Immunometabolism in Obese Asthma

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contribution to the joint publications.

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

Hashim A Periyalil
"I acknowledge the traditional owners and custodians of the land on which we meet today, the Wurundjeri people of the Kulin Nation. I pay my respects to their Elders both past and present".
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This thesis represents not only my work under the guidance of my supervisors; it is an achievement for our research group at HMRI, having established a new method for adipose tissue processing and analysis of adipose tissue macrophages. Undertaking this PhD had been truly a life-changing experience for me and it would not have been possible without the invaluable support and guidance that I received from many people.

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Publications arising from this thesis to date

   
   **Statement of contribution:** Conceptualised the hypothesis, entered, analysed and interpreted the data; and wrote the manuscript.

   
   **Statement of contribution:** Researched the literature and drafted the manuscript.

Abstract list


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Invited seminar presentation

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### Abbreviations

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Full Form</th>
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<tbody>
<tr>
<td>ACQ</td>
<td>Asthma Control Questionnaire</td>
</tr>
<tr>
<td>AHR</td>
<td>Airway hyperresponsiveness</td>
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<tr>
<td>ANOVA</td>
<td>Analysis of variance</td>
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<tr>
<td>AT</td>
<td>Adipose tissue</td>
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<tr>
<td>ATM</td>
<td>Adipose tissue macrophage</td>
</tr>
<tr>
<td>AQLQ</td>
<td>Asthma Quality of Life Questionnaire</td>
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<tr>
<td>BMI</td>
<td>Body mass index</td>
</tr>
<tr>
<td>CDC</td>
<td>Centres for Disease Control</td>
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<tr>
<td>CI</td>
<td>Confidence interval</td>
</tr>
<tr>
<td>CLS</td>
<td>Crown-like structures</td>
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<tr>
<td>CRP</td>
<td>C-reactive protein</td>
</tr>
<tr>
<td>CT</td>
<td>Computed tomography</td>
</tr>
<tr>
<td>DALY</td>
<td>Disability-adjusted life year</td>
</tr>
<tr>
<td>DEXA</td>
<td>Dual-energy x-ray absorptiometry</td>
</tr>
<tr>
<td>DMEM</td>
<td>Dubelcco’s modified Eagle Medium</td>
</tr>
<tr>
<td>DPBS</td>
<td>Dulbecco’s Phosphate-Buffered Saline</td>
</tr>
<tr>
<td>ELISA</td>
<td>Enzyme-linked immunosorbent assay</td>
</tr>
<tr>
<td>eNO</td>
<td>Exhaled nitric oxide</td>
</tr>
<tr>
<td>FA</td>
<td>Fatty acid</td>
</tr>
<tr>
<td>ER</td>
<td>Endoplasmic reticulum</td>
</tr>
<tr>
<td>ERV</td>
<td>Expiratory reserve volume</td>
</tr>
<tr>
<td>FACS</td>
<td>Fluorescence-activated cell sorting</td>
</tr>
<tr>
<td>FEV&lt;sub&gt;1&lt;/sub&gt;</td>
<td>Forced expiratory volume in one second</td>
</tr>
<tr>
<td>FFA</td>
<td>Free fatty acid</td>
</tr>
<tr>
<td>FRC</td>
<td>Functional residual capacity</td>
</tr>
<tr>
<td>FSC</td>
<td>Forward scatter</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Definition</td>
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<tr>
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<tr>
<td>FVC</td>
<td>Forced vital capacity</td>
</tr>
<tr>
<td>GINA</td>
<td>Global Initiative for Asthma</td>
</tr>
<tr>
<td>hsCRP</td>
<td>High sensitivity C-reactive protein</td>
</tr>
<tr>
<td>ICS</td>
<td>Inhaled corticosteroid</td>
</tr>
<tr>
<td>IFN-γ</td>
<td>Interferon gamma</td>
</tr>
<tr>
<td>IgE</td>
<td>Immunoglobulin E</td>
</tr>
<tr>
<td>IHC</td>
<td>Immunohistochemistry</td>
</tr>
<tr>
<td>IL</td>
<td>Interleukin</td>
</tr>
<tr>
<td>IQR</td>
<td>Interquartile range</td>
</tr>
<tr>
<td>LPS</td>
<td>Lipopolysaccharide</td>
</tr>
<tr>
<td>MFI</td>
<td>Mean fluorescence intensity</td>
</tr>
<tr>
<td>MGG</td>
<td>May-Grunwald-Giemsa staining</td>
</tr>
<tr>
<td>MCP</td>
<td>Monocyte chemotactic protein</td>
</tr>
<tr>
<td>MMP-9</td>
<td>Matrix metalloproteinase-9</td>
</tr>
<tr>
<td>NF-κB</td>
<td>Nuclear factor-kappa B</td>
</tr>
<tr>
<td>NLRP3</td>
<td>Nucleotide-binding domain, leucine-rich-containing family, pyrin domain containing 3</td>
</tr>
<tr>
<td>ORO</td>
<td>Oil Red O</td>
</tr>
<tr>
<td>PBMC</td>
<td>Peripheral blood mononuclear cells</td>
</tr>
<tr>
<td>PD15</td>
<td>Provocation dose required to induce a drop in FEV$_1$ of 15%</td>
</tr>
<tr>
<td>PPAR</td>
<td>Peroxisome proliferator activated receptors</td>
</tr>
<tr>
<td>RV</td>
<td>Residual volume</td>
</tr>
<tr>
<td>SAT</td>
<td>Subcutaneous adipose tissue</td>
</tr>
<tr>
<td>SD</td>
<td>Standard deviation</td>
</tr>
<tr>
<td>SDS</td>
<td>Standard deviation scores</td>
</tr>
<tr>
<td>SSC</td>
<td>Side scatter</td>
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<tr>
<td>SVF</td>
<td>Stromal vascular fraction</td>
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<tr>
<td>Acronym</td>
<td>Definition</td>
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<td>----------------------------------</td>
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<tr>
<td>TACE</td>
<td>TNF-α converting enzyme</td>
</tr>
<tr>
<td>TAG</td>
<td>Triacylglycerol</td>
</tr>
<tr>
<td>T2DM</td>
<td>Type 2 Diabetes mellitus</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-α</td>
<td>Tumour necrosis factor-alpha</td>
</tr>
<tr>
<td>VAT</td>
<td>Visceral adipose tissue</td>
</tr>
<tr>
<td>WAT</td>
<td>White adipose tissue</td>
</tr>
<tr>
<td>WHO</td>
<td>World Health Organisation</td>
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Synopsis

The prevalence of asthma and obesity has risen significantly to epidemic proportions. Obese asthmatics represent a unique clinical phenotype, characterised by worse asthma control, increased risk of hospitalisation and treatment-related side effects. Despite a well-established association between obesity and asthma, the inflammatory mechanisms and consequences of asthma in obese individuals remain unclear. It is therefore essential to have a greater understanding of the multi-level interactions in the inflammometry of obese asthma to develop targeted treatment for a better outcome. This thesis aims to examine the altered immunometabolism in obese asthma, the potential role of CRP and sCD163 as biomarkers of systemic and adipose tissue inflammation and the inflammatory link between adipose tissue, systemic and airway inflammation in obesity and asthma.

Obesity is characterised by infiltration of adipose tissue by activated macrophages and mast cells, which further potentiate a pro-inflammatory microenvironment, systemic inflammation and negative clinical effects (immunometabolism). However, the role of macrophages and mast cells in obese asthma is unclear. Furthermore, the systemic inflammatory profile across age and sex in obese asthma is unknown. In chapter 3, soluble ectodomain of CD163 (sCD163) and CRP were utilised as biomarkers of macrophage and mast cell activation respectively, to examine age and sex-specific effects on these innate immune pathways in obese asthma. We noted a heterogeneous inflammatory profile in obese asthmatics with obese female children characterised by significantly higher levels of circulating sCD163 and obese
female adults having significantly higher levels of circulating CRP. In obese female children, we also noted associations between sCD163 and percentage of android fat, lung function and asthma control. These findings indicate macrophage activation is the predominant innate immune pathway in obese female children and has potential clinical implications in this cohort.

Obesity is associated with macrophage infiltration and functional polarisation in adipose tissue. The role of adipose tissue macrophage (ATM) phenotypes (ie M1 pro-inflammatory and M2 anti-inflammatory macrophages) in obese asthma is unclear. We developed a method to isolate and perform functional phenotyping of ATMs, as described in Chapter 4. In Chapter 5, we compared macrophage infiltration and functional phenotypes across subcutaneous and visceral adipose tissue depots. Obese asthmatics were characterised by a significantly higher macrophage infiltration in the visceral adipose tissue depot, particularly the pro-inflammatory M1 macrophage phenotype. In obese subjects, BMI and waist circumference were positively correlated with the ratio of M1:M2 ATMs in VAT. Furthermore, the negative relationship between M1:M2 ATM ratio in VAT and %FEV₁ highlights the potential clinical implication of this finding.

In Chapter 6, we explored the mechanistic basis of adipose tissue inflammometry-driven systemic inflammation in obese asthma, by examining the relationships between CRP and sCD163 and ATM phenotypes. CRP was positively associated with percentage of M1 ATMs in VAT of obese asthmatics. Furthermore, among all subjects, CRP was negatively associated with sputum macrophage count. These findings suggest CRP as a potential biomarker of macrophage activation in obese asthma and a plausible
relationship between systemic and altered airway inflammation in obese asthma.

The data presented in this thesis highlights the potential role of macrophage-mediated inflammatory pathways in obese asthma. Further work in this area may enable identification of newer therapeutic targets, which could facilitate better clinical outcomes, in terms of morbidity and mortality in obese asthma.