, referred for an overnight polysomnography

<table>
<thead>
<tr>
<th>Sleep Indices</th>
<th>Non-asthma (n=17)</th>
<th>Asthma (n=17)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>PDSS Total; mean(SD)</td>
<td>15.3(5.2)</td>
<td>16.2(5.7)</td>
<td>0.620</td>
</tr>
<tr>
<td>Total sleep time (mins); mean(SD)</td>
<td>428.5(38.8)</td>
<td>415.3(53.5)</td>
<td>0.416</td>
</tr>
<tr>
<td>Total awake time (mins); median[IQR]</td>
<td>44.0[35.0, 54.5]</td>
<td>54.5[32.0, 66.5]</td>
<td>0.642</td>
</tr>
<tr>
<td>Sleep efficiency (%) ; mean(SD)</td>
<td>81.6(6.9)</td>
<td>78.2(7.5)</td>
<td>0.181</td>
</tr>
<tr>
<td>Sleep latency (mins); mean(SD)</td>
<td>40.9(16.9)</td>
<td>56.6(25.5)</td>
<td>0.042</td>
</tr>
<tr>
<td>Stage 1 (%) ; median[IQR]</td>
<td>2.0[0.7, 2.6]</td>
<td>0.6[0.3, 1.6]</td>
<td>0.094</td>
</tr>
<tr>
<td>Stage 2 (%) ; median[IQR]</td>
<td>48.9[43.3, 52.6]</td>
<td>49.0[43.5, 55.8]</td>
<td>0.931</td>
</tr>
<tr>
<td>Stage 3 (%) ; median[IQR]</td>
<td>31.4[29.0, 37.1]</td>
<td>32.7[26.1, 38.1]</td>
<td>0.904</td>
</tr>
<tr>
<td>REM latency (mins); median[IQR]</td>
<td>166.5[139.0, 237.5]</td>
<td>149.0[100.5, 192.0]</td>
<td>0.294</td>
</tr>
<tr>
<td>REM sleep (%) ; mean(SD)</td>
<td>17.9(8.5)</td>
<td>16.9(6.4)</td>
<td>0.717</td>
</tr>
<tr>
<td>AI (n/hr); median[IQR]</td>
<td>3.2[1.9, 10.1]</td>
<td>3.7[2.7, 6.4]</td>
<td>0.836</td>
</tr>
<tr>
<td>RDI Total (n/hr); median[IQR]</td>
<td>0.1[0.0, 1.3]</td>
<td>0.2[0.0, 0.4]</td>
<td>0.986</td>
</tr>
</tbody>
</table>

*Significantly different between asthma and non-asthma group (p<0.05); PDSS, Paediatric Daytime Sleepiness Scale; REM, rapid eye movement; AI, arousal index; RDI, respiratory disturbance index
There was a significantly higher plasma triglyceride level in the asthmatic group versus the non-asthmatic group, while HOMA and hsCRP levels were not significantly different between groups (Table 5.3). Morning plasma ghrelin (p=0.328) and leptin (p=0.959) levels were also similar between the asthmatic and non-asthmatic group (Figure 5.3).

Table 5.3: Metabolic biomarkers in children with and without asthma, referred for an overnight polysomnography

<table>
<thead>
<tr>
<th>Systemic biomarkers</th>
<th>Non-asthma (n=14)</th>
<th>Asthma (n=13)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cortisol (nmol/L); median[IQR]</td>
<td>363[310, 424]</td>
<td>326[310, 403]</td>
<td>0.865</td>
</tr>
<tr>
<td>IGF-1 (U/ml); median[IQR]</td>
<td>1.04[0.74, 1.94]</td>
<td>0.73[0.66, 1.08]</td>
<td>0.190</td>
</tr>
<tr>
<td>hsCRP (mg/L); median[IQR]</td>
<td>1.1[0.5, 1.5]</td>
<td>0.7[0.2, 0.9]</td>
<td>0.113</td>
</tr>
<tr>
<td>HOMA-IR; median[IQR]</td>
<td>1.4[0.9, 2.4]</td>
<td>1.4[1.0, 1.7]</td>
<td>0.961</td>
</tr>
<tr>
<td>Triglycerides (mmol/L); median[IQR]*</td>
<td>0.7[0.7, 0.8]</td>
<td>1.0[0.8, 1.2]</td>
<td>0.013</td>
</tr>
<tr>
<td>Cholesterol (mmol/L); mean(SD)</td>
<td>4.4(0.4)</td>
<td>4.8(0.2)</td>
<td>0.380</td>
</tr>
<tr>
<td>TC: HDL (ratio); mean(SD)</td>
<td>3.2(1.0)</td>
<td>3.3(0.9)</td>
<td>0.786</td>
</tr>
</tbody>
</table>

*Significantly different between asthma and non-asthma group (p≤0.05); IGF-1, insulin growth factor-1; hsCRP, high-sensitivity C-Reactive Protein; HOMA-IR, homeostatic model assessment of insulin resistance; TC: HDL, total cholesterol: high-density lipoprotein cholesterol

Figure 5.3. Fasting plasma levels of appetite hormones in children referred for an overnight polysomnography
Food cravings did not differ between groups, with a similar mean total FCI-II score between the asthmatic and the non-asthmatic group (2.1(0.6) vs 2.1(0.8), p=0.930).

Dietary energy tended to be higher in the asthmatic group, but this was not statistically significant (Table 5.4). The proportion of energy from macronutrients was similar between the two groups, as was the quality of fat intake (Table 5.4).

Table 5.4: Dietary intake measured by the Australian Child and Adolescent Eating survey in children with and without asthma, referred for an overnight polysomnography

<table>
<thead>
<tr>
<th>Dietary intake measures</th>
<th>Non-asthma (n=17)</th>
<th>Asthma (n=17)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Energy (kJ); mean(SD)</td>
<td>8945.5(2314.5)</td>
<td>9365.4(2722.7)</td>
<td>0.631</td>
</tr>
<tr>
<td>Protein (%energy); mean(SD)</td>
<td>16.4(2.5)</td>
<td>17.2(2.5)</td>
<td>0.380</td>
</tr>
<tr>
<td>Total fat (%energy); mean(SD)</td>
<td>33.4(4.1)</td>
<td>31.5(3.7)</td>
<td>0.183</td>
</tr>
<tr>
<td>Saturated fat (%fat); mean(SD)</td>
<td>51.0(3.5)</td>
<td>49.1(4.4)</td>
<td>0.177</td>
</tr>
<tr>
<td>PUFA (%fat); mean (SD)</td>
<td>11.7(2.1)</td>
<td>12.7(3.1)</td>
<td>0.276</td>
</tr>
<tr>
<td>MUFA (%fat); mean(SD)</td>
<td>37.2(2.3)</td>
<td>38.2(2.2)</td>
<td>0.197</td>
</tr>
<tr>
<td>Carbohydrate (%energy); mean(SD)</td>
<td>51.6(5.3)</td>
<td>52.0(4.5)</td>
<td>0.809</td>
</tr>
<tr>
<td>Sugars (g); mean(SD)</td>
<td>146.4(47.7)</td>
<td>159.4(72.6)</td>
<td>0.542</td>
</tr>
<tr>
<td>Fibre (g); mean(SD)</td>
<td>23.5(6.4)</td>
<td>27.2(8.4)</td>
<td>0.151</td>
</tr>
<tr>
<td>ACARFS; mean(SD)</td>
<td>26.6(8.1)</td>
<td>27.9(10.1)</td>
<td>0.683</td>
</tr>
</tbody>
</table>

*Significantly different between asthma and non-asthma group (p≤0.05); ACARFS, Australian Child and Adolescent Recommended Food Score; MUFA, mono-unsaturated fatty acid; PUFA, poly-unsaturated fatty acid

The average weekly time spent in sedentary behaviours (1590[1275, 3040] vs 2160[1755, 3000]mins/ week, p=0.318) and the average weekly time spent in physical activities (490[263, 680] vs 390[210, 513]mins/ week, p=0.174) was similar in the asthmatic group compared to the non-asthmatic group. The average metabolic equivalent (MET) value was also similar between the asthmatic and non-asthmatic group (4.5(1.2) vs 4.6(1.5) MET, p=0.701).

No associations between sleep variables and appetite hormones or food cravings score were detected. TST was positively associated with the Australian Child and Adolescent Recommended Food Score (ACARFS) (r=0.36, p=0.04). The PDSS score
was positively associated with TC: HDL ($r=0.61$, $p=0.001$) and average weekly sedentary time ($r=0.39$, $p=0.02$). No association was detected between average weekly physical activity time or METS and sleep variables.

5.4 Discussion

The current study compared sleep quantity and sleep quality in asthmatic and non-asthmatic children, referred for an overnight polysomnography for a suspected sleep disorder. This study also compared the prevalence of modifiable lifestyle risk factors for obesity between asthmatic and non-asthmatic children, and investigated if such risk factors were associated with measures of sleep quantity and/ or quality. Sleep quality, but not sleep quantity, was marginally reduced in children with well-controlled intermittent-mild asthma compared to non-asthmatic children, as indicated by a longer sleep onset latency. Appetite markers, dietary intake and activity levels were similar amongst the two groups. However, the data suggest that sleep duration and daytime sleepiness may be associated with altered lipid levels, dietary intake, and activity levels in children, with and without asthma.

Sleep quality was slightly poorer in asthmatic children compared to non-asthmatic children, with a statistically significant difference in sleep latency of approximately 16mins. A similar difference has previously been reported between asthmatic and non-asthmatic children and adults. However, the clinical significance of this difference is unclear and further studies in a large sample of children are warranted. Sleep latency did not correlate with any measure of appetite, dietary intake or activity level in the present study, suggesting that a delay in sleep onset may not have a significant impact on risk for weight gain via adversely affecting dietary intake and activity patterns in this group of children. In contrast, a previous study has indicated that sleep latency is inversely associated with physical activity, but positively
associated with sedentary activity, in non-asthmatic children\textsuperscript{181}. In fact, a recent study showed that moderate-vigorous intensity physical activity in the evening reduced sleep latency that night\textsuperscript{234}. In addition, a cross-sectional study of children with a mean sleep latency of 56.3mins, reported an association between increased sleep latency and increased emotional eating, but found that this relationship was mediated by anxiety\textsuperscript{176}.

In adults, asthma severity has been shown to be associated with poorer sleep quality outcomes, including reduced sleep efficiency and increased arousals\textsuperscript{155, 156}. In fact, longer sleep latency and poorer sleep quality overall has been associated with worse asthma control in adults\textsuperscript{155}. Poorer sleep efficiency\textsuperscript{162}, shorter sleep duration\textsuperscript{166}, increased awakenings\textsuperscript{162, 166}, and increased daytime sleepiness\textsuperscript{162} in children with asthma have been reported, which may be associated with asthma severity\textsuperscript{157, 162}. Indeed, a previous study in children found increased awakenings and poorer sleep efficiency in children with uncontrolled nocturnal asthma, compared to controls\textsuperscript{162}. A study using a validated sleep questionnaire in asthmatic children aged 4-10yrs, found that the sleep disturbance score was well above clinically normal, and that sleep disturbance increased in parallel with nocturnal asthma symptoms\textsuperscript{158}. There was no significant difference in sleep duration, sleep efficiency, arousals or daytime sleepiness between the asthmatic and non-asthmatic children in the presented study. Notably, the children in our study had well-controlled intermittent-mild asthma and therefore the magnitude of the between-group differences for sleep variables may have been attenuated.

Increased fasting plasma triglyceride levels were detected in the asthmatic group compared to non-asthmatic children, despite no significant difference in BMI $z$-score. Notably, BMI $z$-score does not indicate adiposity level and a measure of body
composition may have provided further insight, given that higher adiposity levels have been reported in asthmatic children, compared to controls of a similar BMI \( z \)-score\(^{146} \). A previous large study found a higher prevalence of raised triglyceride levels amongst asthmatic children, compared to non-asthmatic children, and reported triglyceride levels were associated with asthma prevalence, independent of BMI status\(^{72} \). Although the triglyceride levels in the presented study were not clinically abnormal, this cross-sectional analysis may suggest that unfavourable changes in metabolic markers may occur in asthmatic children, irrespective of the participants being of a healthy weight. As only 17.6\% of the children in this study were taking ICS medications, it is unlikely that this is driving the effect. In addition, HOMA-IR was similar between groups and therefore it is unlikely that the difference in triglycerides is due to impaired glucose tolerance or insulin resistance. Intake of saturated fat was considerably high in both asthmatic and non-asthmatic children, comprising approximately 50\% of total fat intake compared to the recommendation of no more than 30\% of total fat intake for cardiovascular health\(^{235} \). However, there was no difference in fat quantity or quality intake, as estimated from the validated FFQ, which may explain the difference in triglyceride levels between the two groups. Furthermore, the lack of correlation between plasma triglycerides and sleep quality/quantity suggests that altered sleep patterns aren’t contributing to this potential metabolic impairment. Further investigation is required.

The presented study did not detect a difference in fasting morning plasma levels of appetite hormones or food cravings. There was also no association detected between appetite measures and sleep variables. Previous studies have indicated that sleep disturbance may be associated with alterations in appetite. Food cravings, as measured by FCI-II, have been associated with increased daytime sleep in a group of
non-asthmatic adolescents. One study has reported on appetite hormones and sleep in non-asthmatic children and found self-reported sleep duration to correlate negatively with leptin levels in females, while no association was detected for males. This is similar to a study in women that reported a single night of sleep restriction to 3 hours was associated with increased morning leptin levels which were not associated with measures of hunger or food cravings. Likewise, a small study in males found that acute sleep restriction did not affect energy intake, appetite, hunger, or ghrelin and leptin levels, but did note a significant reduction in daytime activity counts and intensity of activity. However, there are reports in adults that acute and habitual sleep deprivation is associated with depressed leptin levels, increased ghrelin levels, and increased ratings of hunger, and appetite, particularly for carbohydrate and fat-rich foods. Our data indicates that a single night measure of sleep quantity and quality is not associated with appetite hormone concentrations or food cravings in children. However, assessment of chronic sleep disturbance may reveal different results.

Supporting the hypothesis that sleep duration modifies diet quality, we found that a higher TST was associated with a greater Australian Child and Adolescent Recommended Food Score. This indicates that sleep duration is positively associated with a dietary intake pattern that closely mirrors National Dietary Guidelines for Australia. Previous studies in non-asthmatic children have indicated that self-reported sleep duration is positively associated with favourable dietary practices, including higher fruit and vegetable consumption and reduced soft drink consumption. A recent longitudinal study found short sleep duration in children was associated with unfavourable dietary intake patterns in both girls and boys. Notably, eating patterns were found to partially mediate the relationship between sleep and overweight/
obesity in this group of children\textsuperscript{240}. In contrast, two recent studies found no change in energy or macronutrient intake with decreasing sleep duration\textsuperscript{241, 242}.

We did not detect a difference between time spent in physical activity or sedentary activity between asthmatic and non-asthmatic children. However, in this group of children, increases in daytime sleepiness paralleled an increase in sedentary behaviour and TC: HDL levels. Increases in sedentary activity and a decrease in physical activity time and exertion level, has been reported to occur with reductions in sleep duration, in adults and children\textsuperscript{173, 243, 244}. In children, a sleep duration <8hrs has been associated with increased sedentary behaviour and more TV viewing, even after adjusting for BMI\textsuperscript{239}. In contrast, a recent study reported that increased duration and intensity of physical activity during the day was associated with reduced sleep duration and sleep efficiency that night, as measured by actigraphy\textsuperscript{234}. Sleep duration and sleep efficiency were also inversely associated with physical activity the following day\textsuperscript{234}. The heterogeneity of study results indicate that more research is needed using objective measures of activity and sleep.

The current study was conducted in a sample of children with suspected sleep disorders, which may have impacted the results. However, the PDSS score, AI and RDI were clinically normal, indicating that these children were without excessive daytime sleepiness and sleep disordered breathing. It should be noted that in this group of children, mean sleep duration was clinically significantly lower than the recommended ≥9hours per night\textsuperscript{150}. However, this agrees with previous reports that worldwide sleep durations are decreasing in children and adolescents\textsuperscript{149, 153}, with the difference in sleep requirement and actual self-reported sleep time each night as large as 2hrs\textsuperscript{150}. A limitation of this study is the difference in the female: male ratio, with a higher number of females in the non-asthmatic group. Gender has been identified as
an important factor contributing to differences in sleep duration and quality\textsuperscript{245-247} and the difference in gender between the two groups may have impacted the results obtained. However, this study was not able to undertake gender analysis due to the small sample size. This study is also limited by its cross-sectional nature, in that it is unable to establish the direction of associations or indicate the effects of chronic sleep disturbance. Chronic sleep disturbance may be more closely associated with changes in lifestyle patterns and further research in this area is required. The relationship between objectively-measured sleep and lifestyle risk factors for obesity has not been investigated in children with and without asthma. A key strength of this study is the utilisation of a population undergoing PSG, which has enables an objective assessment of sleep quantity and quality, appetite, dietary intake and activity measures in a clearly defined group of asthmatic and non-asthmatic children.

Sleep quality, but not sleep quantity, was marginally worse in well-controlled intermittent-mild asthmatic children, compared to children without asthma. In addition, triglyceride levels were mildly higher in the asthmatic compared to the non-asthmatic children. However, there was no difference in lifestyle risk factors for obesity between the asthmatic and non-asthmatic children in this study. Daytime sleepiness correlated with adverse changes in lipid levels and increased sedentary activity, which if chronically sustained, could lead to a positive energy balance. This warrants further investigation, particularly in groups with excessive daytime sleepiness. Sleep duration was also correlated with dietary consumption patterns in accordance with national dietary guidelines. Further investigation into sleep disturbance in asthmatic children and the lifestyle implications, with the consideration of gender, is warranted.
6. Chapter VI: Diet-induced weight loss in obese children with asthma: a pilot randomized controlled trial
6.1 Introduction

Obesity is highly prevalent in the asthmatic population, with almost one in two children with asthma carrying excess weight \(^{68}\), compared to approximately one quarter of the general population \(^{4}\). Addressing the high prevalence of obesity in children with asthma is of critical importance. Once a child becomes overweight or obese the risk of obesity tracking into adulthood is dramatically increased \(^9\). The detrimental effects of obesity upon adult respiratory status has been well documented \(^{92}\), with significant lung restriction \(^{112}\), steroid resistance \(^{105}\), altered airway inflammation \(^{89, 91}\), and raised systemic inflammation \(^{91}\) characterising factors. There is also increasing evidence to suggest that excess weight in children with asthma is associated with worse asthma control and increased risk of exacerbations \(^{92, 209}\), reduced static lung function \(^{114, 115}\) and reduced steroid efficacy \(^{102}\), complicating their management.

Despite the heterogeneity in interventions and measurement outcomes, weight loss in asthmatic populations consistently demonstrates a significant improvement in asthma outcomes \(^{33, 183}\), notably lung function \(^{131, 184-186}\), asthma control and severity \(^{131, 184-186}\), airway responsiveness \(^{184-186}\), medication use \(^{184, 185}\), and quality of life \(^{185, 186}\). With the exception of one uncontrolled study \(^{131}\), previous weight loss interventions in the asthmatic population have been conducted in adults. The majority of these studies have investigated surgically-induced weight loss \(^{183}\), while few have used very low to low calorie diets \(^{33}\) or combination therapy \(^{131, 186}\). Surgical intervention has achieved up to 35-45\% weight loss within 1-3yr follow-up in asthmatic adults \(^{183, 184}\), with associated improvements in dynamic and static lung function, airway reactivity and medication usage \(^{183-185}\). Dietary interventions conducted in asthmatic adults have ranged from 8-26weeks in duration and achieved approximately 8-19\% weight loss,
with associated improvements in asthma control, lung function, bronchodilator response, quality of life, self-reported dyspnea and rescue medication use\textsuperscript{33, 186}.

Evidence suggests that interventions including a dietary component are effective in achieving weight loss in non-asthmatic children\textsuperscript{248}. Only one weight loss study has been conducted in obese asthmatic adolescents, which used a 12-month combined dietary, physical activity, psychological and medical intervention\textsuperscript{131}. This uncontrolled pre-post study reported significant improvements in lung function, asthma severity and symptoms, with approximately 13\% weight loss\textsuperscript{131}. However, whether an energy-restricted diet can induce acute weight loss in obese children with asthma and achieve significant improvements in asthma outcomes has not been investigated in a randomised controlled trial (RCT).

Therefore, the aim of this study was to investigate whether: a) acute weight loss can be achieved in children with asthma in a 10-week RCT using dietary intervention alone; and b) dietary-induced weight loss is associated with changes in asthma outcomes, including systemic and airway inflammation, lung function and asthma control.

6.2 Methods

6.2.1 Study Participants

Obese children (BMI $z$-score $\geq 1.64$ standard deviation score (SDS)), aged 8-17 years, with a physician diagnosis of asthma were recruited from the John Hunter Children’s Hospital (JHCH) outpatients, local medical centres and the general community in Newcastle, Australia. Participants were randomised by a statistician to one of two groups: (1) Dietary intervention (DIG); or (2) Wait-list control (WLC), who received the intervention after the initial 10-week control period (Figure 6.1). Exclusion
criteria included unexplained weight change during the past 3 months, inflammatory or endocrine disorders, and respiratory disorders other than asthma. Informed participant assent (Appendix 7) and guardian consent (Appendix 8) were obtained. The study was registered with the Australian New Zealand Clinical Trials Registry (ACTRN12610000955011) and approved by the Hunter New England and University of Newcastle Human Research Ethics Committees (09/05/20/5.08).

6.2.2 Study Intervention

Participants randomised to the intervention group underwent a 10 week dietary intervention, which targeted a 500-kcal/day energy reduction from individually calculated age- and gender-appropriate energy requirements (Schofield equation to estimate basal metabolic rate using activity factor of 1.55)\(^235\). Participants were required to attend counselling sessions with an Accredited Practising Dietitian (MEJ) in weeks 0, 1, 2, 4, 6, 8, and 10 and were contacted via telephone in alternate weeks. Sessions involved theoretical and practical education on selection of foods and

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**Figure 6.1.** Participant flow through randomised controlled trial
appropriate serving sizes to optimise macronutrient and micronutrient intakes within an energy-restricted diet; identification and resolution of barriers to dietary change; and goal-setting. Materials included individually adapted meal plans (Appendix 9) and a commercial calorie counter, and additional handouts used during nutrition education sessions (Appendix 10). Participants were encouraged to self-monitor energy intake using a food diary throughout the study period.

6.3 Clinical visits

6.3.1 Clinical assessment

Participants attended JHCH after an overnight fast (≥12hours) and withholding antihistamines and asthma medications (≥24hours). Asthma stability was confirmed, defined as no exacerbation, respiratory tract infection or oral corticosteroid use in the past 4 weeks. Clinical asthma pattern, current asthma status, and quality of life were assessed using Global Initiative for Asthma (GINA) guidelines, Juniper Asthma Control Questionnaire (ACQ), and Paediatric Asthma Quality of Life Questionnaire (standardised) (PAQLQ(s)), respectively, in their original unmodified form. Atopy was determined by positive skin prick test to common allergen(s) (Aspergillus fumigatus, Alternaria tenius, Dermatophagoides Pteronyssinus, Cockroach mix, Grass mix). Tobacco exposure was measured by urinary cotinine (NicAlert, Nymox Pharmaceutical Corp, USA NJ). Dynamic and static lung function was measured using spirometry (Windows KoKo PFT System Version 4.9 2005, PDS Inc Louisville USA) and plethysmography (MedGraphics Elite Series Plethysmograph, USA; Breeze Suite 6.4.1.14 Version 510 2008, MedGraphics Corp., USA). FEV₁ and FVC values were expressed as a percentage of the predicted values, and obstruction as FEV₁/TLC(%).
6.3.2 Anthropometry

Weight and height were measured using 150 kg max scales (EB8271 NuWeigh, Newcastle Weighing Services NSW, Australia) and 2 m wall-suspended measuring tape with wall stop (Surgical and Medical Supplies Pty Ltd SA, Australia). BMI was calculated (weight (kg) / height (m)^2) and converted to BMI z-scores. Total body and thoracic fat and lean mass were measured as a percentage (%) of total body weight using dual energy X-ray absorptiometry (GE Lunar Prodigy, Medtel; GE Healthcare encore 2007 software Version 11.40.004, Madison USA).

6.3.3 Airway biomarkers

Participants underwent exhaled Nitric Oxide (eNO) measurement (NiOX chemiluminescent Detector, Aerocrine, Australian Supplier Zynergy Medical) and combined bronchial provocation testing and sputum induction with hypertonic saline (4.5%) (ULTRA-NEB™ ultrasonic nebuliser, DeVilbiss, Model 2000). Airway hyperresponsiveness (AHR) was defined as a fall in FEV₁ ≥15% of their baseline FEV₁. The dose response slope (DRS) and the log-transformed provocation dose (LogPD₁₅) were calculated. Opaque mucocellular sputum portions were selected from saliva, processed using dithiothreitol, and a total cell count of leukocytes and viability performed. Cytospins were prepared, stained (May-Grunwald Geimsa) and a differential cell count obtained from 400 non-squamous cells.

6.3.4 Systemic biomarkers

Fasting blood samples were centrifuged at 3000 rpm, 4°C for 10 minutes. All samples underwent duplicate testing. Plasma IL-6 (R&D Systems, Minneapolis MN USA), and serum leptin and adiponectin (Bio-Rad, Hercules CA USA) were measured using commercial ELISAs, with respective sensitivity of 0.039pg/ml, 3.1pg/ml and
32.7pg/ml. Serum high sensitivity C-Reactive Protein (CRP), and plasma cholesterol, high-density lipoprotein cholesterol (HDL-C), triglyceride and glucose were measured using commercial assays (CRP Flex reagent cartridge, CHOL Flex reagent cartridge, HDLC Flex reagent cartridge, TRIG Flex reagent cartridge & GLU Flex reagent cartridge, Dimension Vista System, Siemans Healthcare Diagnostics Inc. 2008, Newark USA). Plasma insulin was measured using commercial immunoassay (Access Ultrasensitive Insulin assay, Beckman Coulter Inc. 2008, CA USA). Low-density lipoprotein cholesterol (LDL-C) (total cholesterol–(HDL-C)–(0.4545*triglycerides)) and homeostasis model assessment of insulin resistance (HOMA-IR) (glucose (mmol/L)*insulin (mlU/L) / 22.5) were calculated.

6.3.5 Statistical analysis

Data are presented as mean (standard deviation, SD), median [interquartile range, IQR], or proportion (n, (%)). Outcome data are reported as change (∆) from baseline and analysed as intention-to-treat. Continuous data were assessed using a paired mean-comparison t-test or Wilcoxon sign-rank test for within-group comparisons, and a two-group mean-comparison t-test or Wilcoxon rank-sum test for between-group comparisons. Associations between ∆BMI z-score and 5 key pathways were explored using spearman-rank correlation coefficients: mechanical function (∆ERV), airway inflammation (∆%sputum inflammatory markers, ∆eNO), systemic inflammation (∆CRP), endocrine function (∆leptin), and metabolic function (∆HOMA-IR). All tests were of size alpha = 0.05 and unadjusted for multiple comparisons. Statistical analysis was performed using Intercooled Stata Version 11.0 for Windows (StataCorp, College Station, Texas, USA 1984-2005).
6.4 Results

Group characteristics were similar at baseline (Table 6.1), except for a significantly higher ACQ score in the DIG compared to the WLC.

Table 6.1: Subject characteristics at baseline, randomised to dietary intervention or wait-list control group for ten weeks

<table>
<thead>
<tr>
<th>Subject Characteristics</th>
<th>Dietary Intervention</th>
<th>Wait-list Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>number; n</td>
<td>13</td>
<td>15</td>
</tr>
<tr>
<td>Age (years); mean(SD)</td>
<td>11.5(2.1)</td>
<td>12.4(2.4)</td>
</tr>
<tr>
<td>Gender (female); n (%)</td>
<td>3(23.1)</td>
<td>8(53.3)</td>
</tr>
<tr>
<td>Height (cm); mean(SD)</td>
<td>1.6(0.1)</td>
<td>1.6(0.1)</td>
</tr>
<tr>
<td>Weight (kg); median[IQR]</td>
<td>59.9[56.1, 78.6]</td>
<td>71.2[66.5, 82.4]</td>
</tr>
<tr>
<td>BMI z-score (SDS); median[IQR]</td>
<td>2.1[1.9, 2.3]</td>
<td>2.2[1.8, 2.4]</td>
</tr>
<tr>
<td>Total body fat mass (%); mean(SD)</td>
<td>44.7(5.9)</td>
<td>44.8(7.3)</td>
</tr>
<tr>
<td>Total body lean mass (%); mean(SD)</td>
<td>53.3(5.6)</td>
<td>53.3(6.8)</td>
</tr>
<tr>
<td>Atopic (Y/N); n (%Y)</td>
<td>9(69.2)</td>
<td>10(66.7)</td>
</tr>
<tr>
<td>FEV₁ % predicted (%); mean(SD)</td>
<td>90.5(13.6)</td>
<td>96.0(7.6)</td>
</tr>
<tr>
<td>FVC % predicted (%); mean(SD)</td>
<td>100.8(10.2)</td>
<td>101.4(6.9)</td>
</tr>
<tr>
<td>FEV₁/TLC (%); mean(SD)</td>
<td>59.1(9.0)</td>
<td>64.0(9.0)</td>
</tr>
<tr>
<td>ACQ Score; median[IQR]</td>
<td>1.14[0.43, 1.57]</td>
<td>0.57[0.29, 0.86]</td>
</tr>
<tr>
<td>PAQLQ; median[IQR]</td>
<td>5.52[4.65, 6.26]</td>
<td>6.00[5.65, 6.52]</td>
</tr>
<tr>
<td>Airway hyperresponsiveness (%true)†; n (%)</td>
<td>8(72.7)</td>
<td>10(76.9)</td>
</tr>
<tr>
<td>LogPD₁₅ (ml); mean(SD)</td>
<td>1.4[0.4, 2.0]</td>
<td>1.1[-0.3, 2.4]</td>
</tr>
<tr>
<td>DRS (%fall/ml); median[IQR]</td>
<td>2.6[1.4, 9.2]</td>
<td>1.8[0.5, 14.3]</td>
</tr>
<tr>
<td>SABA (true)†; n (%)</td>
<td>11(84.6)</td>
<td>13(86.7)</td>
</tr>
<tr>
<td>ICS (true)†; n (%)</td>
<td>7(53.9)</td>
<td>3(20.0)</td>
</tr>
<tr>
<td>Beqs; median[IQR]</td>
<td>400[275, 400]</td>
<td>400[233, 400]</td>
</tr>
</tbody>
</table>

ACQ, Asthma Control Questionnaire (Juniper); Beqs, beclomethasone equivalents; BMI, body mass index; DRS, dose response slope; FEV₁, forced expiratory volume in 1 second; FVC, forced vital capacity; ICS, inhaled corticosteroid; LogPD₁₅, log-transformed provocation dose; PAQLQ, Paediatric Asthma Quality of Life Questionnaire (Juniper); SABA, short acting β-agonist; SDS, standard deviation score; TLC, total lung capacity; *p =0.026 vs control group; †Pearson’s Chi-squared test.

Following the intervention period, a significant reduction in all adiposity indicators occurred in the DIG (Table 6.2), with a clinically important reduction in BMI z-score (Figure 6.2a) and a reduction in %body fat (Figure 6.2b). However, metabolic markers remained unchanged in the DIG. No significant change in anthropometric or
metabolic markers was observed for the WLC, except for a statistically significant increase in body weight and fasting glucose levels (Table 6.2). The change in BMI z-score and %body fat was significantly different between groups (Figure 6.2a, Figure 6.2b). The change in weight was also significantly different between groups, while changes in metabolic markers were not significantly different (Table 6.2).

**Figure 6.2.** Change (Δ) from baseline in a) Body mass index (BMI) z-score and b) Total body fat (%) following 10 week dietary intervention in obese asthmatic children.

‡p-value<0.05 within group; §p-value <0.05 between groups.

There was no significant change in dynamic lung function, within or between groups (Table 6.3). Static lung function, including ERV (Figure 6.3a), was significantly different within the DIG compared to baseline. However, the difference between groups was not statistically significant. ACQ score improved significantly within the DIG, compared to the WLC (Figure 6.3b). PAQLQ symptom (0.6[-0.1, 1.2], p<0.05) and emotional (0.4[0.3, 1.7], p<0.05) domain scores significantly improved in the DIG, but this was not different compared to the change in the WLC (0.1[-0.4, 0.6] and -0.1[-0.4, 0.8], respectively). The change in PAQLQ total and activity domain scores were not statistically significant within the DIG (0.7(1.2) and 0.6[-0.3, 1.7], respectively) or compared to the WLC (0.1(0.7) and 0.1[-0.2, 0.4], respectively).
Table 6.2: Change in anthropometric & metabolic variables in obese children with asthma, following randomisation to diet-induced weight loss intervention or no intervention for ten weeks

<table>
<thead>
<tr>
<th>Anthropometric &amp; metabolic markers</th>
<th>Intervention group</th>
<th>Control group</th>
<th>Change (Δ) between groups: p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Baseline</td>
<td>Change (Δ) vs. Baseline</td>
<td>Baseline</td>
</tr>
<tr>
<td>Weight (kg); median[IQR]</td>
<td>59.9[56.1, 78.6]</td>
<td>-3.4[-4.8, -2.9]*</td>
<td>71.2[66.5, 82.4]</td>
</tr>
<tr>
<td>Total body lean mass (%) ; mean(SD)</td>
<td>53.3(5.6)</td>
<td>2.1(3.0)*</td>
<td>53.3(6.8)</td>
</tr>
<tr>
<td>Glucose (mmol/L); median[IQR]</td>
<td>4.3[3.9, 4.4]</td>
<td>0.0[-1.0, 0.3]</td>
<td>4.4[4.0, 4.7]</td>
</tr>
<tr>
<td>Insulin (mU/L); median[IQR]</td>
<td>7.3[5.3, 12.0]</td>
<td>-0.7[-6.4, 3.7]</td>
<td>10.1[7.1, 16.2]</td>
</tr>
<tr>
<td>Total cholesterol (mmol/L); median[IQR]</td>
<td>3.9[3.8, 4.1]</td>
<td>-0.1[-0.3, 0.2]</td>
<td>4.1[3.8, 4.7]</td>
</tr>
<tr>
<td>LDL-C (mmol/L); median[IQR]</td>
<td>2.2[2.0, 2.7]</td>
<td>-0.1[-0.3, 0.2]</td>
<td>2.2[1.9, 2.8]</td>
</tr>
<tr>
<td>HDL-C (mmol/L); median[IQR]</td>
<td>1.3(0.2)</td>
<td>-0.0[-0.1, 0.0]</td>
<td>1.1(0.2)</td>
</tr>
<tr>
<td>Triglycerides (mmol/L); median[IQR]</td>
<td>1.0[0.7, 1.2]</td>
<td>0.0[-0.4, 0.2]</td>
<td>1.3[0.8, 2.0]</td>
</tr>
<tr>
<td>HOMA-IR; median[IQR]</td>
<td>1.4[0.9, 2.0]</td>
<td>-0.2[-1.0, 1.2]</td>
<td>2.0[1.8, 3.2]</td>
</tr>
</tbody>
</table>

LDL-C, low density lipoprotein cholesterol; HDL-C, high density lipoprotein cholesterol; HOMA-IR, homeostasis model assessment of insulin resistance. *p <0.05 versus baseline value
Table 6.3: Change in lung function & clinical asthma outcomes in obese children with asthma, following randomisation to diet-induced weight loss intervention or no intervention for ten weeks

<table>
<thead>
<tr>
<th>Lung function variables</th>
<th>Intervention group</th>
<th>Control group</th>
<th>Change (Δ) between groups: p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Baseline</td>
<td>Change (Δ) vs. baseline</td>
<td>Baseline</td>
</tr>
<tr>
<td><strong>FEV&lt;sub&gt;1&lt;/sub&gt; (L); median[IQR]</strong></td>
<td>2.4[2.0, 2.9]</td>
<td>0.0[-0.2, 0.1]</td>
<td>2.6[2.2, 2.9]</td>
</tr>
<tr>
<td><strong>FVC (L); median[IQR]</strong></td>
<td>3.4[2.7, 3.5]</td>
<td>0.1(0.2)</td>
<td>3.3[2.9, 3.5]</td>
</tr>
<tr>
<td><strong>FEV&lt;sub&gt;1&lt;/sub&gt;/TLC (%)</strong>: mean(SD)</td>
<td>59.1(9.0)</td>
<td>2.3[-1.3, 14.1]</td>
<td>64.7(8.9)</td>
</tr>
<tr>
<td><strong>TLC (L); median[IQR]</strong></td>
<td>4.4[3.4, 4.8]</td>
<td>0.0[-0.5, 0.0]</td>
<td>4.0[3.6, 4.7]</td>
</tr>
<tr>
<td><strong>FRC (L); median[IQR]</strong></td>
<td>1.9[1.7, 2.1]</td>
<td>0.2(0.5)</td>
<td>1.6[1.5, 2.1]</td>
</tr>
<tr>
<td><strong>RV (L); median[IQR]</strong></td>
<td>0.9[0.8, 1.6]</td>
<td>-0.4(0.5)*</td>
<td>0.9[0.7, 1.1]</td>
</tr>
<tr>
<td><strong>RV/TLC (%)</strong>: mean(SD)</td>
<td>25.8(9.3)</td>
<td>-6.9(9.2)*</td>
<td>20.5(9.0)</td>
</tr>
<tr>
<td><strong>DRS (%fall/ml); median[IQR]</strong></td>
<td>2.6[1.4, 9.2]</td>
<td>0.4[-0.4, 1.7]</td>
<td>1.8[0.5, 14.3]</td>
</tr>
<tr>
<td><strong>LogPD&lt;sub&gt;15&lt;/sub&gt; (ml); mean(SD)</strong></td>
<td>1.4[0.4, 2.0]</td>
<td>-0.4[-0.8, 0.3]</td>
<td>1.1[-0.3, 2.4]</td>
</tr>
</tbody>
</table>

DRS, dose response slope; FEV<sub>1</sub>, forced expiratory volume in 1 second; FRC, functional residual capacity; FVC, forced vital capacity; LogPD<sub>15</sub>, log-transformed provocation dose; RV, residual volume; TLC, total lung capacity. *p <0.05 versus baseline value
Figure 6.3. Change (Δ) from baseline in a) Expiratory Reserve Volume (ERV) and b) Asthma Control Questionnaire (ACQ) score following 10 week dietary intervention in obese asthmatic children.

‡p-value<0.05 within group; $p$-value<0.05 between groups

There was no change in the number or proportion of eosinophils or neutrophils, within or between groups (Table 6.4). However, a non-significant trend towards a reduction in %neutrophils in the DIG was observed. A statistically significant difference in both absolute and %lymphocytes was observed between groups. However, the change within groups was non-significant. A significant increase in CRP levels was detected in the WLC compared to the DIG, while no change was observed in IL-6, leptin or adiponectin levels, within or between groups (Table 6.4).

Table 6.5 presents correlations between ∆BMI z-score and key outcomes. Change in BMI z-score was associated with ∆CRP and ∆eNO (Table 6.5). Change in CRP was positively associated with ∆%body fat (r=0.64, p=0.001) and negatively associated with ∆%lean mass (r=-0.61, p=0.001) and ∆%thoracic lean mass (r=-0.41, p=0.043). Change in eNO was negatively associated with ∆%thoracic lean mass (r=-0.56, p=0.011) only. In addition, ∆HOMA-IR was positively associated with ∆%body fat (r=0.57, p=0.003) and ∆%thoracic fat (r=0.47, p=0.020), while negatively associated with ∆%lean mass (r=-0.61, p=0.001) and ∆%thoracic lean mass (r=-0.51, p=0.012). Only ∆CRP was associated with ∆ACQ (r=0.43, p=0.029).
Table 6.4: Change (Δ) in airway & systemic inflammatory markers in obese children with asthma, following randomisation to diet-induced weight loss intervention or no intervention for ten weeks

<table>
<thead>
<tr>
<th>Airway &amp; systemic inflammatory markers</th>
<th>Intervention group</th>
<th>Control group</th>
<th>Change (Δ) between groups: p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Baseline</td>
<td>Change (Δ) vs. baseline</td>
<td>Baseline</td>
</tr>
<tr>
<td>Exhaled nitric oxide (ppb); median[IQR]</td>
<td>13.1[8.4, 41.8]</td>
<td>-2.6[-11.3, 0.39]</td>
<td>27.2[10.5, 46.7]</td>
</tr>
<tr>
<td>Total cell count (x 10⁶/ml); median[IQR]</td>
<td>3.2[1.3, 4.6]</td>
<td>0.5[-0.5, 2.4]</td>
<td>2.8[1.9, 5.5]</td>
</tr>
<tr>
<td>Neutrophils (%) ; median[IQR]</td>
<td>10.5[8.0, 18.8]</td>
<td>-4.8[-7.5, -0.6]</td>
<td>10.3[2.8, 27.5]</td>
</tr>
<tr>
<td>Eosinophils (%) ; median[IQR]</td>
<td>0.8[0.5, 5.3]</td>
<td>-0.1[-0.5, 5.1]</td>
<td>0.8[0.3, 8.5]</td>
</tr>
<tr>
<td>Macrophages (%) ; mean(SD)</td>
<td>78.8[71.3, 83.5]</td>
<td>3.4[-3.6, 8.6]</td>
<td>78.0[42.5, 84.8]</td>
</tr>
<tr>
<td>Lymphocytes (%) ; median[IQR]</td>
<td>2.3[0.3, 7.0]</td>
<td>-2.4[-4.9, -1.0]</td>
<td>1.0[0.0, 1.3]</td>
</tr>
<tr>
<td>Neutrophils (x 10⁶/ml); median[IQR]</td>
<td>0.53[0.15, 1.50]</td>
<td>-0.09[-0.88, 0.48]</td>
<td>0.49[0.14, 1.39]</td>
</tr>
<tr>
<td>Eosinophils (x 10⁶/ml); median[IQR]</td>
<td>0.02[0.01, 0.12]</td>
<td>-0.01[-0.02, 0.24]</td>
<td>0.03[0.01, 0.12]</td>
</tr>
<tr>
<td>Macrophages (x 10⁶/ml); median[IQR]</td>
<td>2.48[1.25, 4.03]</td>
<td>0.22[-1.69, 3.92]</td>
<td>2.05[1.71, 4.93]</td>
</tr>
<tr>
<td>Lymphocytes (x 10⁶/ml); median[IQR]</td>
<td>0.12[0.05, 0.40]</td>
<td>-0.04[-0.35, 0.00]</td>
<td>0.04[0.02, 0.07]</td>
</tr>
<tr>
<td>C-Reactive Protein (mg/L ); median[IQR]</td>
<td>2.1[1.5, 3.3]</td>
<td>-0.4[-0.5, 0.4]</td>
<td>2.1[0.7, 4.0]</td>
</tr>
<tr>
<td>Interleukin-6 (pg/mL); median[IQR]</td>
<td>1.2[0.7, 2.7]</td>
<td>0.3[-0.3, 0.4]</td>
<td>1.4[0.7, 2.0]</td>
</tr>
<tr>
<td>Leptin (ng/mL); median[IQR]</td>
<td>6.4[0.5, 27.4]</td>
<td>-0.3[-1.8, 0.2]</td>
<td>3.1[0.7, 18.0]</td>
</tr>
<tr>
<td>Adiponectin (ug/L); median[IQR]</td>
<td>4.4[3.6, 6.0]</td>
<td>0.7[-0.5, 2.4]</td>
<td>4.8[4.1, 7.2]</td>
</tr>
</tbody>
</table>

*p <0.05 versus baseline value
Table 6.5: Spearman rank correlation coefficients between change (Δ) in BMI z-score, lung function, and airway and systemic biomarkers.

<table>
<thead>
<tr>
<th>H₀ MOA</th>
<th>Key variable of interest</th>
<th>ΔBMI z-score</th>
<th>r</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mechanical</td>
<td>ΔERV</td>
<td>0.12</td>
<td>0.568</td>
<td></td>
</tr>
<tr>
<td>Systemic inflammation</td>
<td>ΔCRP</td>
<td>0.47</td>
<td>0.012</td>
<td></td>
</tr>
<tr>
<td>Airway inflammation</td>
<td>Δ%eosinophils</td>
<td>-0.49</td>
<td>0.093</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Δ%neutrophils</td>
<td>0.05</td>
<td>0.873</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Δ%macrophages</td>
<td>0.16</td>
<td>0.591</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Δ%lymphocytes</td>
<td>0.05</td>
<td>0.865</td>
<td></td>
</tr>
<tr>
<td></td>
<td>ΔeNO</td>
<td>0.46</td>
<td>0.034</td>
<td></td>
</tr>
<tr>
<td>Endocrine</td>
<td>Δleptin</td>
<td>0.31</td>
<td>0.136</td>
<td></td>
</tr>
<tr>
<td>Metabolic</td>
<td>ΔHOMA-IR</td>
<td>0.33</td>
<td>0.104</td>
<td></td>
</tr>
</tbody>
</table>

Δ, change; BMI, body mass index; CRP, C-Reactive Protein; eNO, exhaled nitric oxide; ERV, expiratory reserve volume; H₀ MOA, hypothesised mechanism of action; HOMA-IR, homeostasis model assessment of insulin resistance. †Spearman’s rank correlation coefficient

6.5 Discussion

The presented study was a pilot RCT aimed at investigating the efficacy of dietary energy restriction to induce acute weight loss in obese children with asthma, and the subsequent effect on asthma outcomes. Results demonstrate that dietary intervention can induce acute weight loss in asthmatic children within 10-weeks. Importantly, acute dietary-induced weight loss was associated with a significant improvement in static lung function, asthma control, and self-reported quality of life in this group of children.

Our data demonstrate that acute weight loss can be achieved in children with asthma, compared to controls, using simple dietary intervention. The presented dietary intervention achieved a median 5.7% reduction in body weight within 10-weeks, comparable with the 5.4% weight loss achieved at 6-months using interdisciplinary
therapy in a previous study\textsuperscript{131}. Notably, in the presented study, the dietary intervention was effective in reducing BMI $z$-score by a statistically significant 0.2BMI-SDS, which is comparable to previous studies. Few weight loss intervention studies conducted in children and adolescents have reported age- and sex-standardised BMI, which limits comparability between studies. Nonetheless, of previous weight loss interventions in non-asthmatic children which included a dietary component and reported standardised BMI, reductions of approximately 0.2-0.4BMI-SDS have been reported over time frames ranging from 4-6mths\textsuperscript{249-252}, while others have found no significant change\textsuperscript{253, 254}. Hence, the weight loss achieved in the current study, a median BMI $z$-score reduction of 0.2SDS, suggests our intervention was very successful. This may be due to the frequency of dietetic contact and/or the delivery of the dietary intervention, or that it was motivated by evaluating the impact on asthma outcomes. Participants were required to focus on one aspect of lifestyle modification which may make adjusting to beneficial changes easier than addressing multiple aspects simultaneously. Participation in a weight loss study designed to evaluate the effect upon asthma outcomes may also have motivated adherence to the dietary prescription. Investigation of whether greater weight loss can be achieved with a longer intervention, and follow-up to evaluate weight loss maintenance, is a consideration for future studies.

In both asthmatic and non-asthmatic adults, obesity is associated with reduced static lung function, namely FRC and ERV, while ventilatory function is only moderately affected\textsuperscript{112}. A dose-response relationship has been reported with weight loss in adults, whereby greater improvements in FEV$_1$, FVC and TLC are seen with greater amounts of weight loss\textsuperscript{190}. However, the most notable increases in lung function following weight loss have been reported for ERV, with increases of approximately 20% and
60% reported in asthmatic adults following diet-induced\textsuperscript{186} and surgery-induced\textsuperscript{184} weight loss, respectively. Recently, Boulet et al reported significant improvements in %predicted dynamic and static lung function variables, with the greatest improvement seen in %ERV, which more than doubled the baseline value by 12-months post-surgery in asthmatic adults\textsuperscript{184}. Recent reports describe RV and FRC, expressed as both %predicted and relative to TLC, to be significantly lower in overweight and obese asthmatic children compared to non-obese counterparts\textsuperscript{114, 115}. We recently reported that obesity in children with asthma is also associated with a reduced ERV (Jensen et al, unpublished). Conversely, childhood FEV\textsubscript{1} and FVC appear largely unaffected by the presence of obesity, with reports of increased ventilatory function in overweight and obese children compared to non-obese children with asthma\textsuperscript{92, 114, 115}. A recent uncontrolled weight-loss study did not measure static lung function, but did demonstrate significant improvements in %predicted spirometry values at 6-months and 12-months post-intervention in asthmatic adolescents\textsuperscript{131}. In contrast to this study, our participants had relatively normal baseline %FEV\textsubscript{1} and %FVC, which may explain why a change in spirometry measurements was not observed following acute weight loss. However, similar to adult studies, a significant improvement in ERV for the intervention group was detected, following weight loss. In addition, there was a significant reduction in RV and %RV/TLC, suggesting a reduction in obstruction.

Importantly, the improvement in asthma control in the intervention group approached clinical significance. There was also a clinically significant improvement in self-reported quality of life for the intervention group. However, only the change in the PAQLQ symptom and emotion domains reached statistical significance. Improved quality of life and asthma control has been demonstrated in asthmatic adults, 6 and 12-months following surgically-induced weight loss\textsuperscript{184, 185} and 10-weeks following
dietary-induced weight loss. Our results demonstrate that acute weight loss can achieve clinically significant improvements in static lung function, and significant improvements in asthma control and quality of life in obese children. Follow-up studies are needed to investigate the effect of long-term weight loss intervention on lung function and asthma control in this group of children.

Non-eosinophilic asthma, characterised by significant neutrophilia, has been described as a defining feature of adult obese asthma, and recently, Scott et al reported a significant association between %weight loss and reduced sputum %neutrophils in asthmatic females following a 10-week dietary and/or exercise intervention. Although a recent cluster analysis in asthmatic children identified a distinct cluster characterised by a greater BMI, elevated peripheral neutrophils and poorer FEV	extsubscript{1}, the presence of airway neutrophilia in paediatric obese asthma has not been described. The presented study is the first paediatric weight loss trial to report airway inflammation. No significant change in eNO or induced sputum inflammatory cells was detected within groups. However, there was a non-significant trend towards a reduction in %neutrophils in the intervention group. Likewise, there was no significant difference in the change in airway inflammatory markers between groups, with the exception of absolute and %lymphocytes. Interestingly, in asthmatic adults, an increase in %lymphocytes in broncho-alveolar lavage (BAL) samples 12-months post-bariatric surgery has also been reported, while no change in neutrophils or eosinophils were observed. Future studies including a follow-up period are important to assess whether airway inflammation may decline significantly over time if weight loss is continued or maintained.

Elevated systemic inflammation has been described in adult obese asthma. Following weight loss, significant increases in adiponectin levels, and significant
reductions in CRP\textsuperscript{184}, leptin\textsuperscript{186} and IL-6\textsuperscript{186} have been reported. Previous weight loss interventions in non-asthmatic children have produced variable changes in systemic inflammation\textsuperscript{33}. Both short (3-6 weeks) and long term (3-12 months) weight-loss studies have observed significant reductions in CRP, IL-6 and leptin, and increases in adiponectin\textsuperscript{33}, while other studies have reported no difference in CRP, TNF-α or adiponectin levels over a 6-12 month period, despite weight loss of up to 0.4BMI-SDS\textsuperscript{33, 249, 251}. These studies also failed to detect a significant change in metabolic markers\textsuperscript{249, 251}. The recent weight loss intervention by da Silva et al reported modest but statistically significant improvements in CRP, leptin and adiponectin in obese asthmatic and non-asthmatic adolescents at 12-months, where there was no significant change at 6-months\textsuperscript{131}. Furthermore, a 12-month longitudinal study in non-asthmatic children demonstrated that improvements in TNF-α, CRP, leptin and adiponectin levels did not occur in children with weight loss <0.5BMI-SDS compared to children with weight loss ≥0.5 BMI-SDS\textsuperscript{43, 256}. Although, the presented study achieved a significant reduction in BMI z-score in 10-weeks, the median reduction of 0.2BMI-SDS may not have been sufficient to achieve favourable changes in systemic biomarkers. Indeed, systemic inflammatory and metabolic biomarkers remained stable in this group of children, despite acute weight loss. However, moderate but significant increases in glucose and CRP were observed in the control group, even in this short period of time. This may suggest that although this group of children appeared metabolically healthy at baseline, changes in body weight, specifically body composition, may have adverse effects upon the metabolic profile, which increases risk for other chronic conditions, including cardiovascular disease and diabetes mellitus. This is supported by the positive correlations between ΔCRP and ΔHOMA-IR versus fat mass and negative associations versus lean mass.
In an endeavour to understand the mechanism of weight loss in asthma, we explored the associations between $\Delta$BMI $z$-score and key outcomes. Previous reports have suggested that obesity adversely impacts asthma outcomes via mechanical restriction of the chest wall and diaphragm$^{106}$. It is also suggested that obesity alters the airway inflammatory phenotype in asthma, altering management needs$^{89, 91}$. Our results indicate that weight change after 10 weeks is associated with airway and systemic inflammation, as indicated by $\Delta$CRP and $\Delta$eNO. This may suggest that weight loss, and more specifically, reductions in adiposity and increases in lean mass, can lead to reductions in both systemic and airway inflammation. However, increased systemic inflammation was the only outcome associated with poorer asthma control, supported by a positive association between $\Delta$CRP and $\Delta$ACQ. This may suggest that the adverse effect of obesity may operate via alterations in systemic inflammation, and that body composition is an important consideration, as previously hypothesised$^{33, 92}$. However, further investigation is required.

This was the first RCT, designed as a pilot study, to investigate: a) the feasibility of dietary-induced weight loss in asthmatic children; and b) whether improvements in asthma outcomes are observed with acute weight loss. The presented study is limited by the sample size, which may have reduced the likelihood of detecting a change in outcome measures. Although this was a randomised trial, the intervention group tended to have a greater proportion of males and a greater proportion were reportedly using ICS. The intervention group also had a significantly poorer ACQ score at baseline, compared to the WLC, which may have confounded the results. In addition, a potential source of bias is improved patient-directed asthma awareness, such as improved medication adherence, purely due to enrolment in a study in which the primary end-points are asthma outcomes. Although there was no significant change in
reported medication use during the course of the study in either group, the reliability of this information is subject to the limitations of self-report. Furthermore, the nature of the intervention precluded blinding of participants and research officers to group allocation. However, the key outcomes of the study were objective measurements, which would have minimised bias. Lastly, our study did not include a follow-up period. Recent studies including a follow-up period have indicated that weight loss is maintained in children$^{249, 250, 252}$. Therefore, further investigation into the efficacy of diet-induced weight loss in asthmatic children is needed, which includes an adequate follow-up period.

The presented study demonstrates that rapid weight loss can be achieved safely in obese asthmatic children with dietary intervention alone, resulting in improvements in static lung function, asthma control and quality of life. On the other hand, changes in airway and systemic inflammation were not detected following acute dietary-induced weight loss in this group of children. However, exploratory analysis suggests that body composition changes, specifically greater adiposity and lesser lean mass, are associated with adverse changes in systemic and airway inflammation. In addition, systemic inflammation was associated with poorer asthma control in children. Our data indicates that weight loss can produce beneficial changes in childhood asthma outcomes and supports the need for larger RCTs, with an appropriate intervention and follow-up period, to investigate further the efficacy of weight loss intervention in asthmatic children.
7. Chapter VII: General Discussion & Future Directions
This thesis has examined the relationship between obesity and asthma in children, looking at inflammatory, physiological and clinical aspects of paediatric obese asthma. At the time the research in Chapter III and IV was commenced, there were no studies published internationally that assessed lung volumes and airway inflammation by induced sputum, in obese children with asthma. In addition, there were no weight loss studies published that had been conducted in children with asthma, specifically to evaluate the impact on asthma outcomes, at the time that the research in Chapter VI was commenced.

7.1 Characterising Paediatric Obese Asthma

Chapter III & IV aimed to characterise the obese phenotype in asthmatic children. Given the extent of information available in adults and the significant differences noted between obese and non-obese asthmatic adults, particularly with regard to static lung function deficits, increased systemic inflammation and an altered airway inflammatory profile, such investigations in children were warranted.

In Chapter III, examination of airway inflammation by sputum inflammatory cell counts found no difference in airway neutrophils or eosinophils between obese and non-obese children with asthma. This is different to adults where there is a distinct pattern of airway inflammation associated with obese asthma, characterised by increased airway neutrophils.\(^{89, 91}\). We did find that obese asthmatic female children had lower sputum %eosinophils, lower eNO concentrations, and a lower prevalence of eosinophilic asthma, compared to obese asthmatic male children. These results indicate that there may also be a propensity for female children to display a non-eosinophilic pattern of airway inflammation, however it is less pronounced than in obese adults. This difference in airway inflammation between males and females was not seen in non-obese asthmatic children.
Systemic inflammation did not differ between obese and non-obese children with asthma. This is considerably different to obese asthma in adults, where significant elevations in systemic inflammation compared to non-obese asthmatics are noted, including leptin, CRP and IL-6\(^91, 96, 98\). The relative absence of inflammation from obesity in our cross-sectional study may indicate that obesity is operating via alternative mechanisms, and/or can be viewed as a co-morbidity as opposed to a cause of asthma in children. Given that research has shown systemic inflammation is raised in adult obese asthma, our findings may indicate that the timing of adipose tissue becoming pathological is important to the inflammatory environment related to obesity. That is, the adverse changes in inflammatory markers seen in adults may not occur in young children. Consideration of age, growth stage (considering lean: fat mass deposition), duration of obesity, the deposition (number of adipocytes, size of adipocytes) and distribution (visceral versus sub-cutaneous) of fat mass, and the degree/ severity of obesity (i.e. mild/ moderate/ morbid obesity)\(^23, 26, 214\) are important to our understanding of the inflammatory differences in obese asthma between adults and children.

Chapter III revealed that, similar to adults, obesity has mechanical effects relevant to respiratory outcomes, as obesity was found to be associated with a reduction in ERV in asthmatic children. This is an important lung function measure to report, as it is the most significantly depressed lung function measure in obese adults\(^112\) and has not been previously reported in children. In Chapter IV, the mechanical effects of obesity on lung function in children was investigated further by exploring linear associations between body composition and dynamic and static lung function in children with and without asthma. This chapter revealed important differences between fat and lean mass associations with lung function in children. Inverse associations were found
between fat mass and FRC, but also between lean mass and ERV, indicating that increases in body mass, regardless of the composition, may be detrimental to lung function in children with and without asthma. Interestingly, lean mass had positive associations with dynamic lung function, after controlling for age, height, gender and asthma. In asthmatic adults, lean mass has been positively associated with lung function in males, including ERV, but in females, both lean mass and fat mass have been inversely associated with ERV\textsuperscript{144}. In addition, lean mass has been shown to be more strongly associated with asthma than fat mass in female adults, whereas no association was shown for males\textsuperscript{145}. In non-asthmatic adults, fat mass is inversely related to lung function in females and males, particularly static lung function\textsuperscript{142}. However, lean mass has been positively associated with lung function, specifically FEV\textsubscript{1} and TLC, in males only\textsuperscript{142}. These adult studies indicate there is a gender influence on body composition and lung function associations\textsuperscript{142, 144, 145}, but this is yet to be explored in children.

7.2 Risk Factors for Obesity in Asthma

In order to understand why obesity is higher in asthma, Chapter V investigated whether lifestyle risk factors for obesity, including sleep characteristics, were more prevalent in non-obese children with asthma, compared to children without asthma. This area of research has not previously been reported in asthmatic children or adults, despite reports of poor sleep in the asthmatic population\textsuperscript{79, 154, 156}, and despite the known associations between sleep and overweight/obesity risk in both children and adults\textsuperscript{14, 151}. This cross-sectional study revealed that sleep quality is slightly poorer in asthmatic children, despite well-controlled asthma. However, no differences were seen in plasma appetite hormones, food cravings, dietary intake or activity levels as measured subjectively by validated questionnaires. There was an association between
dietary intake and sleep duration, whereby increased total sleep time paralleled dietary intake patterns that closely mirrored Australian Dietary Guidelines for Children and Adolescents. This would indicate that less sleep is associated with a poorer dietary intake pattern. In addition, an association between increasing daytime sleepiness and increasing sedentary behaviour was also noted. The data in Chapter V suggests that poor sleep and daytime sleepiness potentially increase the prevalence of risk factors for weight gain in children, with and without asthma, via changing dietary intake and activity, although the exact mechanism has not been confirmed. This area requires further research. Daytime sleepiness also paralleled increases in TC: HDL, indicating a potential impact of daytime sleepiness on CVD risk outcomes. Furthermore, we found higher triglyceride levels in non-obese asthmatic children, despite a minority taking low dose ICS, and having a similar dietary intake, and glucose tolerance and insulin sensitivity, compared to non-asthmatic children.

7.3 Weight loss in Paediatric Obese Asthma

In Chapter VI, the feasibility and efficacy of a dietary intervention to induce acute weight loss and subsequently improve asthma outcomes was investigated. This is the first weight-loss RCT conducted in obese asthmatic children. This ten week pilot study revealed that acute weight loss can be achieved in a short period of time. Importantly, reductions in weight were predominantly due to reductions in fat mass. This weight loss was associated with a significant improvement in static lung function, quality of life and asthma control. Relevant to the clinician, this weight loss was achieved using simple dietary changes that can be implemented in the clinical setting in consultation with a dietitian, and weight loss should be suggested for overweight/ obese asthmatic children as part of their management. Furthermore,
knowing that improved asthma control may be an outcome, this offers a new motivator for weight loss in children and further research is needed.

A reduction in airway or systemic inflammation was not detected with acute weight loss in Chapter VI. This is not surprising given the cross-sectional study in Chapter III found no significant elevation in airway or systemic inflammation in obese asthma. However, there was an attenuation of the rise in CRP and glucose in the intervention group. In contrast, the control group had a significant increase in weight, CRP and glucose, indicating that weight gain and metabolic impairment can commence within a short period of time in this group of children. That is, without an intervention there is a worsening in metabolic outcomes for obese asthmatic children. Exploratory analysis of the RCT change data indicated that change in weight, specifically reductions in adiposity and increases in lean mass, were associated with decreases in both airway (eNO) and systemic inflammation (CRP), indicating that weight gain may still be contributing to increases in local and systemic inflammation in asthma, albeit to a small extent. In addition, only the change in systemic inflammation (CRP) paralleled a change in asthma control (ACQ), indicating that the improvements seen in asthma control with weight loss may be mediated via a change in systemic inflammation.

### 7.4 Clinical and Scientific Implications

In Chapter III & IV we indicated that measurement of lung volumes by plethysmography may reveal important deficits in lung function in obese children, otherwise not detected by routine spirometry. In the clinical setting, it appears important to measure lung volumes in order to assess the respiratory deficits of the obese child with asthma. In addition, body composition appears important to lung function in children. Encouraging a healthy weight, with a focus on promoting lean
mass retention, is an area of research that requires further exploration in children with asthma. Further studies are needed to examine whether intervention with resistance training/weight-bearing activity, with the aim of promoting lean tissue preservation and accretion, is beneficial to children with asthma, specifically lung function.

Chapter III indicated that there may be less eosinophilic inflammation in obese female asthmatics. Given that ICS (and oral steroids during exacerbations) are the most common preventer medication used in childhood asthma and work by reducing eosinophilic inflammation, this information has important implications for the pharmaceutical management of the obese asthmatic child. Further studies are needed to investigate the existence of gender-based differences in asthma outcomes, particularly airway inflammation and medication use, in paediatric obese asthma. Furthermore, steroid insensitivity has been suggested by previous literature. However, given that airway inflammation was not altered in obese asthma compared with non-obese asthma, future studies may also consider alternate mechanisms of steroid insensitivity in obese children with asthma.

Chapter V has indicated sleep is an important lifestyle measurement to be included in the clinical and research setting, as sleep quality was observed to be poorer in asthmatic children, despite well-controlled intermittent-mild asthma. Assessment of daytime sleepiness (using a validated scale such as the PDSS) may identify patients at increased risk of poor lifestyle habits, such as increased sedentary activity. Early intervention is the preferable method to tackle the obesity epidemic in children. However, the prevalence of modifiable risk factors for obesity – sleep duration, dietary intake and activity levels – were similar between asthmatic and non-asthmatic children and further investigation as to why obesity rates are higher in the asthmatic population is needed.
Weight loss should be recommended by the clinician and included as part of the routine management for overweight/obese children with asthma. In Chapter VI, we demonstrated that weight loss is feasible in obese children with asthma using simple dietary modification. Importantly, weight loss, specifically fat loss, demonstrates benefit to asthma outcomes and should be encouraged in the clinical setting, and may suggest that overweight/obese asthmatic children should be prioritised on waiting lists for weight loss services. Most weight loss studies in asthma have used bariatric surgery for obese adults\textsuperscript{183-185}. Surgical intervention is typically indicated in extreme obesity cases and in children, would not generally be indicated. Therefore, intervening with dietary modifications that can be implemented with the aid of a dietitian is beneficial and feasible in the clinical setting and general community. Also, in opposition to lifestyle interventions that have used multi-disciplinary programs\textsuperscript{131}, our research indicates that dietary intervention alone, i.e. asking the patient to modify one lifestyle aspect, can achieve significant weight loss. This intervention achieved acute weight loss in a short period of time, comparable to that achieved in non-asthmatic children in longer term studies (4-6 months)\textsuperscript{249-252}, and this may suggest that the frequency of consults focusing on one lifestyle modification was beneficial to the participant. It is also possible that the success of the weight loss programme may at least partly be due to increased motivation to lose weight in this group at the prospect it may help their disease, and this warrants further investigation.

7.5 Future Directions
The pilot RCT in Chapter VI demonstrated the efficacy and feasibility of weight loss in obese asthmatic children. Future trials with a larger sample size are needed to replicate the results, and to investigate whether a longer term intervention produces greater weight loss and greater benefit to asthma outcomes. Also, the combination of
resistance training and energy restriction to preserve lean mass while encouraging fat loss may yield greater improvements in lung function. Trials involving a follow-up period are also needed to assess whether the effects of weight loss, and benefit to asthma outcomes, are sustained over a period of time. In addition, future trials may assess whether declines in airway inflammation become apparent with greater weight loss and maintenance of weight loss.

Asthma appears to affect the quality of sleep and this may be associated with increasing asthma severity. Sleep quality was marginally poorer in asthmatic children. However, these children had intermittent-mild asthma, and investigation into associations between sleep and lifestyle in children with poorly controlled/moderate-severe asthma may reveal more dramatic differences between asthmatic and non-asthmatic children. In addition, the association between daytime sleepiness and sedentary activity requires investigation, particularly in groups with excessive daytime sleepiness. Future studies should include objective measurement of sleep, activity and dietary intake. In addition, longitudinal measurement of modifiable lifestyle risk factors for obesity, including sleep habits, dietary intake and activity patterns, and accounting for potential gender disparities, may reveal more information regarding chronic sleep disturbance and obesity risk in the asthmatic population.

Investigation into the progression of obese asthma through the life stages is needed. The changes in clinical, physiological and inflammatory aspects of obese asthma from childhood to adulthood may be a product of the degree and duration of obesity, as well as body composition, and may be influenced by the hormonal environment which alters with age, growth stage and gender. In addition, the distribution of adiposity may be important to asthma outcomes, for example intra-thoracic versus extra-thoracic adipose tissue and android versus gynoid adipose tissue. Longitudinal
studies, including direct measures of body composition, lung function and airway and systemic inflammation are required to investigate the progression of the obese asthma phenotype from childhood, through to adolescence and adulthood. Given the information on obese asthma in adulthood, at some point the airway inflammatory profile becomes predominantly non-eosinophilic in obese asthma, and longitudinal studies are needed to identify the factors contributing to these changes. This may provide some indication of when and what factors contribute to the airway and systemic inflammatory alterations in obese asthma seen in adults, but not in children. In addition, investigation into the gender disparities in paediatric obese asthma is warranted. Indeed, adult studies have indicated a gender-based difference in obese asthma, specifically airway inflammation and lung function, and this remains to be investigated in children. Our data indicated that there may be a difference in the airway inflammatory profile between males and females, and perhaps associations between body composition and asthma outcomes would also reveal different outcomes for males and females.

7.6 Final conclusion

The research conducted as part of this thesis has contributed to improving our understanding of paediatric obese asthma, which is important to the clinical management of this population. This thesis has identified important differences between obese asthma in children and adults. We have characterised the airway and systemic inflammatory profile in obese asthmatic children and determined that the extent of inflammation in children is much less than in adults. We have ruled out lifestyle risk factors, including sleep and dietary intake, as significant contributors to the higher prevalence rate of overweight and obesity in asthmatic children, compared to non-asthmatic children. As part of this thesis, we have also established the
feasibility and efficacy of a short term dietary intervention in obese asthmatic children to induce acute weight loss, improve body composition, and improve asthma outcomes.
8. Reference List


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9. Appendices
Asthma and Lifestyle

PARTICIPANT INFORMATION SHEET

Invitation to participate
We would like to invite you to take part in a research project investigating whether body weight is associated with inflammation and asthma. This study is being undertaken as part requirement for a PhD at the University of Newcastle by Megan Jensen, under the supervision of Dr Lisa Wood, Professor Peter Gibson and Associate Professor Clare Collins. It is hoped that this study will increase our knowledge about weight status and asthma.

Why are we doing this research?
Asthma is the most common chronic disease in children and adolescents. It is believed that there may be an association between body weight and asthma. Extra body weight is associated with increased inflammation throughout the body, while asthma is associated with inflammation in the lungs. This study will examine whether this inflammation is different between people who have asthma and people who do not have asthma.

Who can participate in the research?
We are seeking people 8-17 years of age, with or without doctor diagnosed stable asthma.

If you follow a special diet, have a lung disease other than asthma, have had a recent respiratory tract infection, a visit to the GP or hospital due to a worsening of asthma symptoms, use of oral steroids or a change in asthma medication this study is not suitable for you.

What does the study involve?
If you agree to participate in the study, you will be required to visit the clinic at John Hunter Hospital to undergo a series of tests and to consult with an Accredited Practising Dietitian. You will be required to attend the clinic at the John Hunter Hospital a total of two times. The study is described in more detail below.
Clinic Visit 1:
If you agree to participate you will need to visit the clinic for a number of tests, described below. This visit will take approximately 2-3 hours. Before this visit you will need to fast for 12 hours and withhold your asthma reliever medication (if you use them) for 6-24 hours.

1. **Allergy skin prick test:** You will have a skin prick allergy test during the visit. A small amount of fluid is put on your skin then tiny pricks are made on the skin at this location (this doesn’t break the skin and is not painful). If you are allergic to the fluid, a small itchy lump will occur. This usually lasts for an hour or so and if it is annoying we can give you some cream which takes the itch away.

2. **Lung function tests:** We wish to measure how well your lungs work and this involves asking you to breathe different ways, such as deep breathing and blowing out rapidly. Some of these breathing tests may be performed whilst sitting in a plethysmograph, which looks a bit like a high-tech telephone booth. Your lung function will also be assessed by blowing into a spirometer, a machine that measures the amount of air expelled from your lungs. These are routine breathing tests that may cause some breathlessness and/or dizziness which usually lasts for a few seconds only. If you feel uncomfortable during the test and want to stop, you can.

3. **Saline Challenge:** You will be asked to inhale a mist of salty water. This will involve using a snorkel-like mouthpiece to breathe the mist for consecutive periods of 30 seconds, 1 minute, 2 minutes and then 3 lots of 4 minutes. A breathing test will be done at the end of each period and you will be asked to cough up a specimen of sputum. This is a routine test. The test will be stopped at your request or if your breathing test results worsen. You will be given salbutamol (Ventolin) if you develop any problems with your breathing. Salbutamol is a medication that immediately relieves constriction of the airways and is inhaled by mouth through a puffer device.

4. **Blood test:** Approximately 20mL (4 teaspoons) of blood will be taken from a vein in your forearm to measure levels of inflammation, fatty acids, insulin, glucose, and cholesterol. If any of the blood tests are not normal, we will call you to tell you and with your permission, send this information to your GP. We will also tell you whether the study is still suitable for you.

5. **Questionnaires:** You will be asked to answer questions about your general background, asthma, the foods you eat and how active you are. These questionnaires will take approximately 40 minutes to complete.

6. **Height and weight:** Your height, weight and waist circumference will also be measured.

Your mum and/or dad can be with you the entire time if you would like. We can stop the tests at any time if you feel uncomfortable.

Clinic Visit 2:
We will need you to come into the clinic to complete a couple more tests, which are described below. This visit will take approximately 2 hours. Before this visit we will ask you to record your food intake for four days. We will give you cups and spoons to measure how much food and fluid you are eating and drinking over four days. We will also ask you to record your steps for seven days. We will provide you with a pedometer to count your steps.
1. **Body composition**: We will also measure your body composition using a dual energy x-ray absorptiometry (DEXA) machine. This is a routine procedure that involves the use of very low levels of radiation. This research study involves exposure to a very small amount of radiation (Total Body Scan). The effective dose from this study is less than 0.010mSv. At this dose, no harmful effects of radiation have been demonstrated and the risk is negligible. It is equal to the amount of radiation we are all exposed to on a daily basis. This test does not hurt. It will provide us with a picture of your body on the inside.

Since DEXA should not be used on pregnant women, we are required to confirm that female participants are not pregnant. To do this we will ask you to give us a small sample of urine and we will conduct a pregnancy test prior to conducting the DEXA scan.

2. **Other tests**: We will also measure the level of cotinine in your body. This is a simple test that indicates exposure to cigarette smoke and requires a small sample of urine.

Your mum and/or dad can be with you the entire time if you would like. We can stop the tests at any time if you feel uncomfortable.

3. **Dietary consult**: During the visit you will receive dietary advice from a qualified dietitian to help improve the quality of their diet. The session will look at your 4-day food record and answer any questions you or your family may have about your current diet. This session will take approximately 60 mins.

**What are the benefits and risks of the study?**
If this study is suitable for you and you agree to participate, you will receive advice about your diet from a dietitian, free of charge. The sputum test can cause difficulty breathing, coughing, some discomfort in your chest and wheezing. This is brief and responds promptly to reliever medication (Ventolin). The side effects of having blood collected may include bleeding or bruising at the injection site and possible dizziness and/or fainting. Please advise the research team if you normally feel dizzy or faint when you have blood collected. The risk associated with the DEXA scan is negligible and is equal to the natural background radiation we receive on a daily basis from our environment. The effective dose from this study is less than 0.010mSv. At this dose level, no harmful effects of radiation have been demonstrated and the risk is negligible. All radiation exposures will be carried out in accordance with the ARPANSA Code of Practice “Exposure of Humans to Ionizing Radiation for Research Purposes” (RPS8). We would like you to tell us if you have participated in any research studies in the previous 5 years that have involved the use of radiation, as we need to make sure that you do not exceed a safe level of radiation.

**What happens at the end of the study?**
At the end of the study or if you decide to withdraw from the study, you will be referred back to your GP. A copy of your results from the study will also be sent to your GP.

**Costs**
Participation in this study will not cost you anything, nor will you be paid. However, you will receive a parking voucher to cover parking costs while attending the hospital. You will also receive individual dietary counselling by an Accredited Practicing Dietitian.

**What choice do I have?**
Your participation in this study is voluntary. If you don’t want to participate in the study this will not affect your current or future medical care. If, during the study, you wish to withdraw, you are free to do so. All information is kept strictly confidential and your name will not
appear in any reports. All participants will be given a study number from which the participant cannot be identified. Your permission will be sought before accessing your medical records. Your personal information will be accessed, used and stored in accordance with Commonwealth Privacy Laws and the NSW Health Records and Information Privacy Act 2002. The results of this study will be shared with other people in the scientific community. They may be compared to results from other studies.

If you decide to withdraw from the study, you can withdraw all data relating to you and have any samples destroyed. An exception to this is in the case of an adverse event where data needs to be retained for regulatory reporting. However, all data that is collected will be beneficial to the research study.

Who do I call if I have any questions or problems?
If you have any questions about the study, your child’s results or treatment, you can contact the staff listed on the following page:

Dr Lisa Wood Business hours 02 4985 5677
Miss Megan Jensen Business hours 02 4985 5649

Chief Investigator:
Dr Lisa Wood.

Co-Investigators:
Professor Peter Gibson
Associate Professor Clare Collins
Dr. Jodi Hilton

Student Investigator:
Megan Jensen

Complaints about this research
This research has been approved by the Hunter New England Human Research Ethics Committee of Hunter New England Health, Reference number 09/05/20/5/09. Should you have concerns about your rights as a participant in this research, or you have a complaint about the manner in which the research is conducted, it may be given to the researcher, or, if an independent person is preferred, to Dr Nicole Gerrand, Professional Officer (Research Governance and Ethics), Hunter New England Human Research Ethics Committee, Hunter New England Health, Locked Bag 1, New Lambton NSW 2305. Phone: 02 4921 4950, Email hnehrec@hnehealth.nsw.gov.au
I agree to my participation in the above research project and give my consent freely. I understand that the project will be conducted as described in the Information Sheet, a copy of which I have retained. I have carefully read the information provided to me and understand its content. All questions raised by me have been answered to my satisfaction. I give permission for the members of the research team to access my medical records. I also give permission for the members of the research team to contact my GP and/or respiratory physician and for relevant information to the study and/or my clinical management to be shared if necessary. I understand that I am free to withdraw my consent at any time without reason and without disadvantage to my future care.

**PARTICIPANT CONSENT FORM**

I consent to my participation in this study

Participant name (please print) ________________________________

Participant signature ___________________________ Date ______________
Asthma and Lifestyle

PARENTAL INFORMATION SHEET

Invitation to participate
We would like to invite your child to take part in a research project investigating whether body weight is associated with inflammation and asthma. This study is being undertaken as part requirement for a PhD at the University of Newcastle by Megan Jensen, under the supervision of Dr Lisa Wood, Professor Peter Gibson and Associate Professor Clare Collins. It is hoped that this study will increase our knowledge about weight status and asthma.

Why are we doing this research?
Asthma is the most common chronic disease in children and adolescents. It is believed that there may be an association between body weight and asthma. Extra body weight is associated with increased inflammation throughout the body, while asthma is associated with inflammation in the lungs. This study will examine whether this inflammation is different between people who have asthma and people who do not have asthma.

Who can participate in the research?
We are seeking people 10-17 years of age, with or without doctor diagnosed stable asthma.

If your child follows a special diet, has a lung disease other than asthma, has had a recent respiratory tract infection, a visit to the GP or hospital due to a worsening of asthma symptoms, uses oral steroids or has had a change in asthma medication this study is not suitable for your child.

What does the study involve?
If you and your child agree to their participation in the study, they will be required to visit the clinic at John Hunter Hospital to undergo a series of tests and to consult with an Accredited Practising Dietitian. Your child will be required to attend the clinic at the John Hunter Hospital a total of two times.

The study is described in more detail below.

Clinic Visit 1:
If you and your child agree to their participation they will need to visit the clinic for a number of tests, described below. This will take approximately 2-3hrs. Prior to this visit your child...
will need to fast for 12 hours and withhold their asthma reliever medication (if applicable) for 6-24 hours.

1. **Allergy skin prick test:** Your child will have a skin prick allergy test during the visit. A small amount of fluid is put on their skin then tiny pricks are made on the skin at this location (this doesn’t break the skin and is not painful). If your child is allergic to the fluid, a small itchy lump will occur. This usually lasts for an hour or so and if it is annoying we can apply a cream which takes the itch away.

2. **Lung function tests:** We wish to measure how well your child’s lungs work and this involves performing various breathing efforts such as deep breathing and blowing out rapidly into lung function equipment. Some breathing efforts may be performed whilst sitting in a plethysmograph - specialised lung testing equipment which looks a bit like a high-tech telephone booth. Your child’s lung function will also be assessed by blowing into a spirometer, a machine that measures the amount of air expelled from their lungs. These are routine breathing tests with no known adverse effects, except for perhaps some breathlessness and/or dizziness which usually lasts for a few seconds only. We can stop this test at any time if your child feels uncomfortable.

3. **Saline Challenge:** Your child will be asked to inhale a mist of salty water. This will involve using a snorkel-like mouthpiece to breathe the mist for consecutive periods of 30 seconds, 1 minute, 2 minutes and then 3 lots of 4 minutes. A breathing test will be done at the end of each period and your child will be asked to cough up a specimen of sputum. This is a routine test. The test will be stopped at your child’s request or if their breathing test results worsen. If any problems develop with their breathing, your child will be given salbutamol (Ventolin). Salbutamol is a medication that immediately relieves constriction of the airways and is inhaled by mouth through a puffer device.

4. **Blood test:** Approximately 20mL (4 teaspoons) of blood will be taken from a vein in your child’s forearm to measure levels of inflammation, fatty acids, insulin, glucose, and cholesterol. If any abnormalities are detected from the blood test, we will inform you / your child via telephone and with your / your child’s permission, refer to your GP.

5. **Questionnaires:** Your child will be asked to answer questions about their general background and asthma, their medication use and medical history, the foods they eat and how active they are. These questionnaires will take approximately 40 minutes to complete. You may assist your child with some of these questions as they may not remember certain details e.g. their medications and medical history.

6. **Anthropometry:** During this visit we will measure their height, weight and waist circumference.

If you wish, you can be with your child the entire time. We can stop the tests at any time if your child feels uncomfortable.

**Clinical Visit 2:**

We will need your child to come into the clinic to complete body composition analysis and receive an individual dietary consult with an Accredited Practising Dietitian (APD), described below. This visit will take approximately 2hrs.

Prior to this visit we will ask your child to record their food intake for four days. We will give your child cups and spoons to measure how much food and fluid they are eating and drinking over four days. We will also ask your child to record their steps for seven days. We will provide your child with a pedometer to count their steps.
1. **Body composition:** We will measure your child’s body composition using a dual energy x-ray absorptiometry (DEXA) machine. This is a routine procedure that involves the use of very low levels of radiation. This research study involves exposure to a very small amount of radiation (Total Body Scan). The effective dose from this study is less than 0.010mSv. At this dose, no harmful effects of radiation have been demonstrated and the risk is negligible. It is equal to the amount of radiation we are all exposed to on a daily basis. It will provide us with a picture of your child’s body on the inside. Since DEXA should not be used on pregnant women, we are required to confirm that female participants are not pregnant. To do this we will ask your daughter to give us a small sample of urine and we will conduct a pregnancy test prior to conducting the DEXA scan.

2. **Other tests:** We will also measure the level of cotinine in your child’s body. This is a simple test that indicates exposure to cigarette smoke and requires a small sample of urine.

If you wish, you can be with your child the entire time. We can stop the tests at any time if your child feels uncomfortable.

3. **Dietary consult:**
During the visit you and your child will receive individualised dietary advice from a qualified dietitian to help improve the quality of their diet. The session will review their 4-day food record and address any questions you or your child may have with their current diet. This session will take approximately 60mins.

**What are the benefits and risks of the study?**
If this study is suitable for your child and you and your child agree to their participation in this study, your child will receive specialised advice about their diet from a dietitian, free of charge. The sputum test can cause difficulty breathing, coughing, some discomfort in their chest and wheezing. This is brief and responds promptly to reliever medication (Ventolin). The side effects of having blood collected may include bleeding or bruising at the injection site and possible dizziness and/or fainting. Please advise the research team if your child normally feels dizzy or faint when they have blood collected. The risk associated with the DEXA scan is negligible and is equal to the natural background radiation we receive on a daily basis from our environment. The effective dose from this study is less than 0.010mSv. At this dose level, no harmful effects of radiation have been demonstrated and the risk is negligible. All radiation exposures will be carried out in accordance with the ARPANSA Code of Practice “Exposure of Humans to Ionizing Radiation for Research Purposes” (RPS8). We would like you to tell us if your child has participated in any research studies in the previous 5 years that have involved the use of radiation, as we need to make sure that a safe level of radiation is not exceeded.

**What happens at the end of the study?**
At the end of the study or if your child decides to withdraw from the study, they will be referred back to their GP. A copy of their results from the study will also be sent to their GP.

**Costs**
Participation in this study will not cost you / your child anything, nor will you / your child be paid. However, you will receive a parking voucher to cover parking costs when attending the hospital. Your child will also receive individual dietary counselling by an Accredited Practicing Dietitian.
What choice do I have?
Your child’s participation in this study is voluntary. If your child does not want to participate in the study, this will not affect their current or future medical care. If during the study your child wishes to withdraw or you wish to withdraw consent, you and your child are free to do so. All information is kept strictly confidential and your child’s name will not appear in any reports. All participants will be given a study number from which the participant cannot be identified. You and your child’s permission will be sought before accessing their medical records. Your child’s personal information will be accessed, used and stored in accordance with Commonwealth Privacy Laws and the NSW Health Records and Information Privacy Act 2002. The results of this study will be shared with other people in the scientific community. They may be compared to results from other studies.

If your child decides to withdraw from the study or you wish to withdraw consent, all data relating to your child can be withdrawn and any samples can be destroyed. An exception to this is in the case of an adverse event where data needs to be retained for regulatory reporting. However, all data that is collected will be beneficial to the research study.

Who do I call if I have any questions or problems?
If you have any questions about the study, your child’s results or treatment, you can contact the staff listed on the following page:

Dr Lisa Wood
Business hours 02 4985 5677
Miss Megan Jensen
Business hours 02 4985 5679

Chief Investigator:
Dr Lisa Wood.

Co-Investigators:
Professor Peter Gibson
Associate Professor Clare Collins
Dr. Jodi Hilton

Student Investigator:
Megan Jensen

Complaints about this research
This research has been approved by the Hunter New England Human Research Ethics Committee of Hunter New England Health, Reference number 09/05/20/5/09. Should you have concerns about your rights as a participant in this research, or you have a complaint about the manner in which the research is conducted, it may be given to the researcher, or, if an independent person is preferred, to Dr Nicole Gerrand, Professional Officer (Research Governance and Ethics), Hunter New England Human Research Ethics Committee, Hunter New England Health, Locked Bag 1, New Lambton NSW 2305. Phone: 02 4921 4950, Email hnehrec@hnehealth.nsw.gov.au
Asthma and Lifestyle

Chief Investigator: Dr Lisa Wood
Ph: 02 4985 5677

Student Investigator: Megan Jensen
Ph: 02 4985 5679

PARENTAL CONSENT FORM

I agree to the participation of my child in the above research project and give my consent freely. I understand that the project will be conducted as described in the Information Sheet, a copy of which I have retained. I have carefully read the information provided to me and understand its content. All questions raised by me have been answered to my satisfaction. I give permission for the members of the research team to access my child’s medical records. I also give permission for the members of the research team to contact my child’s GP and/or respiratory physician and for relevant information to the study and/or my child’s clinical management to be shared if necessary. I understand that I am free to withdraw my consent at any time without reason and without disadvantage to the future care of my child.

I consent to my child’s participation in this study

Participant name (please print) __________________________________________

Parent / Guardian name (please print)
____________________________________________

Parent / Guardian signature _________________________________________
Date _______________
## Appendix 3: Medications withheld prior to appointments

At your FIRST VISIT, I plan on performing the following test(s):

- □ Skin Prick Allergy test
- □ Saline Challenge test

If you require either **prednisone or antibiotics** for your chest or you feel unwell prior to either appointment please let me know by calling 4985 5679. If this occurs, I may need to reschedule your appointment(s).

*Please do not eat or drink anything (except water) for 12hrs prior to your appointment.*

I would like you to try to **withhold** your puffers and antihistamines for your visit. Please attempt to **withhold** the following medications for the stated times before your visit:

<table>
<thead>
<tr>
<th>6 Hours</th>
<th>12 Hours</th>
<th>24 Hours</th>
<th>5 Days</th>
<th>6 weeks</th>
</tr>
</thead>
<tbody>
<tr>
<td>Airomir</td>
<td>Austyn</td>
<td>Foradile</td>
<td>Aller G</td>
<td>Phenergan</td>
</tr>
<tr>
<td>Asmol</td>
<td>Neulin</td>
<td>Neulin SR</td>
<td>Andrumin</td>
<td>Polaramine</td>
</tr>
<tr>
<td>Atrovent</td>
<td>Oxis</td>
<td>Avil</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Atrovent Forte</td>
<td>Seretide</td>
<td>Avil Retard</td>
<td>Sudagesic</td>
<td></td>
</tr>
<tr>
<td>Bricanyl</td>
<td>Serevent</td>
<td>Benadryl</td>
<td>Teldane</td>
<td></td>
</tr>
<tr>
<td>Combivent</td>
<td>Singular</td>
<td>Claratyne</td>
<td>Telfast</td>
<td></td>
</tr>
<tr>
<td>Epaq</td>
<td>Slo-bid</td>
<td>Demazin</td>
<td>Vallergan</td>
<td></td>
</tr>
<tr>
<td>Intal</td>
<td>Spiriva</td>
<td>Disolyn</td>
<td>Zadine</td>
<td></td>
</tr>
<tr>
<td>Intal Forte</td>
<td>Symbicort</td>
<td>Dramamin</td>
<td>Zyrtec</td>
<td></td>
</tr>
<tr>
<td>Respolin</td>
<td>Theo-Dur</td>
<td>Panadol Sinus</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tilade</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ventolin</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

If you find it too difficult to withhold your medications, please take them as needed, as your comfort is more important. You may continue to use ‘preventer’ medications such as Flixotide, Pulmicort and QVAR as usual. If you have any questions, please call Megan on 4985 5679.
9.4 Appendix 4: Participant information sheet and assent form for obesity risk factors study: 7-12 years

Asthma and Sleep Invitation

Dear ______________________

*We would like to invite you to take part in our study!*

**What is the study about?**
Our study is about asthma and sleep. It is also about sleep, food and activity. A lot of kids have asthma and some of them do not sleep well. Our study is to find out if asthma makes it harder to sleep. You may not have asthma, but we would still like you to take part in our study. This will help us see if kids without asthma sleep differently to kids with asthma. Poor sleep can change the way you eat and the types of activities that you like to do. So, we also want to know about the foods you like to eat, and what kind of sports and activity you like to do.

**What happens in the study?**
If you would like to take part in our study, we will visit you while you are having your sleep study done. We will ask you and your mum / dad some questions. We will also do some tests. These are listed below. You only have to take part if you want to and your mum and dad are happy for you to do so. Even if your mum and dad say YES now to take part in the study, they can always change their mind and say NO if you decide you don’t want to take part later on. You do not have to do anything that makes you feel uncomfortable.
What questions will be asked?
We will ask you questions about:

- the foods you like to eat and how hungry you feel
- the kind of sports and activities you like to do e.g. watching TV, playing on the computer
- how happy you are

There are no wrong or right answers. Only we will see your answers. No one else will see your answers.

What tests will be done?

1. Breathing test:
   - We want to know how well your lungs work.
   - We will ask you to blow into a machine.
   - The machine will measure how much air you blow out.
   - This test does NOT hurt. You may feel dizzy or breathless but this lasts for a few seconds only.

2. Blood test:
   - There are ‘hormones’ in your blood that make you hungry.
   - We want to measure how much of these ‘hormones’ you have.
   - We will take a small amount of blood from a vein in your forearm when you wake up in the morning after your sleep study.
   - You will feel a ‘sting’ on your arm when we take your blood. This will not last long. We can put a special cream on your arm before the blood test so you don’t feel it.
What other test will be done?

3. The ‘Rest Test’:

We will ask you to come back and visit us on another day. We will do one test, called the ‘rest test’. This will measure your breathing while you are not doing anything.

- We will ask you to lie quietly on a bed for 15-20 minutes.

- You will have a large plastic hood over your head that you can see through.

- This test does NOT hurt. You will be able to breathe easily.

Questions?

Please ask us if you have any questions at all about the study or the tests. We are more than happy to talk to you!

If something happens that you don’t like when you are participating in the study, tell your Mum or Dad and they will know what to do.

Happy to take part in the study?

Please tick the box below if your questions have been answered and you have had the study explained to you.

☐ This study has been explained to me by my parent or the research officer
(child to tick box)

Print Name: _____________________ Signature: _____________________
Date: _________________
9.5 Appendix 5: Participant information sheet and assent form for obesity risk factors study: 12-17 years

Department of Respiratory and Sleep Medicine
John Hunter Hospital
Level 3, HMRI
Ph: 02 4985 5766
Fax: 02 4985 5850
Email: Lisa.Wood@newcastle.edu.au

Sleep, Asthma and Lifestyle Risk Factors

INFORMATION SHEET

Invitation to participate
We would like to invite you to take part in a research project investigating whether asthma reduces sleep quantity and/or sleep quality. We are also looking at whether poor sleep is linked to certain lifestyle factors that may cause weight gain, such as reduced physical activity and increased appetite for foods high in excess kilojoules (energy). This study is being undertaken as part requirement for a PhD at the University of Newcastle by Megan Jensen, under the supervision of Dr Lisa Wood, Professor Peter Gibson and Professor Clare Collins. It is hoped that this study will increase our knowledge about the impact of sleep and asthma upon risk factors for excess weight gain.

Why are we doing this research?
Asthma is the most common chronic disease in children and adolescents. It is believed that there may be an association between body weight and asthma. Children with asthma are reported to have poorer sleep than children without asthma. It is also believed that poor sleep is associated with excess weight gain. This study will examine whether sleep is different between children who have asthma and children who do not have asthma and whether poor sleep increases the risk for excess weight gain.

Who can participate in the research?
We are seeking healthy weight children and adolescents, aged 7-17 years, with or without doctor diagnosed stable asthma. If you have a lung disease other than asthma or use medications that alter their sleep, this study is not suitable for you.

What does the study involve?
If you agree to participate in the study, you will be visited by a research officer during your overnight stay at the John Hunter Hospital sleep unit. The research officer will collect information on your appetite, diet, physical activity, and quality of life in addition to your sleep study results. If you agree to also participate in the second part of the study, you will be required to attend the human physiology lab at the University of Newcastle for one visit only. You can consent to part or all of the following tests. You can withdraw your consent at anytime. The study is described in more detail below.
Sleep Study (Part 1):
If you agree to participate, a research officer will visit the sleep unit during your stay at the John Hunter Hospital.
The research officer will collect the following information during you stay in the sleep unit:

1. **Questionnaires**: You will be asked to answer questions about your general background and asthma, medication use and medical history, your appetite, the foods you eat and how active you are. These questionnaires will take approximately 60 minutes to complete. Your parent can help you with some of these questions if you do not remember certain details, such as your medications and medical history.

2. **Lung function testing**: We wish to measure how well your lungs work. We do this by asking you to blow into a spirometer, a machine that measures the amount of air expelled from their lungs. This is a routine breathing test with no known adverse effects, except for perhaps some breathlessness and/or dizziness which usually lasts for a few seconds only. We can stop this test at any time if you feel uncomfortable.

3. **Blood test**: Approximately 4.5 teaspoons of blood will be taken from a vein in your forearm the morning after your sleep study. This will require you to fast for 12 hours prior. This means you do not eat or drink anything after your dinner the night before the blood test. You will still be able to drink plenty of water. We will use the blood sample to measure levels of appetite hormones and inflammatory markers. These markers control your hunger. If any abnormalities are detected from the blood test, we will inform you via telephone and with your permission, refer to your GP. If you wish, we can put a special cream on your arm before the blood test, which will numb the area so you do not feel the blood test.

4. **Anthropometry**: During this visit we will measure your height, weight and waist circumference. All measurements will be taken while you are fully clothed.

5. **Polysomnography (PSG)**: We will also collect the information from your sleep study. The PSG looks at the way your brain works, and how you breathe and move whilst you are sleep. This will tell us how well you sleep. The sleep lab staff will be conducting this test.

If you wish, your mum/dad can be with you the entire time. We can stop the tests at any time if you feel uncomfortable.

**Human Physiology Lab (Part 2):**
We will need you to come into the human physiology lab at The University of Newcastle to complete resting energy expenditure analysis. This is measured using special equipment. You will be asked to lie quietly on a bed while the amount of air you breathe will be measured. You will have a hood you can see through over your head and you will be able to breathe normally. This will take 15-20 minutes but we will ask you to allow 30mins at the lab. This test causes no known adverse effect.

If you wish, your parent can be with you the entire time. We can stop the tests at any time if you feel uncomfortable.

**What are the benefits and risks of the study?**
There are minimal risks involved in this study. The lung function test may cause breathlessness and/or dizziness, which usually lasts for a few seconds only. The side effects of having blood collected may include bleeding or bruising at the injection site and possible dizziness and/or fainting. Please advise the research team if you normally feel dizzy or faint when you have blood collected.
**What happens at the end of the study?**
At the end of the study, or if you decide to withdraw from the study, you will be referred back to your GP / respiratory physician.

**Costs**
Participation in this study will not cost your parent anything, nor will your parent be paid. However, your parent will receive a $20 reimbursement to cover travel and parking costs when attending the visits.

**What choice do I have?**
Your participation in this study is voluntary. If you do not want to participate in the study, this will not affect your current or future medical care. If during the study you wish to withdraw, you are free to do so. All information is kept strictly confidential and your name will not appear in any reports. All participants will be given a study number from which the participant cannot be identified. Your permission will be sought before accessing your medical records. Your personal information will be accessed, used and stored in accordance with Commonwealth Privacy Laws and the *NSW Health Records and Information Privacy Act 2002*. The results of this study will be shared with other people in the scientific community. They may be compared to results from other studies.

If you decide to withdraw from the study, all data relating to you can be withdrawn and any samples can be destroyed. An exception to this is in the case of an adverse event where data needs to be retained for regulatory reporting. However, all data that is collected will be beneficial to the research study.

**Who do I call if I have any questions or problems?**
If you have any questions about the study or your results, you can contact the staff listed below:

- **Dr Lisa Wood**  
  Business hours 02 4985 5677

- **Miss Megan Jensen**  
  Business hours 02 4985 5679

**Chief Investigator:**
Dr Lisa Wood.

**Co-Investigators:**
- Professor Peter Gibson
- Dr. Jodi Hilton
- Professor Clare Collins
- Professor Robin Callister

**Student Investigator:**
Megan Jensen

**Complaints about this research**
This research has been approved by the Hunter New England Human Research Ethics Committee of Hunter New England Health, Reference number 10/08/18/5.04. Should you have concerns about your rights as a participant in this research, or you have a complaint about the manner in which the research is conducted, it may be given to the researcher or, if an independent person is preferred, to Dr Nicole Gerrand, Manager Research Ethics and Governance, Hunter New England Health, Hunter New England Human Research Ethics Committee, Hunter New England Health, Locked Bag 1, New Lambton NSW 2305. Phone: 02 4921 4950, Email hnehrec@hnehealth.nsw.gov.au
PARTICIPANT CONSENT FORM: Sleep, Asthma and Lifestyle Risk Factors

Chief Investigator: Dr Lisa Wood Ph: 02 4985 5677
Student Investigator: Megan Jensen Ph: 02 4985 5679

Please indicate the tests that you consent to by marking the appropriate tick box and signing below:

PART 1
- Study questionnaires, lung function testing, anthropometry
- Collection of a blood sample (20ml)

PART 2
- Resting energy expenditure analysis

I ______________________ (please print name in full) agree to participate in the above study. I have placed a tick in the box for the tests I am willing to do. I understand that the project will be conducted as described in the Information Sheet, a copy of which I have retained. I have carefully read the information provided to me / had the information explained to me and understand what is required from me. All questions raised by me have been answered to my satisfaction. I understand that I am free to withdraw from the study at any time without reason and without disadvantage to my future care.

I agree to participate in this study

Your name (please print) ______________________  Date ____________

Your signature ______________________
9.6 Appendix 6: Guardian information sheet and assent form for obesity risk factors study

Sleep, Asthma and Lifestyle Risk Factors

PARENTAL INFORMATION SHEET

Invitation to participate
We would like to invite your child to take part in a research project investigating whether asthma reduces sleep quantity and/or sleep quality. We are also looking at whether poor sleep is linked to certain lifestyle factors that may cause weight gain, such as reduced physical activity and increased appetite for foods high in excess kilojoules (energy). This study is being undertaken as part requirement for a PhD at the University of Newcastle by Megan Jensen, under the supervision of Dr Lisa Wood, Professor Peter Gibson and Professor Clare Collins. It is hoped that this study will increase our knowledge about the impact of sleep and asthma upon risk factors for excess weight gain.

Why are we doing this research?
Asthma is the most common chronic disease in children and adolescents. It is believed that there may be an association between body weight and asthma. Children with asthma are reported to have poorer sleep than children without asthma. It is also believed that poor sleep is associated with excess weight gain. This study will examine whether sleep is different between children who have asthma and children who do not have asthma and whether poor sleep increases the risk for excess weight gain.

Who can participate in the research?
We are seeking healthy weight children and adolescents, aged 7-17 years, with or without doctor diagnosed stable asthma. If your child has any lung disease other than asthma or uses medications that alter their sleep, this study is not suitable for them.

What does the study involve?
If you agree to your child’s participation in the study, they will be visited by a research officer during their overnight stay at the John Hunter Hospital sleep unit. The research officer will collect information on their appetite, diet, physical activity, and quality of life in addition to their sleep study results. If you also agree to their participation in the Part 2 of the study, your child will be required to attend the human physiology lab at the University of Newcastle for one visit only.

You can consent to part or all of the following tests. You can withdraw your consent at anytime. The study is described in more detail below.
Sleep Study (Part 1):
If you agree to your child’s participation, a research officer will visit the sleep unit during their stay at the John Hunter Hospital. The research officer will collect the following information during your child’s stay in the sleep unit:

1. Questionnaires: You and your child will be asked to answer questions about their general background (and asthma), medication use and medical history, their appetite, the foods they eat and how active they are. These questionnaires will take approximately 60 minutes to complete. You may help your child with some of these questions if they do not remember certain details, such as medications and medical history.

2. Lung function testing: We wish to measure how well your child’s lungs work. We do this by asking your child to blow into a spirometer, a machine that measures the amount of air expelled from their lungs. This is a routine breathing test with no known adverse effects, except for perhaps some breathlessness and/or dizziness which usually lasts for a few seconds only. We can stop this test at any time if your child feels uncomfortable.

3. Blood test: Just over 20mL (4.5 teaspoons) of blood will be taken from a vein in your child’s forearm the morning after their sleep study. This will require you to fast for 12 hours prior. This means they do not eat or drink anything after their dinner the night before the blood test. Your child can drink plenty of water. We will use the blood sample to measure levels of appetite hormones and inflammatory markers, which indicates your child’s hunger levels. If any abnormalities are detected from the blood test, we will inform you via telephone and with your permission, refer to your GP. If your child would like, we can put a special cream on their arm before the blood test, which will numb the area so they do not feel the blood test.

4. Anthropometry: During this visit we will measure your child’s height, weight and waist circumference. All measurements will be taken while they are fully clothed.

5. Polysomnography (PSG): We will also collect the information from your child’s sleep study. The PSG looks at their brainwaves, and their breathing and movement whilst they are asleep. This will tell us how well your child sleeps. The sleep lab staff will be conducting this test. If you wish, you can be with your child the entire time. We can stop the tests at any time if your child feels uncomfortable.

Human Physiology Lab (Part 2):
We will need you to come into the human physiology lab at The University of Newcastle to complete resting energy expenditure analysis. This is measured using specialised equipment called indirect calorimetry. You will be asked to lie quietly on a bed in the laboratory while the amount of oxygen you use will be measured. You will have a hood you can see through over your head and you will be able to breathe normally. This will take 15-20 minutes but we will ask you to allow 30mins at the lab. This test causes no known adverse effect.

What are the benefits and risks of the study?
There are minimal risks involved in this study. The lung function test may cause breathlessness and/or dizziness, which usually lasts for a few seconds only. The side effects of having blood collected may include bleeding or bruising at the injection site and possible dizziness and/or fainting. Please advise the research team if you normally feel dizzy or faint when you have blood collected.
What happens at the end of the study?
At the end of the study, or if you decide to withdraw from the study, you will be referred back to your GP / respiratory physician.

Costs
Participation in this study will not cost you anything, nor will you be paid. However, you will receive a $20 reimbursement to cover travel and parking costs when attending the visits.

What choice do I have?
Your participation in this study is voluntary. If you do not want to participate in the study, this will not affect your current or future medical care. If during the study you wish to withdraw, you are free to do so. All information is kept strictly confidential and your name will not appear in any reports. All participants will be given a study number from which the participant cannot be identified. Your permission will be sought before accessing your medical records. Your personal information will be accessed, used and stored in accordance with Commonwealth Privacy Laws and the NSW Health Records and Information Privacy Act 2002. The results of this study will be shared with other people in the scientific community. They may be compared to results from other studies.

If you decide to withdraw from the study, all data relating to you can be withdrawn and any samples can be destroyed. An exception to this is in the case of an adverse event where data needs to be retained for regulatory reporting. However, all data that is collected will be beneficial to the research study.

Who do I call if I have any questions or problems?
If you have any questions about the study or your results, you can contact the staff listed below:

Dr Lisa Wood Business hours 02 4985 5677
Miss Megan Jensen Business hours 02 4985 5679

Chief Investigator:
Dr Lisa Wood.

Co-Investigators:
Professor Peter Gibson
Dr. Jodi Hilton
Professor Clare Collins
Professor Robin Callister

Student Investigator:
Megan Jensen

Complaints about this research
This research has been approved by the Hunter New England Human Research Ethics Committee of Hunter New England Health, Reference number 10/08/18/5.04. Should you have concerns about your rights as a participant in this research, or you have a complaint about the manner in which the research is conducted, it may be given to the researcher or, if an independent person is preferred, to Dr Nicole Gerrand, Manager Research Ethics and Governance, Hunter New England Health, Hunter New England Human Research Ethics Committee, Hunter New England Health, Locked Bag 1, New Lambton NSW 2305. Phone: 02 4921 4950, Email hnehrec@hnehealth.nsw.gov.au
PARENTAL CONSENT FORM: Sleep, Asthma and Lifestyle Risk Factors

Chief Investigator: Dr Lisa Wood
Student Investigator: Megan Jensen

Please indicate the tests that you consent to by marking the appropriate tick box and signing below:

PART 1

- Study questionnaires, lung function testing, anthropometry
- Collection of a blood sample (20ml)

PART 2

- Resting energy expenditure analysis

I __________________________ (please print name in full) agree to the participation of my child in the above research project and give my consent freely. I have indicated the tests to which I give my consent by marking the appropriate tick box. I understand that the project will be conducted as described in the Information Sheet, a copy of which I have retained. I have carefully read the information provided to me and understand its content. All questions raised by me have been answered to my satisfaction. I give permission for the members of the research team to access my child’s medical records. I also give permission for the members of the research team to contact my child’s GP and/or respiratory physician and for relevant information to the study and/or my child’s clinical management to be shared if necessary. I understand that I am free to withdraw my consent at any time without reason and without disadvantage to my child’s future care.

I consent to my child’s participation in this study

Participant name (please print) __________________________ Date __________________
Guardian name (please print)
Guardian signature __________________________

Dr Lisa Wood
Department of Respiratory and Sleep Medicine
John Hunter Hospital
HMRI, Level 3
Ph: 02 4985 5766
Fax: 02 4985 5850
Email: Lisa.Wood@newcastle.edu.au
9.7 Appendix 7: Participant information sheet and assent form for

weight loss study

Department of Respiratory and Sleep Medicine
John Hunter Hospital
Level 3, HMRI
Ph: 02 4985 5766
Fax: 02 4985 5850
Email: Lisa.Wood@newcastle.edu.au

PARTICIPANT INFORMATION SHEET

Invitation to participate
We would like to invite you to take part in a research project investigating whether lifestyle changes improve asthma symptoms. This study is being undertaken as part requirement for a PhD at the University of Newcastle by Megan Jensen, under the supervision of Dr Lisa Wood, Professor Peter Gibson, and Associate Professor Clare Collins. It is hoped that this study will increase our knowledge about asthma.

Why are we doing this research?
Asthma is the most common chronic diseases in children and teenagers. It is believed that there may be an association between body weight and asthma. Extra body weight is associated with increased inflammation throughout the body, while asthma is associated with inflammation in the lungs. This study will examine whether lifestyle changes reduce this inflammation and improve asthma symptoms.

Who can participate in the research?
We are seeking people 8-17 years of age, with doctor diagnosed stable asthma and who would like to lose weight. We need you to be a certain weight to be eligible for this study. If you know your weight and height, we can work out over the phone for you whether you fall into this certain weight range.

If you follow a special diet, have a lung disease other than asthma, have had a recent respiratory tract infection, a visit to the GP or hospital due to a worsening of asthma symptoms, use of oral steroids or a change in asthma medication this study is not suitable for you.

What does the study involve?
If you agree to participate in the study, you will be randomised by a computer to one of two groups. You will have an equal chance of allocation to each group but we cannot place you in the group of your choice. This intervention lasts for ten weeks and involves a number of visits to the clinic at John Hunter Hospital during this time. The study is described in more detail below.

Study programs:
(i) Group 1
If you are randomised to participate in Group 1, you will receive a nutritionally adequate meal plan from a qualified dietitian. This diet will include healthy meal and snack ideas. A list of suitable foods will be provided so you can pick and choose which foods you would like to eat. You will also be given a calorie counter which has the nutrition information on a range of foods. This will allow you to choose foods you like while still following the dietary plan.
This nutrition program contains fewer calories than you are currently consuming and may cause you to feel hungry. This is required to shift your energy balance so that you can lose weight. As part of this study, you will receive advice and support from a dietitian, either in person (week 0, 1, 2, 4, 6, 8, 10) or over the telephone (week 3, 5, 7, 9). These counselling sessions will go for approximately 60 minutes. You will also be asked to keep a food diary for the length of the study.

We will also provide you with a pedometer for the duration of the study. A pedometer measures how many steps you take each day. You will be asked to take a certain number of steps each day.

If you are allocated to Group 1 you will be required to visit the clinic a total of 8 times over a 3mth period.

(ii) Group 2
If you are randomised to Group 2, you will be asked to continue your current diet and activity levels as normal for approximately 10wks. After the 10wks you will receive the same nutrition program as above.
If you are allocated to Group 2 you will be required to visit the clinic a total of 9 times over a 5-6mth period.

Screening visit:
If you agree to participate you will need to visit the clinic for a screening visit to make sure this study is suitable for you. This will take approximately 30-60 minutes. During this visit we will ask you about your medical and medication history. We will also measure your blood pressure, your height and your weight.
We will call you to tell you if the study is suitable for you and make a time for you to begin the study. If the study is not suitable for you we will, with your permission, send the information to your doctor for follow-up if required.

Weeks 0 and 10 visit:
Before you begin the study and during your final week (week 10) we will also ask you to record your food intake for four days. We will give you cups and spoons to measure how much food and fluid you are eating and drinking over four days. We will also need you to come in to the clinic on the first and last day of the study to complete a number of tests, which are described in detail below. This first and last visit will take approximately 3-4 hours. Before these visits you will need to fast for 12 hours and withhold your asthma reliever medication for 6-24 hours.

1. Questionnaires: You will be asked to answer questions about your general background, asthma, the foods you eat and how active you are. These questionnaires will take approximately 40 minutes to complete.

2. Body composition: Your height and weight will be measured. We will also measure your body composition using a dual energy x-ray absorptiometry (DEXA) machine. This is a routine procedure that involves the use of very low levels of radiation. This research study involves exposure to a very small amount of radiation (Total Body Scan). The effective dose from this study is less than 0.010mSv. At this dose, no harmful effects of radiation have been demonstrated and the risk is negligible. It is equal to the amount of radiation we are all exposed to on a daily basis. This test does not hurt. It will provide us with a picture of your body on the inside.
Since DEXA should not be used on pregnant women, we are required to confirm that female participants are not pregnant. To do this we will ask you to give us a small sample of urine and we will conduct a pregnancy test prior to conducting the DEXA scan.

3. Allergy skin prick test: You will have a skin prick allergy test during the visit. A small amount of fluid is put on your skin then tiny pricks are made on the skin at this location (this
doesn’t break the skin and is not painful). If you are allergic to the fluid, a small itchy lump will occur. This usually lasts for an hour or so and if it is annoying we can give you some cream which takes the itch away.

4. Lung function tests: We wish to measure how well your lungs work and this involves asking you to breathe different ways, such as deep breathing and blowing out rapidly. Some of these breathing tests may be performed whilst sitting in a plethysmograph, which looks a bit like a high-tech telephone booth. Your lung function will also be assessed by blowing into a spirometer, a machine that measures the amount of air expelled from your lungs. These are routine breathing tests that may cause some breathlessness and/or dizziness which usually lasts for a few seconds only. If you feel uncomfortable during the test and want to stop, you can.

5. Saline Challenge: You will be asked to inhale a mist of salty water. This will involve using a snorkel-like mouthpiece to breathe the mist for consecutive periods of 30 seconds, 1 minute, 2 minutes and then 3 lots of 4 minutes. A breathing test will be done at the end of each period and you will be asked to cough up a specimen of sputum. This is a routine test. The test will be stopped at your request or if your breathing test results worsen. You will be given salbutamol (Ventolin) if you develop any problems with your breathing. Salbutamol is a medication that immediately relieves constriction of the airways and is inhaled by mouth through a puffer device.

6. Blood test: Approximately 30mL (6 teaspoons) of blood will be taken from a vein in your forearm to measure levels of inflammation, fatty acids, insulin, glucose, cholesterol and liver and renal function. If any of the blood tests are not normal, we will call you to tell you and with your permission, send this information to your GP. We will also tell you whether the study is still suitable for you.

7. Other tests: We will also measure the level of cotinine in your body. This is a simple test that indicates exposure to cigarette smoke and requires a small sample of urine.

Your mum and/or dad can be with you the entire time if you would like. We can stop the tests at any time if you feel uncomfortable.

Weeks 0, 1, 2, 4, 6, & 8:
During these visits you will be given nutrition information to help you lose weight. You will be provided with a food diary and asked to record what and when you eat and drink each day. You will also receive a calorie counter. Each session will review your food diary and address any problems you may be having. These sessions will take about 1hr.

Weeks 3, 5, 7, & 9:
You will receive a phone call during these weeks to see how you are going with the diet and whether there are any questions or problems you are having. This will take from as little as 10mins, depending on how many questions you have about the dietary plan.

What are the benefits and risks of the study?
If this study is suitable for you and you agree to participate in this study you will receive specialised advice about your diet from a dietitian, free of charge. The sputum test can cause difficulty breathing, coughing, some discomfort in your chest and wheezing. This is brief and responds promptly to reliever medication (Ventolin). The side effects of having blood collected may include bleeding or bruising at the injection site and possible dizziness and/or fainting. Please advise the research team if you normally feel dizzy or faint when you have blood collected. The risk associated with the DEXA scan is negligible and is equal to the natural background radiation we receive on a daily basis from our environment. The effective dose from this study is less than 0.010mSv. At this dose level, no harmful effects of radiation
have been demonstrated and the risk is negligible. All radiation exposures will be carried out in accordance with the ARPANSA Code of Practice “Exposure of Humans to Ionizing Radiation for Research Purposes” (RPS8). We would like you to tell us if you have participated in any research studies in the previous 5 years that have involved the use of radiation, as we need to make sure that you do not exceed a safe level of radiation.

**What happens at the end of the study?**
At the end of the study or if you decide to withdraw from the study, you will be referred back to your GP. A copy of your results from the study will also be sent to your GP.

**Costs**
Participation in this study will not cost you anything, nor will you be paid. However, you will receive a parking voucher to cover parking costs while attending the hospital. You will also receive individual dietary counselling by an Accredited Practicing Dietitian.

**What choice do I have?**
Your participation in this study is voluntary. If you don’t want to participate in the study this will not affect your current or future medical care. If, during the study, you wish to withdraw, you are free to do so. All information is kept strictly confidential and your name will not appear in any reports. All participants will be given a study number from which the participant cannot be identified. Your permission will be sought before accessing your medical records. Your personal information will be accessed, used and stored in accordance with Commonwealth Privacy Laws and the *NSW Health Records and Information Privacy Act 2002*. The results of this study will be shared with other people in the scientific community. They may be compared to results from other studies.

If you decide to withdraw from the study, you can withdraw all data relating to you and have any samples destroyed. An exception to this is in the case of an adverse event where data needs to be retained for regulatory reporting. However, all data that is collected will be beneficial to the research study.

**Who do I call if I have any questions or problems?**
If you have any questions about the study, your results, or your treatment, you can contact the staff listed on the following page:

<table>
<thead>
<tr>
<th>Name</th>
<th>Business hours</th>
<th>Contact Number</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dr Lisa Wood</td>
<td>Business hours</td>
<td>02 4985 5677</td>
</tr>
<tr>
<td>Miss Megan Jensen</td>
<td>Business hours</td>
<td>02 4985 5679</td>
</tr>
</tbody>
</table>

**Chief Investigator:**
Dr Lisa Wood

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**Co-Investigators:**
Professor Peter Gibson
Associate Professor Clare Collins
Dr. Jodi Hilton

**Student Investigator:**
Megan Jensen

**Complaints about this research**
This research has been approved by the Hunter New England Human Research Ethics Committee of Hunter New England Health, Reference number 09/05/20/5/09. Should you have concerns about your rights as a participant in this research, or you have a complaint about the manner in which the research is conducted, it may be given to the researcher, or, if an independent person is preferred, to Dr Nicole Gerrand, Professional Officer (Research Governance and Ethics), Hunter New England Human Research Ethics Committee, Hunter New England Health, Locked Bag 1, New Lambton NSW 2305. Phone: 02 4921 4950, Email hnehrec@hnehealth.nsw.gov.au
PARTICIPANT ASSENT FORM

I agree to participate in the above research project and agree to participate freely. I understand that the project will be conducted as described in the Information Sheet, a copy of which I have kept. I have carefully read the information provided to me and understand its content. All questions raised by me have been answered to my satisfaction. I understand that I am free to withdraw from the study at any time without reason and without disadvantage to my future care.

I give permission for the members of the research team to access my medical records. I also give permission for the members of the research team to contact my GP and / or respiratory physician and for relevant information to the study and / or my clinical management to be shared if necessary.

If you agree to participate in this study please sign below

Participant name (please print) ____________________________________________

Participant signature __________________________ Date __________

To be completed by person conducting assent discussion:

☐ The participant is capable of reading and understanding the assent form and has signed above as documentation of assent to take part in this study

☐ The participant is not capable of reading the assent form, however, the information was explained verbally to the subject who signed above to acknowledge the verbal explanation and his/her assent to take part in this study.

Name of Person Obtaining Assent (Print) ____________________________________________

Signature of Person Obtaining Assent __________________________ Date __________
9.8 Appendix 8: Guardian information sheet and assent form for weight loss study

Dietary Intervention Study

PARENTAL INFORMATION SHEET

Invitation to participate
We would like to invite your child to take part in a research project investigating whether lifestyle changes improve asthma symptoms. This study is being undertaken as part requirement for a PhD at the University of Newcastle by Megan Jensen, under the supervision of Dr Lisa Wood, Professor Peter Gibson and Associate Professor Clare Collins. It is hoped that this study will increase our knowledge about asthma.

Why are we doing this research?
Asthma is the most common chronic disease in children and adolescents. It is believed that there may be an association between body weight and asthma. Extra body weight is associated with increased inflammation throughout the body, while asthma is associated with inflammation in the lungs. This study will examine whether lifestyle changes reduces this inflammation and improves asthma symptoms.

Who can participate in the research?
We are seeking people 8-17 years of age, with doctor diagnosed stable asthma and who would like to lose weight. We need your child to be a certain weight to be eligible for this study. If you know your child’s weight and height, we can work out over the phone whether your child falls into this certain weight range.

If your child follows a special diet, has a lung disease other than asthma, has had a recent respiratory tract infection, a visit to the GP or hospital due to a worsening of asthma symptoms, uses oral steroids or has had a change in asthma medication this study is not suitable for your child.

What does the study involve?
If you and your child agree to their participation in the study, they will be randomised by a computer to one of two groups. Your child will have an equal chance of allocation to each group but we cannot place your child in the group of your / their choice. This intervention lasts for ten weeks and involves a number of visits to the clinic at John Hunter Hospital during this time. The study is described in more detail below.

Study programs:
(i) Group 1
If your child is randomised to participate in Group 1, they will receive a nutritionally adequate meal plan from a qualified dietitian. This diet will include healthy meal and snack ideas. A list of suitable foods will be provided so you and your child can pick and choose which foods they would like to eat. Your child will also receive a calorie counter which has
the nutrition information on a range of foods. This will allow your child to choose foods they like while still following the dietary plan. This nutrition program contains fewer calories than your child is currently consuming and may cause them to feel hungry. This is required to shift their energy balance so that you can lose weight. As part of this study, you and your child will receive advice and support from a dietitian, in person (week 0, 1, 2, 4, 6, 8, 10) and over the telephone (week 3, 5, 7, 9). These counselling sessions will go for approximately 60 minutes. Your child will also be asked to keep a food diary for the length of the study. We will also provide your child with a pedometer for the duration of the study. A pedometer measures how many steps are taken each day. Your child will be asked to take a certain number of steps each day.

If your child is allocated to Group 1 they will be required to visit the clinic a total of 8 times over a 3mth period.

(ii) Group 2
If your child is randomised to Group 2, they will be asked to continue their current diet and activity levels as normal for approximately 10wks. After the 10wks they will receive the same nutrition program as above.

If your child is allocated to Group 2 they will be required to visit the clinic a total of 9 times over a 5-6mth period.

**Screening visit:**
If you and your child agree to their participation they will need to visit the clinic for a screening visit to make sure this study is suitable for them. This will take approximately 30-60 minutes. During this visit we will ask about your child's medical and medication history and will measure their blood pressure, their height and their weight.

We will advise you and your child of their suitability for the study via telephone and organise a convenient time for them to begin the study. If the study is not suitable for your child we will, with you / your child’s permission, forward the information to your GP for follow-up if required.

**Weeks 0 and 10 visit:**
Prior to commencing the study and during the final week (week 10) of the study, we will ask your child to record their food intake for four days. We will give your child cups and spoons to measure how much food and fluid they are eating and drinking over four days. We will also need you child to come into the clinic on the first and last day of the study to complete a number of tests, which are described in detail below. This first and last visit will take approximately 3-4 hours. Before these visits your child will need to fast for 12 hours and withhold their asthma reliever medication for 6-24 hours.

1. **Questionnaires:** Your child will be asked to answer questions about their general background and asthma, the foods they eat and how active they are. These questionnaires will take approximately 40 minutes to complete. You may assist your child with some of these questions as they may not remember certain details e.g. their medications and medical history.

2. **Body composition:** Your child’s height and weight will be measured. We will also measure their body composition using a dual energy x-ray absorptiometry (DEXA) machine. This is a routine procedure that involves the use of very low levels of radiation. This research study involves exposure to a very small amount of radiation (Total Body Scan). The effective dose from this study is less than 0.010mSv. At this dose, no harmful effects of radiation have been demonstrated and the risk is negligible. It is equal to the amount of radiation we are all exposed to on a daily basis. It will provide us with a picture of your child’s body on the inside.
Since DEXA should not be used on pregnant women, we are required to confirm that female participants are not pregnant. To do this we will ask your daughter to give us a small sample of urine and we will conduct a pregnancy test prior to conducting the DEXA scan.

3. **Allergy skin prick test**: Your child will have a skin prick allergy test during the visit. A small amount of fluid is put on their skin then tiny pricks are made on the skin at this location (this doesn’t break the skin and is not painful). If your child is allergic to the fluid, a small itchy lump will occur. This usually lasts for an hour or so and if it is annoying we can apply a cream which takes the itch away.

4. **Lung function tests**: We wish to measure how well your child’s lungs work and this involves performing various breathing efforts such as deep breathing and blowing out rapidly into lung function equipment. Some breathing efforts may be performed whilst sitting in a plethysmograph - specialised lung testing equipment which looks a bit like a high-tech telephone booth. Your child’s lung function will also be assessed by blowing into a spirometer, a machine that measures the amount of air expelled from their lungs. These are routine breathing tests with no known adverse effects, except for perhaps some breathlessness and/or dizziness which usually lasts for a few seconds only. We can stop this test at any time if your child feels uncomfortable.

5. **Saline Challenge**: Your child will be asked to inhale a mist of salty water. This will involve using a snorkel-like mouthpiece to breathe the mist for consecutive periods of 30 seconds, 1 minute, 2 minutes and then 3 lots of 4 minutes. A breathing test will be done at the end of each period and your child will be asked to cough up a specimen of sputum. This is a routine test. The test will be stopped at your child’s request or if their breathing test results worsen. If any problems develop with their breathing, your child will be given salbutamol (Ventolin). Salbutamol is a medication that immediately relieves constriction of the airways and is inhaled by mouth through a puffer device.

6. **Blood test**: Approximately 30mL (6 teaspoons) of blood will be taken from a vein in your child’s forearm to measure levels of inflammation, fatty acids, insulin, glucose, cholesterol and liver and renal function. If any abnormalities are detected from the blood test, we will inform you / your child via telephone and with your / your child’s permission, refer to your GP. We will also inform you / your child whether the study is still suitable for them.

7. **Other tests**: We will also measure the level of cotinine in your child’s body. This is a simple test that indicates exposure to cigarette smoke and requires a small sample of urine.

If you wish, you can be with your child the entire time. We can stop the tests at any time if your child feels uncomfortable.

**Weeks 0, 1, 2, 4, 6, & 8**:  
During these visits you and your child will be given nutrition information to help them lose weight. A food diary will be provided for your child to record what and when they eat and drink each day. They will also receive a calorie counter. Each session will review their food diary and address any problems you or your child may be having with the intervention. These sessions will take approximately 60mins.

**Weeks 3, 5, 7, & 9**:  
We will contact you and your child via telephone during these weeks to see whether there are any questions / problems you or your child are having with the dietary plan. This will take from as little as 10mins, depending on how many questions you and your child have about the dietary plan.
What are the benefits and risks of the study?
If this study is suitable for your child and you and your child agree to their participation in this study, your child will receive specialised advice about their diet from a dietitian, free of charge. The sputum test can cause difficulty breathing, coughing, some discomfort in their chest and wheezing. This is brief and responds promptly to reliever medication (Ventolin). The side effects of having blood collected may include bleeding or bruising at the injection site and possible dizziness and/or fainting. Please advise the research team if your child normally feels dizzy or faint when they have blood collected. The risk associated with the DEXA scan is negligible and is equal to the natural background radiation we receive on a daily basis from our environment. The effective dose from this study is less than 0.010mSv. At this dose level, no harmful effects of radiation have been demonstrated and the risk is negligible. All radiation exposures will be carried out in accordance with the ARPANSA Code of Practice “Exposure of Humans to Ionizing Radiation for Research Purposes” (RPS8). We would like you to tell us if your child has participated in any research studies in the previous 5 years that have involved the use of radiation, as we need to make sure that a safe level of radiation is not exceeded.

What happens at the end of the study?
At the end of the study or if your child decides to withdraw from the study, they will be referred back to their GP. A copy of their results from the study will also be sent to their GP.

Costs
Participation in this study will not cost you / your child anything, nor will you / your child be paid. However, you will receive a parking voucher to cover parking costs while attending the hospital. Your child will also receive individual dietary counselling by an Accredited Practicing Dietitian.

What choice do I have?
Your child’s participation in this study is voluntary. If your child does not want to participate in the study this will not affect their current or future medical care. If during the study your child wishes to withdraw or you wish to withdraw consent, you and your child are free to do so. All information is kept strictly confidential and your child’s name will not appear in any reports. All participants will be given a study number from which the participant cannot be identified. You and your child’s permission will be sought before accessing their medical records. Your child’s personal information will be accessed, used and stored in accordance with Commonwealth Privacy Laws and the NSW Health Records and Information Privacy Act 2002. The results of this study will be shared with other people in the scientific community. They may be compared to results from other studies.

If your child decides to withdraw from the study or you wish to withdraw consent, all data relating to your child can be withdrawn and any samples can be destroyed. An exception to this is in the case of an adverse event where data needs to be retained for regulatory reporting. However, all data that is collected will be beneficial to the research study.

Who do I call if I have any questions or problems?
If you have any questions about the study, your child’s results or treatment, you can contact the staff listed on the following page:

Dr Lisa Wood  Business hours  02 4985 5677
Miss Megan Jensen  Business hours  02 4985 5679

Chief Investigator:
Dr Lisa Wood.
Co-Investigators:
Professor Peter Gibson
Associate Professor Clare Collins
Dr. Jodi Hilton

Student Investigator:
Megan Jensen

Complaints about this research
This research has been approved by the Hunter New England Human Research Ethics Committee of Hunter New England Health, Reference number 09/05/20/5/09. Should you have concerns about your rights as a participant in this research, or you have a complaint about the manner in which the research is conducted, it may be given to the researcher, or, if an independent person is preferred, to Dr Nicole Gerrand, Professional Officer (Research Governance and Ethics), Hunter New England Human Research Ethics Committee, Hunter New England Health, Locked Bag 1, New Lambton NSW 2305. Phone: 02 4921 4950, Email hnehrec@hnehealth.nsw.gov.au
Asthma and Lifestyle

Chief Investigator: Dr Lisa Wood  Ph: 02 4985 5677
Student Investigator: Megan Jensen  Ph: 02 4985 5679

PARENTAL CONSENT FORM

I agree to the participation of my child in the above research project and give my consent freely. I understand that the project will be conducted as described in the Information Sheet, a copy of which I have retained. I have carefully read the information provided to me and understand its content. All questions raised by me have been answered to my satisfaction. I give permission for the members of the research team to access my child’s medical records. I also give permission for the members of the research team to contact my child’s GP and/or respiratory physician and for relevant information to the study and/or my child’s clinical management to be shared if necessary. I understand that I am free to withdraw my consent at any time without reason and without disadvantage to the future care of my child.

I consent to my child’s participation in this study

Participant name (please print) __________________________________________

Parent / Guardian name (please print) ______________________________________

Parent / Guardian signature ______________________________________________

Date ___________________
9.9 Appendix 9: Meal plans used in weight loss study

😊 Nutrition & You 😊

Your body needs food to give it energy to function and grow properly. Food also provides nutrients needed by your body. 😊

However, not all foods are equal. Some foods are better than others and should be eaten more frequently. We call these foods everyday foods.

**Everyday foods** 😊 include wholegrain breads and cereals, fruit and vegetables, low-fat dairy products and lean meat, legumes (e.g. red kidney beans, lentils, chickpeas) and nuts. These foods provide moderate energy, fibre, and a range of nutrients that your body needs each day. 😊

Other foods we should eat occasionally. We call these foods *sometimes foods*. **Sometimes foods** include chips, lollies, doughnuts, cakes, biscuits, chocolate, ice-cream, cordial and soft drink and crumbed / fried / greasy foods e.g. chicken nuggets, crumbed fish and hot chips, Pluto pups, pizza, pies, pastries and sausage rolls. These foods provide excess energy, fat, sugar and salt and are low in nutrients.

Your everyday diet should have 3 meals (breakfast, lunch, dinner) and 2-3 snacks (morning tea, afternoon tea, supper). 😊

We have provided an example meal plan for you to follow. We have also included healthy meal and snack ideas so you can pick and choose what you want to eat. 😊
### Example Meal Plan (~7800kJ)

**Breakfast (~1500kJ)**
- Sanitarium Weet-Bix: 2 biscuit, 440kJ
- Milk, Reduced Fat: 200 mL, 350kJ
- Fruit: 1 piece, 320kJ
- Toast: 1 slice, 310kJ
- Philadelphia cheese spread, reduced fat: 3 tsp, 120kJ

**Morning Tea (~1000kJ)**
- Fruit bread: 2 slices, 730kJ
- Margarine: 2 tsp, 250kJ

**Lunch (~1300kJ)**
- Bread, Wholemeal / grain: 2 slices, 620kJ
- Avocado: 10g (2 tsp), 90kJ
- Ham, lite, Pre-Packed/Deli-Sliced: 50g, 230kJ
- Tomato: 1 small, 80kJ
- Lettuce: ½ cup, NA
- Cucumber: 4 slice, NA
- Carrot, grated: ¼ cup, NA
- Popcorn, air-popped, no-fat: 2 cups, 250kJ

**Afternoon Tea (~1000kJ)**
- Cheddar Cheese, 50% Reduced Fat: 40g (2 slices), 440kJ
- Arnotts vita wheat: 4 biscuit, 440kJ
- Vegemite: 5g, 30kJ
- Tomato, Cherry, Raw: ½ cup, 50kJ

**Dinner (~1800kJ)**
- Beef, Steak, Rump, Grilled/Bbq, Fat Trimmed: 80g, 610kJ
- Sweet Potato, baked: ½ cup, 320kJ
- Oil, Canola (for cooking): 1 tsp, 170kJ
- Broccoli, Cauliflower: ½ cup of each, 200kJ
- Sauce, Barbecue / Tomato: 1 tb, 170kJ
- Dinner roll: 1, 350kJ

**Supper (~1200kJ)**
- Fruit, Canned In Natural Juice: 1 cup, 500kJ
- Yoghurt, Skim/Low Fat, Vanilla: 1 tub (200g), 670kJ
Breakfast Ideas (~1500kJ)

Breakfast gives you a kick start to the day and boosts your body’s ability to burn energy and help you lose weight. Try one of the following:

- 1 cup Kellogg’s Sultana Bran OR ¾ Fruity-Bix / Weet-Bix Crunch / Cheerios with 1 cup Low-fat milk and sliced Banana / other fruit

- Porridge made with 1/3 cup rolled oats, 1 cup low-fat milk, sliced banana, cinnamon and 2 tsp honey / brown sugar

- 220g (½ tin) Baked beans on 1 English grain muffin with 1Tb avocado and sliced tomato

- 2 scrambled eggs on 2 slices wholemeal toast and 1 tsp marg / oil

- 220g (½ tin) Creamed corn on 2 slices grain toast with 1 tsp marg

- 40g (2 slices) reduced fat Cheese, tomato and 1Tb avocado on 2 slices wholemeal toast

- 2 slices grain toast with 3 tsp peanut butter and sliced banana

- 2 wholemeal pancakes with ½ cup berries and 3Tb low-fat ricotta and 1Tb honey

- 200g low-fat yoghurt with ¼ cup fruit salad and ½ cup untoasted muesli

- 1 Breakfast bar and 1 Sanitarium Up ‘n’ go

- Smoothie (made with fruit, 100g low-fat yoghurt and 200ml low-fat milk) PLUS slice grain toast with thin spread of light cream cheese

- Milo smoothie (made with 2 tsp milo, 1 banana, 200ml milk, and 100g skim vanilla yogurt) PLUS slice grain toast with thin spread of jam / honey
😊 Lunch (~1500kj) 😊

Make your own healthy lunch by choosing one at each following step:

1. **Start with a base:**
   - 2 slices Mountain bread
   - 2 slices of bread
   - 1 bread roll
   - 1 lavash bread

2. **Add a protein filling:**
   - 60g chicken breast, lite shaved ham, turkey, tuna / salmon (packed in water)
   - OR
   - 1 boiled egg
   - OR
   - 110g (¼ can) Baked Beans
   - OR
   - 40g reduced fat cheddar cheese, 2 slices Kraft free cheese singles, 2 Tb (40g) Light Philadelphia cream cheese
   - OR
   - 2 tsp Peanut butter

3. ☺ **Add as many salad vegetables as possible! ☻** Try:
   - Mixed lettuce leaves / baby spinach leaves, snowpeas, capsicum, sprouts, tomato, cucumber
   - Grated carrot and 2 tsp sultanas taste great with peanut butter or cream cheese
   - Add a few slices of beetroot or 1 pineapple ring for a sweeter flavour

4. **Add a little sauce / spread** if you like:
   - 2 tsp tomato chutney or pickles,
   - 2 tsp sweet chilli sauce
   - 2 tsp mayonnaise
   - 2 tsp cranberry sauce

5. **Finish** with a piece of fresh fruit or a fruit snack pack 😊
Other lunch ideas

✓ 1 cup tabouli, roasted pumpkin, \( \frac{1}{4} \) cup corn, 50g grilled lean red meat or chicken breast and 1Tb sweet chilli sauce 😊

✓ Salad with dry-roasted sweet potato, roasted capsicum, 50g grilled lean meat, 20g reduced-fat fetta, balsamic dressing and a small dinner roll 😊

✓ 2 Nori rolls 😊

✓ Pasta salad made with 1 cup pasta, shaved ham or other lean meat, choice of vegetables e.g. roast pumpkin, eggplant and zucchini and 1Tb seeded mustard or pesto 😊
Dinner (~1500kJ)

Build dinner by following 3 easy steps:

1. **Add one of the following protein foods:**
   - 65-100g cooked chicken breast or turkey breast,
   - 65-100g cooked lean red meat e.g. steak, 2 sml lamb chops, $\frac{1}{2}$ cup mince, 2 slices roast meat
   - 2 Eggs
   - 1/3 cup lentils, baked beans, chickpeas, ked kidney beans or butter beans
   - 80-120g Fish e.g. salmon, tuna, bream, mullet
   - 40g Reduced-fat cheese

2. **Add one of the following:**
   - $\frac{3}{4}$-1 cup Rice, pasta, noodles OR
   - 1 medium potato or $\frac{1}{2}$ medium sweet potato OR
   - 1 pita bread or English muffin

3. **Add lots of non-starchy vegetables!** e.g. cauliflower, broccoli, carrot, zucchini, corn, peas, green beans, capsicum, pumpkin.

4. **Add a little flavour:**
   - Herbs and spices give lots of flavour without adding kilojoules (energy) e.g. fresh or dried basil and oregano on pizza or pasta, cumin and turmeric in Mexican dishes e.g. nachos, dill and lemon on steamed or baked fish
   - Add lemon / lime juice, vinegar, garlic, ginger, chilli, salsa or soy sauce for a strong flavour in a small amount
   - Use tomato sauce, BBQ sauce, mustard and sweet chilli sauce in small amounts as these are higher in energy
Meals ideas (amounts are per person):

- Pasta bake (1 cup cooked pasta) with mixed vegetables and ½ cup tomato based sauce, topped with 40g reduced fat cheese
- Spaghetti Bolognese (1 cup cooked pasta) with ½ cup cooked mince, ¼ cup tomato sauce, mushrooms, grated carrot and zucchini
- Homemade pizza on 1 medium (~15cm) wholemeal pita bread or an English muffin spread with 2 tsp tomato paste, 1 chopped pineapple ring, mushrooms, capsicum, 30g shaved ham and 1/3 cup reduced-fat mozzarella
- 1.5 cups Vegetable-based soup e.g. minestrone, pumpkin, vegetable and barley, chicken and corn plus a medium grain breadroll with 1tsp margarine. Make creamy soups with skim milk or light evaporated milk instead of cream.
- Salmon and rice cakes. Mix 80g drained tinned salmon with 1/3 cup cooked rice and mixed herbs, coat in breadcrumbs, spray lightly with oil and bake in the oven. Serve with one small mashed potato, pumpkin and peas.
- Stir-fry 1 cup of vegetables e.g. baby corn, snow peas, carrot, bean sprouts with 80g lean meat. Add a little stock, soy sauce, garlic, and honey or sweet chilli sauce. Serve with ¾ cup of rice or noodles.
- 2 taco shells, filled with ½ cup red kidney beans or mince cooked in taco seasoning, topped with lettuce, grated carrot and tomato, 1Tb guacamole, 1Tb taco sauce and 30g cheese.
- Easy frittata: beat 2 eggs with 2Tb milk, add 1c vegetables and one rasher of reduced fat bacon trimmed of fat OR 2tspn pesto for flavour and cook over low heat in a medium saucepan. Serve with salad and a small dinner roll. This can also serve as leftovers in the lunchbox if kept cool by a frozen milk popper 😊
😊 Snack Ideas 🌟

Each of the following snacks provide less than or equal to 1000kJ per serve.

You can swap the morning tea, afternoon tea and supper snacks given in the example meal plan for one of the snacks from the following list.

In addition to the 3 snacks in the meal plan, you can choose up to ________ extra snacks each day. You can choose from this list or alternatively choose your own snack, making sure it is no more than 1000kJ using your calorie counter.

✓ McCain Healthy Choice twin pack meals e.g. Beef and Bacon Pasta, Creamy Carbonara, Chicken Bolognaise, Meatballs and Pasta, Chicken and Vegetables Lasagna
✓ St Dalfour range of ready-to-eat meals (excluding 'couscous' and 'wholegrain with beans' variety)
✓ 125g tin baked beans & 4 corn thins / rice thins 😊
✓ Wholemeal crispsbread e.g. Arnotts’ Vitaweat™ or Ryvita™ with thin spread peanut butter (3tsp) OR vegemite and 2 slices low-fat cheese e.g. Kraft Singles Free 😊
✓ ½ English muffin pizza with 1 tsp tomato paste, 20g shaved ham and 20g reduced-fat mozzarella cheese. Try adding tomato, mushroom and capsicum. 😊
✓ 1 English grain muffin with 1Tb Philadelphia™ light cream cheese and 1Tb sultanas 😊
✓ 1 slice Wholemeal bread with 1 boiled egg, 2tsp low-fat mayonnaise and sliced tomato 😊
✓ 2 slices raisin toast with 2tsp margarine 😊
✓ 1 scone (plain, fruit, pumpkin) with 2tsp margarine and 2tsp jam
✓ 1 cup vegetable or pumpkin soup with 1 slice grain bread and 1 tsp margarine ☺️
✓ A cob of corn ☺️
✓ 1 sml can (95g) flavoured or plain tuna with 10 rice crackers 😊
✓ 1 Fruit or Vege Muffin
✓ 1 Nori roll ☻
✓ 2 Arnotts Snack Right™ biscuits OR Paradise Lites™ cookies and 250ml low-fat milk
✓ 1 tetra pack flavoured milk (375ml) e.g. Milo™ or Big M™ ☺
✓ 1 Yoplait LeRice™ ☺
✓ 1 Yoplait GoGurt OR Smackers yoghurt tube
✓ Sakata Minis and a Kraft cheesestick
✓ Uncle Tobys Muesli Bar, Yoghurt Tops, Fruit Breaks or OT’s and 200ml reduced-fat milk 😊
✓ Sanitarium FruityBix™ bar and 200ml reduced-fat milk 😊
✓ Kellogg’s K-Time Twists or Special K™ bar and 200ml reduced-fat milk 😊
✓ Kellogg’s LCMs™ or Nutrigrain™ bar and 200ml reduced-fat milk 😊
✓ 1 Kellogg’s K-Time Muffin Bar or Crunchy Nut bar
✓ 1 cup MiniWheats™ or Fruity-Bites 😊
✓ 1 Nut bar e.g. BeNatural nut bar or Be Natural Trail Bar 😊
✓ 1/3 cup trail mix (mixed seeds, nuts and dried fruit) 😊
✓ 40g snack pack sultanas/dried fruit mix
✓ Sanitarium Up ‘n’ Go or FastBreak 😊
✓ 200g low-fat yoghurt e.g. Yoplait NoFat or Nestle Diet and 1 cup fresh or tinned fruit in natural juice 😎
✓ 200g frozen yogurt and 20g snack pack Sunbeam Fruit Flakes
✓ 2 scoops of low-fat ice-cream with fruit and 1 Tb flavoured topping
✓ 250ml reduced-fat custard OR 200ml plus 1 banana / ¼ cup tinned fruit
✓ 250ml Reduced-fat milk and 2tspn Milo™ 😊
✓ 1 Yogo™ dairy dessert
✓ Smoothie made with 200ml low-fat milk, 100g low-fat yogurt and ½ cup berries & ½ ripe banana 😊
✓ Billabong™ OR Paddle Pop™
✓ 1 packet Arnotts Tiny Teddies™
✓ 50g Pretzels
✓ 4 sml Vitaweats spread with 1Tb Nutella™

😊 Extra Snack Ideas 😊

Each of the following snacks provide around 200kj per serve. If you get really hungry and have already eaten your meals and snacks for the day you can choose one of the following foods or you can use your calorie counter to choose something else that provides no more than 200kj.

✓ 2 cups popcorn, flavoured or plain, no added butter or oil
✓ 2 tbsp salsa or light Tzaziki with mixed vege sticks such as carrot, capsicum, cucumber, snow peas 😊
✓ 1.5 tbsp low-fat cottage cheese or light dip such as Hommus, Corn relish, or Avocado dip (e.g. Black Swan Skinny dips or Chris’ lite and fresh dips) with mixed vege sticks 😊
✓ 2 flavoured corn thins or plain corn / rice thins
✓ 1 cup strawberries 😊
✓ 1 cup watermelon 😊
✓ 1.5 cups cherry tomatoes 😊
✓ 1 Weis™ bar (33g)
✓ 15g mini snack pack Sunbeam sultanas
😊 Tips to a Healthier You 😊

✓ Choose WATER regularly throughout the day.

Did you know that sometimes your body can think you’re hungry when you are just thirsty?

Water is a better choice than soft drink, cordial or juice.

Hint: Try having a glass of water before every meal and sip on water throughout the day.

✓ Choose reduced-fat dairy foods

Dairy products like skim milk, low-fat yogurt and reduced-fat cheese contain essential nutrients for your bones. Try to choose 3 serves from these foods everyday.

1 serve = 250ml milk OR 200g yogurt OR 25g cheese OR 250ml custard

✓ Choose fruit and vegetables as a snack

Did you know that a piece of fruit is better than having juice? Fruit will fill you up and offer you more nutrients than juice.

✓ Choose sometimes foods no more than 2 times per day.

Sometimes foods contain a lot of fat, sugar and salt and do not offer your body a lot of the nutrients it needs. Sometimes foods include cordial, soft drink, chocolate, lollies, chips, pies, and hot dogs. Sometimes foods are also called ‘extra’ foods.

Hint: Try unbuttered air-popped popcorn, rice cakes or pretzels for a savoury snack or try low-fat flavoured milk or a yogurt if you have a sweet tooth.
😊 Tips to a Healthier You 😊

✔ Eat SLOWLY...your food will digest better and you will feel fuller sooner and so you won't need to eat as much. Plus, you can enjoy the flavour of the food better!

✔ WAIT: Wait 15mins before helping yourself to seconds. It takes your stomach about 15mins to work out that it's full!

✔ STOP & SIT DOWN: Eat while you are sitting down and don't do anything else. That means eat at the table with the t.v. and games turned off!

✔ THINK: Think about why you are eating. Are you hungry? Or are you bored? Or is it because everyone else is eating?
Ready-to-Eat Meals are handy when you are rushed and don't have time to cook dinner. However, they are often high in salt so are not recommended to eat everyday.

Tip: Add some vegetables to the side to fill you up even more!

Lean Cuisine Meals
- Sundried tomato chicken with pasta 1690kj
- Beef and mushroom with pasta 1590kj
- Beef Arrabbiata (meatballs and pasta) 1280kj
- Indian style butter chicken with rice 1530kj
- Honey Mustard chicken 1400kj
- Beef Lasagne 1690kj
- Vegetables cannelloni 1620kj
- Chicken Florentine with linguine 1180kj
- Satay chicken noodles 1270kj
- Honey Soy Beef with Wholemeal Noodles 1070kj

Papa Guiseppi’s Tasty Balance frozen pizza 1190kj / quarter pizza

McCain’s Healthy Choice
- Chinese Chicken with Cashews 1380kj
- Creamy Chicken Carbonara 1640kj
- Beef stirfry with Hokkein noodles 1220kj
- Beef Medallions 1050kj
- Honey Sesame Chicken 1240kj

Michelina’s Lean Gourmet
- Creamy Parmesan Spirals 1280kj
- Spaghetti Bolognaise 1180kj

Sunrice flavoured rices
- Oriental style Egg fried rice 995kj (1/2 packet)
- Roast Flavoured Chicken rice 1050kj (1/2 packet)
- Chicken and Mushroom rice 1060kj (1/2 packet)
- Mediterranean Tomato rice 1060kj (1/2 packet)

St Dalfour ready to eat meals
Duck & veges, Chicken & veges, Salmon & veges <1000kj
Ham with potatoes, Three bean with sweetcorn, <1000kj
Tuna and pasta, Vege and pasta, <1000kj
Wholegrain with beans, Couscous <1300kj
You should eat serves of vegetables each day.

You should eat serves of fruit each day.

You should eat serves of low-fat dairy/dairy alternatives each day.

You should eat serves of meat/meat alternatives each day.

You should eat no more than serves of extras or sometimes food each day.

Water is the BEST drink to quench your thirst. It contains NO kilojoules which means you can drink as much as you like!

Enjoy a variety of foods every day

Drink plenty of water

Choose these sometimes or in small amounts
## What is a Serve Size?

### Breads and Cereals
- 2 slices of bread
- 1 medium bread roll
- 1 cup cooked rice, pasta, noodles
- 1 cup porridge, breakfast cereal flakes
- ½ cup muesli
- 4 large crispbreads e.g. Vitaweats / Ryvita

### Dairy and dairy alternatives
- 250 ml glass (1 cup) of milk
- 125 ml (½ cup) evaporated milk
- 40 g (2 slices) of cheese
- 250 ml (1 cup) custard
- 200 g (1 small carton) of yoghurt, plain or fruit

*Alternatives:*
- 1 cup of calcium-fortified soy milk
- 1 cup almonds
- ½ cup pink salmon with bones

### Fruit
- 1 piece medium sized fruit eg apple, orange, mango, mandarin, banana, pear, peach etc
- 2 pieces of smaller fruit eg apricots, kiwi fruit, plums, figs
- About 8 strawberries
- 1 cup diced pieces or canned fruit
- ½ cup fruit juice
- ¼ medium melon (rockmelon, honeydew)
- Dried fruit eg 4 dried apricots 1 ½ tablespoons sultanas
- About 20 grapes or cherries

### Vegetable

#### Starchy vegetables
- 1 medium potato, yam, parsnip
- ½ medium sweet potato

#### Dark green leafy vegetables
- ½ cup cabbage, spinach, silverbeet, broccoli, cauliflower or brussels sprouts

#### Legumes and other vegetables
- 1 cup lettuce or salad vegetables
- ½ cup broad beans, lentils, peas, green beans, zucchini, mushrooms, tomatoes, capsicum, cucumber, sweetcorn, turnips, swede, sprouts, celery, eggplant etc

### Meat and meat alternatives

#### 65-100gm cooked meat or chicken (eg ½ cup mince, 2 small chops or 2 slices roast meat)
- 80-120 g cooked fish fillet

*Alternatives:*
- 2 small eggs
- ½ cup cooked (dried) beans, lentils, chick peas, split peas or canned beans
- 1/3 cup peanuts or almonds

### Extras
- 1 medium piece of plain cake
- 3-4 sweet biscuits
- 60 g jam, honey (1 tablespoon)
- 1 can soft drink or 2 glasses cordial
- 1 sweet bun e.g. iced finger bun
- ½ chocolate bar
- 30 g potato crisps
- 2 scoops ice-cream

*Slice pizza = 2 extras or 1 meat pie / pasty = 3 extras!*
Reading the Nutrition Information Panel

The nutrition information label (or nutrition information panel), is found on the packaging of almost all foods and drinks. Nutrition information labels allow you to make more informed choices about the food that you eat. Nutrition information labels also allow you to compare products and choose the one that is best for you.

Beware: The serve size listed by the food company may be much smaller than the amount you are actually eating. Work out how much you are actually eating.

This column of the table shows the nutrients in each serve of the food. The serve size is decided by the food company and may not be the same amount that you eat. The recommended serve size will differ between products. You usually do not compare foods by the serve size.

This column of the table shows the nutrients in 100g of the food. This column is on all food packaging. You usually compare foods using the ‘Quantity per 100g’ column.

The ingredients list tells you nearly all the ingredients that have been mixed together to make the food or drink. The ingredients added in the largest amounts are listed first and the ingredients added in the smallest amounts are listed last.

As a general rule:
1. Look for foods that do not contain added SUGAR in the first 5 ingredients.
2. Look for foods that do not contain FAT in the first 5 ingredients.

NUTRITION INFORMATION

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<th>Quantity per 100g</th>
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<td>1770 kJ</td>
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<tr>
<td>Protein</td>
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<td>— saturated</td>
<td>0.3 g</td>
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<td>Carbohydrates</td>
<td>14.2 g</td>
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<tr>
<td>— sugars</td>
<td>4.5 g</td>
<td>22.7 g</td>
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<tr>
<td>Sodium</td>
<td>60 mg</td>
<td>305 mg</td>
</tr>
<tr>
<td>Dietary fibre</td>
<td>1.2 g</td>
<td>6.0 g</td>
</tr>
</tbody>
</table>

Contains oats, wheat and soy as indicated in bold type.

Ingredients: rolled oats, sugar, puffed rice, wheat, apricot pieces (8%) [sugar, water, apricot concentrate, dextrose, colour (160(b)), vegetable gum (401), food acid (331), flavour, preservative (202)], glucose syrup, vegetable oil, tapioca starch, salt, emulsifier (soy lecithin), flavour.

Product processed on a line that also processes products containing tree nuts.
**Reading the Nutrition Information Panel**

**NUTRITION INFORMATION**

Servings per package: 8  
Serving size: 20 g (1 bar)

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**Product processed on a line that also processes products containing tree nuts.**

Energy is measured in kilojoules (kJ) or calories (cal). Energy is added to our body when we eat food and taken from our body when we are active e.g. playing sport, walking, doing chores. Some foods add more energy to our body than others. We use the ‘Quantity per 100g’ column to compare different foods and drinks.

Sugar can be ‘natural’ or ‘added’. Natural sugar means it is already present in the food e.g. the sugar found in fruit and milk. Added sugar means it has been added in addition to the sugar already naturally occurring in the food.

As a general rule, look for products that have less than 13g sugar per 100g.

**The catch**? Some food companies use the word natural to market their products. This does not mean that the food or drink is the best choice.

To lose weight we need to increase the amount of energy taken out of our body through activity and decrease the amount taken into our body by making better choices with what we eat and drink.

A ‘reduced fat’ product must have 25% less fat than the original product.

The catch? This does not mean that the ‘reduced fat’ product is low in fat.

A ‘low fat’ food contains less than 3g fat per 100g. A ‘low fat’ liquid e.g. milk, sauce contains less than 1.5g fat per 100ml. We use the ‘Quantity per 100g’ column to see how much fat is in a food.

Fibre is found in plant foods. Fruit, vegetables and wholegrain breads and cereals contain fibre. Fibre helps fill you up for longer and is good for your bowels. Foods that contain more than 10g fibre per 100g are a ‘very good source of fibre’.

Foods that contain more than 6g fibre per 100g are ‘a good source of fibre’.

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Reading the Nutrition Information Panel

Food companies can ‘hide’ sugar and fat in their products under a different name.

Common names for sugar include:
- any word ending in –ose e.g. glucose, dextrose, sucrose, lactose, maltose, fructose
- syrup e.g. high fructose corn syrup, golden syrup
- fruit concentrate, honey
- the term ‘saccharides’ - such as disaccharides or monosaccharide
- molasses
- sugar raw / brown
- malt / malt extract
- xylitol, sorbitol, mannitol

Common names for fat include:
- lard / tallow
- butter fat
- cocoa butter
- baking margarine
- shortening
- chocolate / carob
- vegetable fat / oil
- palm oil
- animal fat
- coconut oil
- hydrogenated vegetable fat
- poly unsaturated fats / oils*
- unsaturated fats /oils*
- safflower / sunflower oil*
- olive / canola oil*
- peanut / soybean / corn oil*

*We need some fats in our diets to keep us healthy. Some fats are better than others. These are better choices for fats than the others listed.
## Reading the Nutrition Information Panel

<table>
<thead>
<tr>
<th>NUTRITION INFORMATION</th>
</tr>
</thead>
<tbody>
<tr>
<td>Servings per package: 8</td>
</tr>
<tr>
<td>Serving size: 20 g (1 bar)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Nutrient</th>
<th>Quantity per serving</th>
<th>Quantity per 100g</th>
</tr>
</thead>
<tbody>
<tr>
<td>Energy</td>
<td>350 kJ</td>
<td>1770 kJ</td>
</tr>
<tr>
<td>Protein</td>
<td>1.5 g</td>
<td>7.7 g</td>
</tr>
<tr>
<td>Fat, total</td>
<td>2.1 g</td>
<td>10.4 g</td>
</tr>
<tr>
<td>— saturated</td>
<td>0.3 g</td>
<td>1.4 g</td>
</tr>
<tr>
<td>Carbohydrates</td>
<td>14.2 g</td>
<td>70.8 g</td>
</tr>
<tr>
<td>— sugars</td>
<td>4.5 g</td>
<td>22.7 g</td>
</tr>
<tr>
<td>Sodium</td>
<td>60 mg</td>
<td>305 mg</td>
</tr>
<tr>
<td>Dietary fibre</td>
<td>1.2 g</td>
<td>6.0 g</td>
</tr>
</tbody>
</table>

Contains oats, wheat and soy as indicated in bold type.

Ingredients: rolled oats, sugar, puffed rice, wheat, apricot pieces (8%) [sugar, water, apricot concentrate, dextrose, colour (160(b)), vegetable gum (401), food acid (331), flavour, preservative (202)], glucose syrup, vegetable oil, tapioca starch, salt, emulsifier (soy lecithin), flavour.

Product processed on a line that also processes products containing tree nuts.

1. What is the main ingredient in this product?
2. Is this food a low fat product?
3. How many times is sugar an ingredient?
4. How much energy is in this food?
5. Compare the following two products. Which is the lowest in energy?