Macrophage activation, age and sex effects of immunometabolism in obese asthma

Hashim A. Periyalil[1,2], Lisa G. Wood[1,2], Hayley A. Scott[1,2], Megan E. Jensen[1,2] and Peter G. Gibson[1,2,3]

Affiliations: [1]Centre for Asthma and Respiratory Diseases, Faculty of Health and Medicine, University of Newcastle, Callaghan, Australia. [2]Hunter Medical Research Institute, New Lambton, Australia. [3]Dept of Respiratory and Sleep Medicine, John Hunter Hospital, New Lambton, Australia.

Correspondence: Peter. G. Gibson, Priority Research Centre for Asthma and Respiratory Diseases, Level-2, Hunter Medical Research Institute, Lot-1, Kookaburra circuit, New Lambton, NSW 2305, Australia.
E-mail: peter.gibson@hnehealth.nsw.gov.au

ABSTRACT Obese asthma is characterised by infiltration of adipose tissue by activated macrophages and mast cells. The aim of this study was to examine the age and sex effects of immunometabolism in obese asthma.

Obese and non-obese asthmatic children and adults underwent spirometry, body composition assessment by dual energy X-ray absorptiometry and measurement of serum soluble CD163 (sCD163), tryptase, C-reactive protein (CRP) and other adipocytokines.

Plasma CRP ($p<0.01$) and leptin ($p<0.01$) were elevated in obese asthmatic adults, and sCD163 ($p=0.003$) was elevated in obese asthmatic children. We observed significantly higher sCD163 in obese female children compared to obese female adults and male children, and higher CRP in obese female adults compared to obese male children and adults. Serum tryptase concentrations were not significantly different across age groups. sCD163 positively correlated with the proportion of android fat in obese female children ($r=0.70$, $p=0.003$) and obese female adults ($r=0.65$, $p=0.003$). In obese female children, sCD163 was inversely associated with forced expiratory volume in 1 s % predicted ($r=-0.55$, $p=0.02$) and was positively associated with the Asthma Control Questionnaire ($r=0.57$, $p=0.02$).

Obese children with asthma have sex-specific macrophage activation, which may contribute to worse asthma control and lung function. The heterogeneous systemic inflammatory profile across age and sex suggests the existence of sub-phenotypes in obese asthma at the molecular level.

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Introduction

Obesity is recognised as a risk factor for asthma across all age groups [1]. Additionally, obesity is related to poor asthma-specific quality of life and worse asthma control in adults and children. Increased systemic inflammation is now recognised as a hallmark of obesity and is related to negative metabolic effects [2]. Immune and inflammatory cells infiltrate adipose tissue and drive systemic inflammation and subsequent end-organ damage [3]. Key infiltrating cells are macrophages [4] and mast cells [5]. The presence of these immune cells in adipose tissue suggests its vital role as a key link between metabolic and immune functions (immunometabolism) in obesity. However, the role of macrophages and mast cells in obese asthma requires further investigation.

Age and sex may have significant effects on obese asthma. Ageing has been shown to have considerable effects on body composition [6] and metabolic dysfunction [7]. Furthermore, LANG *et al.* [8] found age to be an important effect modifier of asthma control and airway function in obese asthma. Various studies have reported that obese adults with asthma had high levels of C-reactive protein (CRP) [9, 10] and markers of macrophage infiltration [11]. Tryptase, a marker of mast cell activation, is also elevated in obese non-asthmatic adults [5]. However, there is conflicting evidence regarding the association of CRP [12, 13] with childhood obese asthma. To date, macrophage activation, measured using markers such as soluble CD163 (sCD163), has not been examined in childhood obese asthma. There is also strong evidence to suggest that, across age groups, the effect of obesity on asthma is more pronounced in females [14]. However, the mechanistic basis of this association is unknown. It is essential to have a greater understanding of the inflammatory and clinical profile of obese asthmatics across age and sex, in order to develop targeted treatment options. Therefore, the aim of this study was to determine whether there are age and sex effects on immunometabolism in obese asthma.

Methods

Subjects

Obese and non-obese children and adults with asthma were recruited from the general community and John Hunter Hospital (Newcastle, Australia) [9, 13]. In this study, we assayed stored samples of plasma from these two previously described populations. Asthma was diagnosed on the basis of current (past 12 months) episodic respiratory symptoms, doctor-diagnosed asthma and in adults, airway hyperresponsiveness (AHR) to hypertonic saline, defined as a >15% decline in forced expiratory volume in 1 s (FEV_1) from baseline. Atopic status was determined by skin-prick allergy testing. Exclusion criteria included unstable asthma, systemic inflammatory diseases, respiratory diseases other than asthma and current smoking in adults. In children, obesity was defined as a body mass index (BMI) z-score $\geqslant$1.64 SD [15]. In adults, obesity was defined as a BMI $\geqslant 30\ kg \cdot m^{-2}$. All subjects gave written informed consent and the study was approved by the Hunter New England Human Research Ethics Committee (New Lambton, Australia; reference no: 12/11/21/5.05).

Clinical assessment

Clinical assessment was undertaken after an overnight fasting and after withholding antihistamines and asthma medications. Clinical asthma pattern and current asthma status were assessed using the Global Initiative for Asthma guidelines and the Asthma Control Questionnaire (ACQ) [16], respectively. Asthma stability was defined as no exacerbation, respiratory tract infection or oral corticosteroid use in the past 4 weeks. In children, BMI was calculated (weight (kg) / height (m^2)) and converted to BMI z-scores. All participants performed spirometry (Windows KoKo PFT System Version 4.9 2005; PDS Inc, Louisville, KY, USA). FEV_1 and forced vital capacity are expressed as a percentage of the predicted value for age, sex and height.

Sputum induction and analysis

Participants underwent combined bronchial provocation testing and sputum induction with hypertonic saline (4.5%) (ULTRA-NEB ultrasonic nebuliser, Model 2000; DeVilbiss Healthcare, Somerset, PA, USA), as described by GIBSON*et al.* [17]. AHR was defined as a >15% decline in FEV_1 from baseline [17]. The dose–response slope (DRS) and log-transformed provocation dose causing a 15% fall in FEV_1 (PD_{15}) were calculated. Sputum was selected, dispersed with dithiothreitol, and total cell counts and viability were determined. Cytospins were prepared, stained (May–Grunwald Giemsa; Sigma-Aldrich, Sydney, Australia) and a differential cell count obtained.

Systemic inflammatory markers

High-sensitivity CRP was measured in children from serum mixed with monoclonal antibody-coated polystyrene particles, specific for human CRP (Dimension Vista System CRP Flex Reagent Cartridge; Siemens Healthcare Diagnostics Inc., Newark, NJ, USA). In adults, ELISA (MP Biomedicals, Orangeburg,

NY, USA) was used to measure CRP. Commercial ELISAs were used to measure plasma interleukin (IL)-6 (R&D Systems, Minneapolis, MN, USA), serum leptin and adiponectin (Bio-Rad, Hercules, CA, USA) in children and adults. Assay sensitivity was 0.039 $pg \cdot mL^{-1}$, 3.1 $pg \cdot mL^{-1}$ and 32.7 $pg \cdot mL^{-1}$ for IL-6, serum leptin and serum adiponectin, respectively. All samples were tested in duplicate. Plasma tryptase was measured by Immuno CAP Tryptase assay (Phadia, Uppsala, Sweden), which measures total tryptase with a reportable range of 1–200 $\mu g \cdot L^{-1}$. Plasma levels of sCD163, a marker of macrophage activation [18], were measured using ELISA (Macro 163 kit; Trillium diagnostics, LLC, Bangor, ME, USA). The lower limit of detection was 0.23 $ng \cdot mL^{-1}$.

Body composition assessment

A total body scan was performed using dual-energy X-ray absorptiometry (Lunar Prodigy Series; GE Medical Systems, Madison, WI, USA). Percentage of android fat distribution was calculated as: android fat (g)/total fat (g) × 100.

Statistical analysis

Data were summarised by descriptive statistics using mean±SD for parametric data and median (interquartile range) for non-parametric data. Group comparisons for continuous data were performed using either ANOVA with Holm–Sidak's *post hoc* test (parametric data) or the Kruskal–Wallis test with Dunn's *post hoc* test (non-parametric data). For correlation analysis, two-tailed Pearson's (parametric data) or Spearman's (non-parametric) correlation coefficient was used (Graph Pad Prism software version 6.0e; GraphPad Software, La Jolla, CA, USA). A p-value $\leqslant 0.05$ was regarded as statistically significant.

Results

Clinical characteristics across age groups when categorised according to BMI

Table 1 describes the subject characteristics of asthmatic children (n=49) and adults (n=158) included in the study. Obese and non-obese adults had significantly greater airflow limitation (FEV_1 % predicted), compared to obese children with asthma (p=0.005). In obese children, the daily dose of inhaled corticosteroids (ICS) was significantly higher than in non-obese children (348±35 *versus* 105±28 $\mu g \cdot day^{-1}$, p<0.01). Similarly, adults required significantly higher doses of ICS compared to their non-obese counterparts (1399±92 *versus* 956±88 $\mu g \cdot day^{-1}$, p=0.002). Baseline atopic status did not differ among groups. AHR, measured by PD_{15}, was significantly lower in obese adults when compared to non-obese children (p=0.004).

TABLE 1 Clinical characteristics of the asthmatic children and adults included in the study

	Children		Adults		p-value
	Obese	Non-obese	Obese	Non-obese	
Subjects n	34	15	85	73	
Age years	12±2	14±2	49±15	51±16	
Females %	38	60	60	59	0.99
BMI	2.1±0.3^{+}	0.3±1.4^{+}	35.4 ±4.1	26.6±3.2	
Atopy	25 (73)	13 (86)	63 (75)	54 (75)	0.78
FEV_1 % predicted	92.8±10.7	87.2±11.6	81.9±19.1*	79.8±20.3*	**0.005**
FVC % predicted	100.8±9.4	96.1±12	93.2±14.7	96.1± 18	0.10
FEV_1/FVC	79.2±6	79.2±8.01	70.9±9.7$^{\#}$	66.8±10.8$^{*,\#,\P}$	**<0.01**
ACQ Score	0.7 (0.4–1.2)	0.7 (0.4–1.3)	1 (0.4–1.6)	0.7 (0.4–1.4)	0.18
GINA pattern					
Intermittent	12 (35)	5 (36)	27 (32)	22 (30)	
Mild persistent	6 (18)	7 (50)	13 (15)	10 (14)	
Moderate persistent	11 (32)	2 (14)	31 (37)	29 (40)	
Severe persistent	5 (15)	0	14 (16)	12 (16)	
PD_{15} mL	3.9 (1.4–12.5)	1.6 (0.4–3.7)	6.7 (3.9–11.7)$^{\#}$	5.56 (0.7–8.6)	**0.004**

Data are presented as mean±SD, n (%) or median (interquartile range), unless otherwise stated. Bold indicates statistical significance. BMI: body mass index; FEV_1: forced expiratory volume in 1 s; FVC: forced vital capacity; ACQ: Asthma Control Questionnaire; GINA: Global Initiative for Asthma; PD_{15}: provocation dose causing a 15% fall in FEV_1. *: p<0.05 *versus* obese children; $^{\#}$: p<0.05 *versus* non-obese children; ¶: p<0.05 *versus* obese adults; $^{+}$: BMI z-scores.

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TABLE 2 Systemic and airway inflammatory markers in asthmatic children and adults when categorised according to body mass index

Variables	Children		Adults		p-value
	Obese	Non-obese	Obese	Non-obese	
Subjects n	34	15	85	73	
Systemic inflammatory markers					
CRP mg·L^{-1}	2.0 (1–3.8)	1.2 (0.8–2.8)	4.7*,#,¶ (1.9–9.6)	1.7 (0.9–5.2)	**<0.01**
Soluble CD163 ng·mL^{-1}	1262#,¶ (1044–1657)	877 (611–1024)	1139¶ (872–1416)	908 (640–1318)	**0.003**
Serum tryptase µg·mL^{-1}	4.4 (3.6–5.9)	3.3 (2.2–4.3)	3.8 (2.8–5.9)	4.5 (3.2–5.7)	0.07
IL-6 pg·mL^{-1}	1.4 (0.8–2.1)	0.8 (0.7–1.1)	1.6 (0.7–2.8)	1.3 (0.9–1.9)	0.08
Leptin pg·mL^{-1}	4736 (656–20793)	5985 (2349–13378)	10937# (6798–21714)	4447 (2252– 8586)	**<0.01**
Adiponectin µg·mL^{-1}	4.4 (3.8–6.6)	6.5 (5.4–1.3)	5.5 (2.9–10.4)	5.7 (5.5–2.3)	0.11
Airway inflammatory markers					
Neutrophils %	10.3 (7.1–28.4); n=21	7.0 (3.4–25.1); n=10	40.9*,¶ (23.6–64.6)	36.5*,¶ (20.3–57.0); n=63	**<0.01**
Macrophages %	78.8#,+ (56.3–84.8); n=21	60.8 (43.9–74.1); n=10	49.8 (28.8–66.4)	51.8 (32.0–67.8); n=63	**0.009**
Eosinophils %	3.0 (0.5–7.3); n=21	9.3+ (4.8–46.8); n=10	2.0 (0.5–4.4)	1.4 (0.5–6.3); n=54	**0.01**
Total cell count ×10^6 cells·mL^{-1}	2.3 (1.4–4.6)	2.8 (1.9–8.7); n=8	2.5 (1.4–4.4); n=40	3.1 (2.3–5.0); n=52	0.23

Data are presented as median (interquartile range), unless otherwise stated. Bold indicates statistical significance. CRP: C-reactive protein; IL: interleukin. *: p<0.05 *versus* obese children; #: p<0.05 *versus* non-obese adults; ¶: p<0.05 *versus* non-obese children; +: p<0.05 *versus* obese adults.

Inflammatory markers across age groups when categorised according to BMI

Systemic and airway inflammatory markers are described in table 2. Obese adults with asthma had significantly higher CRP concentrations than non-obese adults and obese and non-obese children (p<0.01). In contrast, sCD163 was significantly higher in obese children (p=0.003), when compared to non-obese children and non-obese adults. We observed a positive trend for higher plasma tryptase, a biomarker of mast cell activation, among obese children when compared to non-obese children (p=0.014); however, it was not significantly different across age groups (p=0.07). The adipokine leptin was elevated in obese adults with asthma (p<0.01).

Obese asthmatic children had a significantly higher percentage of macrophages in their airways compared to other groups. The percentage of neutrophils was elevated in adults, compared to children with asthma (table 2).

Systemic inflammatory profile in obese asthmatics according to age and sex

Obese female adults with asthma had significantly higher CRP (p<0.01) when compared to obese male adults and children with asthma (fig. 1a). In terms of macrophage activation, sCD163 was significantly higher in obese female children with asthma, when compared to obese female adults (p=0.01) and obese male children (p=0.03) with asthma (fig. 1b).

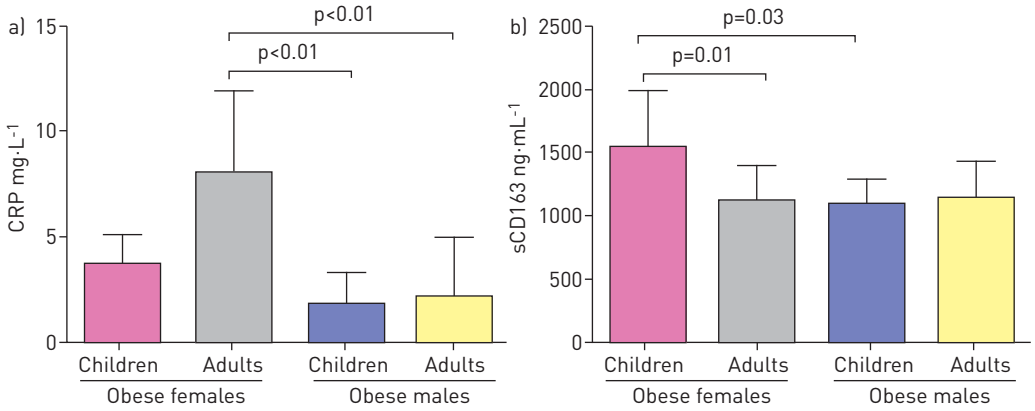

FIGURE 1 Systemic inflammation in obese asthmatics across age and sex. a) C-reactive protein (CRP) and b) soluble CD163 (sCD163).

Associations between systemic inflammatory biomarkers, body composition and clinical asthma outcomes

sCD163 correlated positively with percentage of android fat in obese asthmatic female children (r=0.70, p=0.003) (fig. 2a) and adults (r=0.65, p=0.003). No significant correlation was observed between sCD163 and percentage of android fat in obese male children with asthma (r=0.02, p=0.94) (fig. 2b). CRP correlated positively with percentage of android fat (r=0.67, p=0.002) and negatively with percentage of gynoid fat (r=−0.55, p=0.014) in obese female children with asthma. However, no significant correlations were found between CRP and body composition in female adults. In obese female children with asthma, we observed a positive correlation between sCD163 and ACQ (r=0.57, p=0.02) (fig. 3a) and a negative correlation with measure of airflow limitation (FEV_1) (r=−0.55, p=0.02) (fig. 3b). No significant correlations were observed between tryptase levels and body composition.

Discussion

This is the first study to our knowledge, to report a heterogeneous systemic inflammatory profile across age and sex in obese asthma and to identify macrophage activation as a determinant of this observation. The predominant macrophage activation demonstrated by obese female asthmatic children, when compared to their adult counterparts, suggests age- and sex-specific effects of immunometabolism in obese asthma. Moreover, this novel finding suggests distinct orchestration of immune cell activation and morphogenesis of adipose tissue at a molecular level across age and sex in obese asthma. From a clinical perspective, understanding this phenomenon is critical to enable us to develop age- and sex-specific therapeutic strategies.

Macrophage activation, assessed by measuring sCD163, has been reported to be significantly associated with increased BMI [19, 20] and related comorbidities, such as type-2 diabetes [21] and hypertension [20] in obese adults. sCD163 is the soluble form of CD163 (glycosylated membrane protein expressed exclusively by cells of monocytic lineage), which is cleaved following lipopolysaccharide- or free fatty acid-mediated activation of adipose tissue resident macrophages and released into systemic circulation [18]. The systemic level of sCD163 has now been recognised as a measure of macrophage activation in various obesity-related pro-inflammatory conditions. However, to date, there is little evidence to suggest an association of macrophage activation with obese asthma. Sideleva *et al.* [11] examined adipose tissue inflammation in obese asthma and observed an increased macrophage infiltration of visceral adipose tissue, when controlled for BMI. Our data also suggest that macrophage activation may have a pivotal role in determining increased obesity-related metabolic activity of adipose tissue and translation of these effects to clinical aspects of obese asthma, particularly in obese female children.

We also examined serum tryptase as a marker of mast cell activation in obese asthma [5]. The presence of mast cells noted in adipose tissue in obese mice [5], prior to macrophage recruitment, is suggestive of a pivotal role for mast cells in regulating immunometabolism in obesity. In view of our observation of macrophage activation in obese asthmatic children, we expected a similar trend with mast cell activation. However, tryptase was significantly increased only in obese children, compared to non-obese children and not across all age groups. This may be explained by the effect of age on tryptase, as tryptase levels increase in adults independent of BMI [22], which may have confounded our analysis. In another human study, Ward *et al.* [23] also found no association between BMI and tryptase in obese and non-obese children with and without glucose intolerance and diabetes mellitus (p=0.068). Hence, further assessment of tryptase levels in obese asthmatics is needed. The distinct levels of sCD163, in the absence of a similar

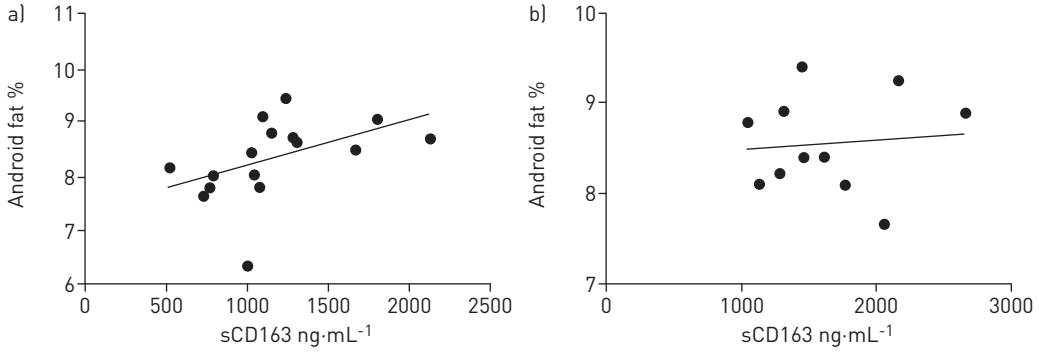

FIGURE 2 Sex-specific effects of central obesity on macrophage activation. Android fat *versus* soluble CD163 (sCD163) in a) obese female children (r=0.70, p=0.003) and b) obese male children (r=0.02, p=0.94).

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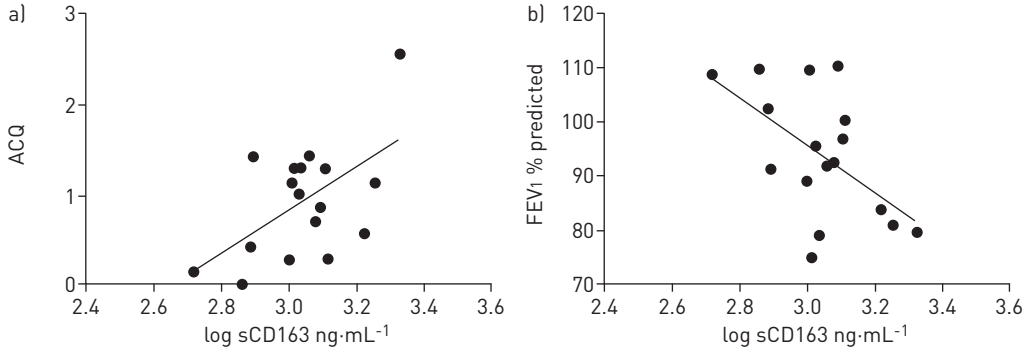

FIGURE 3 Association between macrophage activation and clinical aspects in obese female children with asthma. a) Asthma Control Questionnaire (ACQ) and b) forced expiratory volume in 1 s (FEV1) *versus* log soluble CD163 (sCD163) (r=0.57, p=0.02 and r=−0.55, p=0.02, respectively).

pattern with tryptase, suggest a macrophage-specific immune cell activation pathway in obese asthma across age groups.

In this study, obese female children had significantly higher sCD163 when compared across age and sex. We propose that, in addition to the effects of body composition, a multitude of age- and sex-specific biological mechanisms might contribute to the metabolic activity of adipose tissue. Adipose tissue distribution is heavily influenced by sex, with sex-related patterns becoming apparent during puberty. However, in obese female children, a BMI-related increase in androgens [24, 25] may favour central adiposity. In addition, obese female children have a greater degree of obesity-induced insulin resistance [26], and the compensatory hyperinsulinaemia may further facilitate accumulation of visceral fat [27]. Furthermore, free fatty acid flux, which is an important determinant of metabolic activation of adipose tissue, has been reported to be higher in obese female children when compared to their male counterparts [28]. These complex biological interactions might lead to the development of a unique metabolome favouring macrophage activation in obese female children with asthma.

While there is clear evidence of increased systemic inflammation in obese asthmatic adults [9, 29], there is conflicting evidence regarding systemic inflammation in obese childhood asthma. Cook *et al.* [30] first reported elevated CRP in obese children, defined using the Ponderal index (weight/height^3). However, it is uncertain to what extent the Ponderal index would have reflected the degree of adiposity. Similarly, Khan *et al.* [31], in a cross-sectional study, found significantly elevated high-sensitivity CRP in obese children with asthma, compared to non-obese asthmatic and non-asthmatic children. By further analysis, they observed an independent association between asthma and obesity with high-sensitivity CRP in obese children with asthma. However, in another study [32], IL-6, which is the main inducer of hepatic production of CRP, was not found to be significantly raised in obese children. Moreover, analysis of National Health and Nutrition Examination Survey III data identified elevated CRP levels (>0.22 mg·L^{-1}) in only one in five overweight children [33]. We found that CRP was not elevated in obese children with asthma. In contrast, sCD163 was elevated in obese compared to non-obese children with asthma. Together, these data show age-specific effects of immunometabolism in obese asthma.

Recently, body composition measures have gained greater attention in studies examining obesity-related comorbidities. Most of the association studies reveal that abdominal obesity (waist circumference >88 cm in females and >102 cm in males) has more impact on risk of developing asthma than general obesity. In their large prospective study examining the association of abdominal obesity and incident asthma in adults, Brumpton *et al.* [34] observed that abdominal obesity was a significant risk factor for asthma (OR 1.46, 95% CI 1.52–2.52), particularly in females, even after adjusting for general obesity. We found that body composition was a determinant of inflammatory marker levels. This is the first study to link sCD163 and percentage of android fat (fat distribution around the abdomen). There was a significant correlation between sCD163 and percentage of android fat in obese female children and adults, highlighting increased depot-specific metabolic activity of adipose tissue in obesity. Interestingly, the significantly high systemic levels of CRP observed in obese female adults, when compared across sex, did not correlate with percentage of android fat.

Even though CRP is primarily secreted by the liver, when induced by IL-6, IL-1β and tumour necrosis factor-α, there are other factors that could affect systemic levels of CRP, particularly vascular reactivity [35]. Thus, our data suggest that, CRP, which is an excellent marker of systemic inflammation, may not be a specific reflection of metabolic activity in adipose tissue of obese female adults with asthma.

A large number of cross-sectional and longitudinal studies have reported worse asthma control and increased asthma severity in obese individuals. The significantly higher percentage of macrophages that we observed in the induced sputum of obese children, along with their altered phenotype [36] and activity [37], highlight the potential implication of airway macrophages on clinical aspects of obese asthma. Furthermore, the enhanced reactivity of alveolar macrophages from overweight/obese adult asthmatics to lipopolysaccharide, particularly when they were primed *ex vivo* with high dose of leptin, was demonstrated in an elegant study by Lugogo *et al.* [36]. The authors also observed leptin-induced production of pro-inflammatory cytokines from alveolar macrophages of overweight/obese asthmatics. Together, these findings highlight the potential role of airway macrophages in translating the effects of increased systemic inflammation to the airways. In addition, they also provide a greater understanding of a mechanistic link for previously observed leptin-mediated effects on airway characteristics of obese asthmatics [38]. Recently, Kim *et al.* [39] found mice fed on a high fat diet and *ob/ob* (genetically modified leptin deficient) mice fed on a regular diet developed significantly worse AHR. They have attributed this phenomenon to an inflammasome-mediated pathway triggered by macrophage activation in adipose tissue and involving IL-17. They further observed an increased expression of IL-1β and inflammasome component of NLRP3 in the lung tissue of obese mice, suggestive of an effect of macrophage activation on inflammometry lung tissue in obesity. Similarly, our novel finding of a correlation between sCD163, ACQ and FEV_1 % predicted in obese female children suggests that, indeed, there is an effect of macrophage activation on clinical aspects of obese childhood asthma. Furthermore, increased macrophage activation in obese children with asthma may contribute to their steroid non-responsiveness [40], which ultimately may lead to unwanted side-effects from significantly high doses of steroid therapy.

This study, being designed as cross-sectional and retrospective, cannot fully address all possible interactions between macrophage and mast cell activation and negative metabolic effects in obese asthma. A limitation of this study is the lack of a non-asthmatic control group to determine if these relationships are related to the presence of disease. However, the associations between these biomarkers and asthma control and lung function suggest their direct relevance to asthma. More longitudinal and interventional studies are warranted to address this issue.

Conclusion

In conclusion, we have shown for the first time that there are age- and sex-specific effects on macrophage activation in obese asthma. Our data highlights sCD163 as a potential biomarker of asthma control and disease severity in obese childhood asthma. Macrophage activation in obese childhood asthma may contribute to the steroid non-responsiveness in this population. The association of sCD163 with android fat is in line with the already known increased metabolic activity of visceral adipose tissue. Evidence from this study suggests the need to characterise sub-phenotypes of obese asthma at a molecular level, which may enable us to have a greater understanding of the distinct immunological mechanisms and to identify specific therapeutic targets across age and sex.

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