Trinity College Dublin
Coláiste na Tríonóide, Baile Átha Cliath
The University of Dublin

INFLAMMATORY MARKERS OF ANTIPSYCHOTIC WEIGHT GAIN AND CARDIOMETABOLIC DYSFUNCTION IN YOUTH MENTAL HEALTH DISORDERS

A thesis submitted to Trinity College Dublin for the degree of

Doctor of Philosophy

By

Karen Conlan, B.A., M.B., B.Ch., B.A.O., MRCPsych, MCPsychI, M.Sc.

January 2023

Student Number: 17345459

Supervisors:

Professor Louise Gallagher

Dr Jane McGrath

Dr Andrew Hogan (NUI Maynooth)

Department of Psychiatry, School of Medicine,
Trinity College Dublin, The University of Dublin.

Declaration

I declare that this thesis has not been submitted as an exercise for a degree at this or any other university and it is entirely my own work.

I agree to deposit this thesis in the University's open access institutional repository or allow the Library to do so on my behalf, subject to Irish Copyright Legislation and Trinity College Library conditions of use and acknowledgement.

I consent to the examiner retaining a copy of the thesis beyond the examining period, should they so wish (EU GDPR May 2018).

_____

Karen Conlan

Summary

Second generation antipsychotics (SGAs) are used in children and adolescents to treat psychotic symptoms in Early Onset Psychosis (EOP) and irritability and behaviours that challenge in autism spectrum disorder (ASD). Significant side effects include rapid and excessive weight gain, dyslipidaemia, insulin resistance, hyperglycaemia, and metabolic syndrome, increasing the risk of cardiovascular disease and Type 2 Diabetes Mellitus. Importantly, children appear to be at greater risk of early weight gain and metabolic dysregulation on SGAs compared to adults. Chronic low-grade inflammation has been identified in association with childhood obesity. However, there is limited understanding regarding the impact of SGAs on inflammatory markers. We hypothesized that SGA medications cause inflammation, and that this inflammation drives the associated cardiometabolic side effects.

We recruited nineteen children and adolescents who were commencing SGA medication (risperidone, aripiprazole, olanzapine or quetiapine) and examined their metabolic and inflammatory profile at baseline. We measured height and weight to calculate BMI percentile. We looked at lipid profile, fasting glucose, HbA1c and prolactin levels. In serum we measured leptin, soluble CD163, TNF-α and IL-17. We cultured Peripheral Blood Mononuclear Cells (PBMCs) from the blood samples and measured TNF-α, IL-10, IL-17A and IL-17F from the cell culture supernatants. We also carried out immunophenotyping by flow cytometry to measure the frequency of specific populations of immune cells. We then followed the participants up at three, six and twelve months and repeated these measurements at each time point. A total of ten participants completed the study. Clinically, we found that the participants gained a significant amount of weight within the first three months of commencing medications and BMI percentile increased in 90% of the participants. Weight gain in the first 3 months ranged from 0.5kg to 9kg. We found that the participants with a lower BMI percentile at baseline (<50th percentile) gained more weight on SGA medication than participants with a higher BMI percentile at baseline (>50th percentile). We also found that the SGA risperidone caused a significant increase in prolactin levels of >1000 mIU/L in some participants.

We examined the serum inflammatory profile of the participants at each time point using Enzyme Linked Immunosorbent Assay (ELISA). We found that leptin levels increased significantly at three months in serum. We carried out immunophenotyping by flow cytometry. To get a broad overview of immunophenotype we carried out extracellular staining to examine the frequencies of populations of immune cells. We measured T cells, Monocytes, invariant Natural Killer T (iNKT) cells, Natural Killer (NK) cells and Mucosal Associated Invariant T (MAIT) cells. We found that the frequencies of MAIT cells increased on SGA medication. We examined the cellular inflammatory profile of the participants at each time point. To do this we isolated Peripheral Blood Mononuclear Cells (PBMCs) from whole blood. We set up four conditions for culturing the cells overnight. We cultured the cells in media alone, with Lipopolysaccharide (LPS) (100ng/ml) to stimulate monocytes, with TCR beads (25ng/ml) to stimulate T-cells and with phorbol 12-myristate 13-acetate (PMA) and ionomycin (stock at 500X, used at 1X) to stimulate all immune cells. We harvested the supernatants and measured TNF-α, IL-10, IL17A and IL17F by ELISA. We found that TNF-α increased on SGA treatment in the supernatants of PBMCs stimulated with LPS at 3 months, IL17A increased on SGA treatment in the supernatants of PBMCs stimulated with TCR beads at 3 months and IL-10 increased on SGA treatment in the supernatants of non-treated PBMCs at all time points. We investigated the cellular source of these cytokines using intracellular flow cytometry by staining PBMCs at each time point and we found that MAIT cells were increased and were producing more IL-17 and TNF-α on SGA medication.

As SGAs are dopamine antagonists, we also investigated the effect dopamine had on cytokine production. To do this we used a monocyte cell line and cultured the cells in dopamine hydrochloride (100μM) both with and without LPS (100ng/ml). We found that dopamine alone caused a significant reduction in TNF-α production. We cultured PBMCs in dopamine hydrochloride +/- TCR beads (25ng/ml) +/- PMA (stock at 500X, used at 1X), and in risperidone (1μg/ml) +/- TCR beads +/- PMA. We carried out intracellular flow cytometry and measured TNF-α production by ELISA. We found that both dopamine and risperidone caused a significant reduction in TNF-α production.

Collectively these findings suggest that within three months of starting an SGA these children and adolescents developed an inflammatory phenotype. We found that the same driver of metabolic disease in the form of an inflammatory phenotype that occurs in childhood obesity also occurs during treatment with SGA medications. This indicates that early inflammation could be a possible mechanism for the weight gain and metabolic dysfunction that occurs in patients who are on SGA medications. In conclusion, this study gives us an understanding of some of the immunological underpinnings of the metabolic complications associated with SGAs. Future research could potentially identify novel targets to combat this inflammation and may lead to a reduction in metabolic side effects.

Table of Contents

Declaration i
Summary ii
Table of Contents v
List of Tables xi
List of Figures xii
Acknowledgements xv
Publications arising from this thesis xvii
Statement of work xx
List of Abbreviations xxi
CHAPTER 1: Introduction 1
1.1 Obesity Overview 2
1.1.1 Obesity in children and adolescents 2
1.1.2 Defining obesity in children and adolescents 2
1.1.3 Obesity in Ireland 3
1.1.4 Complications of Obesity 4
1.1.5 Management of childhood obesity 6
1.2 The immune system and inflammation in obesity 6
1.2.1 The immune system 6
1.2.2 Inflammation 9
1.2.3 Obesity and inflammation. Chronic inflammation and immune cell dysregulation in childhood obesity 9
1.3 Second Generation Antipsychotics 13
1.3.1 Overview of Second Generation Antipsychotic medication 13
1.3.2 SGAs and weight gain 14
1.3.3 Factors influencing the risk of SGA-related weight gain 15
1.3.4 Implications of SGA-related weight gain 17
1.3.5 Potential mechanisms for SGA-related weight gain 18
1.3.6 Other complications of SGAs 22
1.4 SGAs and inflammation 22
1.4.1 Inflammation in psychosis and ASD 22
1.4.2 Th1 to Th2 shift theory in psychosis 23
1.4.3 SGAs as both anti-inflammatory and pro-inflammatory agents 24
1.5 Management of SGA related weight gain 25
1.5.1 Monitoring of children and adolescents who are prescribed SGAs 25

1.5.2 Prevention of SGA related weight gain 27
1.5.3 Management options for SGA-related weight gain 27
1.6 Outstanding issues, aims and hypotheses of this thesis 29
1.6.1 Outstanding issues 29
1.6.2 Aims 29
1.6.3 Hypotheses 30
CHAPTER 2: Materials and Methods 31
2.1 Ethical Approval 32
2.2 Study Design 32
2.3 Participant Recruitment 33
2.3.1 SGA group participants 33
2.3.2 Control Participants 34
2.4 Participant interview 34
2.5 Clinical examination: weight, BMI, and metabolic parameters 34
2.6 Metabolic Profiling 34
2.7 Clinical analyses in study 35
2.8 Blood Sampling for Immune Research Work 35
2.8.1 Serum Preparation 36
2.8.2 Peripheral Blood Mononuclear Cell Isolation 36
2.8.3 Cell enumeration 37
2.9 Activation of Immune Cells for Cytokine Production 38
2.10 Harvesting of supernatants after 24 hours 38
2.11 Cryopreservation of peripheral blood mononuclear cells 38
2.12 Thawing of frozen PBMC samples 38
2.13 Setting up cell culture plate for intracellular and extracellular flow cytometry experiment 39
2.14 Immunophenotyping by Flow Cytometry 39
2.14.1 Introduction to Flow Cytometry 39
2.14.2 Extracellular staining 42
2.14.3 Fluorescence Minus One (FMO) Controls 43
2.14.4 Intracellular staining 44
2.14.5 Compensation staining 45
2.14.6 Flow Cytometry Analysis 45
2.15 Enzyme Linked Immunosorbent Assay (ELISA) 45
2.15.1 Introduction to ELISA 45

2.15.2 Leptin and Soluble CD163 Quantification by Enzyme Linked Immunosorbent Assay (ELISA) in serum 46
2.15.3 IL-10, IL-17A, IL-17F and TNF-α Quantification in Cell Culture Supernatants by Enzyme Linked Immunosorbent Assay (ELISA) 48
2.15.4 TNF-α and IL-17 Quantification in serum by High Sensitivity Enzyme Linked Immunosorbent Assay (ELISA) 48
2.16 Method for investigating the effect of dopamine and risperidone on the production of TNF-α and IL17F by PBMCs 49
2.17 Methods for the in-vitro experiments 49
2.18 Statistical Analysis 50
CHAPTER 3: Clinical and metabolic characteristics of the SGA cohort at baseline and during follow up over a 12-month period 53
3.1 Introduction 54
3.1.1 Global increase in SGA prescribing 54
3.1.2 SGA prescribing in Ireland 55
3.1.3 An audit of SGA prescribing in a specialist ASD service 57
3.1.4 Clinical implications of SGA medication 60
3.2 Specific aims of this chapter 61
3.3 Results 62
3.3.1 Study Cohort 62
3.3.2 Study Cohort: Participant Demographics 63
3.3.3 Study cohort: SGAs prescribed 65
3.3.4 Weight gain during treatment with SGAs 66
3.3.5 Lower BMI percentile at baseline resulted in more weight gain 68
3.3.6 Lipid profile during treatment with SGAs 70
3.3.7 Glucose regulation at baseline and on SGAs 72
3.3.8 The influence of medication type, weight gain and BMI at baseline on changes in lipid profile and glucose regulation 74
3.3.9 Correlation between ΔHDL-cholesterol/ΔTriglycerides and ΔHDL-cholesterol/Δweight gain 81
3.3.10 Correlation between ΔBMI/ΔHDL-cholesterol, ΔBMI/ΔLDL-cholesterol, ΔBMI/Δtriglycerides and ΔBMI/Δfasting glucose at 3 months, 6 months and 12 months 83
3.3.11 Effect of SGAs on prolactin 88
3.4 Discussion 90
CHAPTER 4: Investigating the effect of SGAs on immunophenotype 99
4.1 Introduction 100

4.1.1 Obesity and inflammation 100
4.1.2 SGA medication and inflammation 101
4.1.3 Innate immune cell populations that are important in obesity: monocytes, NK cells 102
4.1.3.1 Monocytes 102
4.1.3.2 Natural Killer Cells 102
4.1.4 Adaptive immune cell populations of relevance in obesity: B cells and T cells 103
4.1.4.1 B cells 103
4.1.4.2 T cells 103
4.1.5 Other immune cell populations: MAIT cells, iNKT cells 104
4.1.5.1 MAIT cells 104
4.1.5.2 Invariant Natural Killer T cells 105
4.1.6 Cytokines in the context of obesity 105
4.1.6.1 TNF-α 105
4.1.6.2 IL-1 106
4.1.6.3 IL-17 106
4.1.7 Other signalling molecules of relevance in obesity 106
4.1.7.1 Soluble CD163 106
4.1.7.2 Leptin 107
4.2 Specific aims for this chapter 108
4.3 Results 109
4.3.1 Systemic inflammatory cytokine and adipokine profiling: Leptin Quantification 109
4.3.2 Systemic inflammatory cytokine and adipokine profiling: Soluble CD163 Quantification 112
4.3.3 Systemic inflammatory cytokine and adipokine profiling: Serum IL-17 Quantification 114
4.3.4 Systemic inflammatory cytokine and adipokine profiling: Serum TNF-α Quantification 116
4.3.5 Immunophenotyping: T cell identification 118
4.3.6 T cell profiling before and after treatment with SGA medication 118
4.3.7 Immunophenotyping: MAIT cell identification 121
4.3.8 MAIT Cell profiling before and after treatment with SGA medication 121
4.3.9 Immunophenotyping: Invariant Natural Killer T cell identification ... 124

4.3.10 Invariant Natural Killer T cell profiling before and after treatment with SGA medication 124
4.3.11 Immunophenotyping: Natural Killer cell identification 126
4.3.12 Natural Killer cell profiling before and after treatment with SGA medication 126
4.3.13 Immunophenotyping: Monocyte identification 129
4.3.14 Monocyte frequencies before and after treatment with SGA medication 129
4.4 Discussion 131
CHAPTER 5: Investigating the effects of SGAs on immune cell responses 135
5.1 Introduction 136
5.1.1 Cytokine producing cells and cytokines investigated in this study 136
5.1.2 CD4+ T cells 136
5.1.3 γδ T cells 137
5.1.4 Cytokines 137
5.1.4.1 TNF-α 137
5.1.4.2 IL-10 138
5.1.4.3 IL-17A and IL-17F 138
5.1.5 Dopamine and its effects on inflammation 138
5.2 Specific aims for this chapter 140
5.3 Results 141
5.3.1 The pro-inflammatory cytokine TNF-α increases early on following treatment with SGA medication 141
5.3.2 Dopamine reduces the production of TNF-α by THP-1 cells in vitro 143
5.3.3 IL-10 increases on SGA treatment 145
5.3.4 The production of IL-17A by PBMCs shows an increasing trend on SGA medication 147
5.3.5 The production of IL-17F by PBMCs shows an increasing trend on SGA medication 149
5.3.6 Intracellular immunophenotyping at baseline, 3 months and 6 months in the SGA cohort 152
5.3.7 MAIT cell identification 152
5.3.8 MAIT cell frequencies are increased with SGA treatment and are a source of IL-17 and TNF-α 154
5.3.9 Dopamine causes a reduction in TNF-α production from MAIT cells but does not affect IL-17 production 154
5.3.10 CD4 T Cell identification 157

5.3.11 CD4 T cell frequencies and their production of IL-17 and TNF-α do not change with SGA medication 159
5.3.12 Dopamine does not affect IL-17 or TNF-α production from CD4 T cells 159
5.3.13 Gamma Delta (γδ) cell identification 162
5.3.14 γδ cell frequencies are unchanged with SGA treatment and SGA treatment does not affect IL-17 and TNF-α production by γδ cells 164
5.3.15 Dopamine does not affect IL-17 or TNF production from γδ cells 164
5.4 Discussion 167
CHAPTER 6: Discussion 171
6.1 General Discussion 172
6.1.1 Main results and significance of Study 1 173
6.1.2 Main results and significance of Study 2 175
6.1.3 Main results and significance of Study 3 176
6.2 Strengths of this research work 179
6.3 Study Limitations 180
6.4 The impact of the COVID-19 pandemic on the study 181
6.5 The impact of the study 181
6.6 Future Directions 182
6.7 Conclusion 185
CHAPTER 7: Bibliography 187
APPENDICES 217
Appendix 1 Parent Information Leaflet 218
Appendix 2 Parent Consent Form 226
Appendix 3 Child Information Leaflets broken down by age range 230
Appendix 4 Child Assent Form 241
Appendix 5 Child Information Leaflet and Assent Form (picture based) 242
Appendix 6 Study Assessment Form 247

List of Tables

Table 1.1: Cut-off points for childhood overweight and obesity 3
Table 2.1: Flow cytometry antibodies used in this project 40
Table 2.2: Extracellular flow cytometry staining panel 43
Table 2.3: Intracellular flow cytometry staining panel 44
Table 2.4: Summary of Media for Cultured Cells 50
Table 2.5: ELISA Kits 51
Table 2.6: Antibodies used for Flow Cytometry 51
Table 3.1: Age range and gender of participants recruited to the study (N = 19) 64
Table 3.2: Table depicting number of follow up visits completed by participants 64
Table 3.3: Table depicting age range, gender, and mean BMI of control group 64
Table 3.4: SGA medication prescribed 65
Table 3.5: Reason for commencing SGA medication 65

List of Figures

Figure 1.1: The short and long-term complications of childhood obesity 5
Figure 1.2: Cells of the innate and adaptive immune system 8
Figure 1.3: The relationship between obesity, chronic inflammation and disease 10
Figure 1.4: The changes in adipose tissue from lean to obese..... 12
Figure 1.5: Mechanisms of SGA-related weight gain..... 21
Figure 2.1: Study Overview 32
Figure 2.2: Peripheral Blood Mononuclear Cell Isolation 37
Figure 2.3: Overview of flow cytometry workflow..... 40
Figure 2.4: Overview of ELISA Assay..... 46
Figure 3.1: Pilot data from a specialist ASD in Dublin/Kildare/Wicklow demonstrating overweight and lipid abnormalities in children with ASD who were on SGA medication 59
Figure 3.2: Flow chart of study participants 63
Figure 3.3: BMI percentile pre- and post-treatment with SGA medications 67
Figure 3.4: The effect of baseline BMI and medication type on weight gain..... 69
Figure 3.5: Lipid profiles pre- and post-treatment with SGA medications 71
Figure 3.6: Glucose regulation pre- and post-treatment with SGA medications 73
Figure 3.7: The effect of medication type on lipid profile 75
Figure 3.8: The effect of medication type on glucose regulation..... 76
Figure 3.9: The effect of weight gain on lipid profile 77
Figure 3.10: The effect of weight gain on glucose regulation..... 78
Figure 3.11: The effect of BMI at baseline on lipid profile..... 79
Figure 3.12: The effect of BMI at baseline on glucose regulation 80
Figure 3.13: Correlation between ΔHDL-cholesterol/Triglycerides and ΔHDL-cholesterol /Δweight 82
Figure 3.14: Correlation between ΔHDL and ΔBMI at 3 months, 6 months and 12 months..... 84
Figure 3.15: Correlation between ΔLDL and ΔBMI at 3 months, 6 months and 12 months..... 85
Figure 3.16: Correlation between ΔTriglycerides and ΔBMI at 3 months, 6 months and 12 months 86

Figure 3.17: Correlation between Δfasting glucose and ΔBMI at 3 months, 6 months and 12 months 87
Figure 3.18: Prolactin levels pre- and post-treatment with SGA medications ... 89
Figure 4.1: Leptin levels pre- and post-treatment with SGA medications 110
Figure 4.2: Correlation between Δleptin and Δweight/ΔBMI 111
Figure 4.3: Soluble CD163 Levels pre- and post-treatment with SGA medications 113
Figure 4.4: IL-17 Levels pre- and post-treatment with SGA medications 115
Figure 4.5: TNF-α levels pre- and post-treatment with SGA medications 117
Figure 4.6: CD8 and CD4 T Cell Frequencies pre- and post-treatment with SGA medications 119
Figure 4.7: CD45RA and CD45RO T Cell Frequencies pre- and post-treatment with SGA medications 120
Figure 4.8: MAIT Cell frequencies pre- and post-treatment with SGA medications 122
Figure 4.9: ΔMAIT cell frequency and metabolic parameters 123
Figure 4.10: Invariant Natural Killer T Cell frequencies pre- and post-treatment with SGA medications 125
Figure 4.11: Natural Killer Cell frequencies pre- and post-treatment with SGA medications 127
Figure 4.12: ΔNK cell frequency and metabolic parameters 128
Figure 4.13: Monocyte Cell frequencies pre- and post-treatment with SGA medications 130
Figure 5.1: TNF-α levels in cell culture supernatants pre- and post-treatment with SGA medications 142
Figure 5.2: TNF-α levels in cell culture supernatants of THP-1 cells 144
Figure 5.3: IL-10 levels in cell culture supernatants pre- and post-treatment with SGA medications 146
Figure 5.4: IL-17A levels in cell culture supernatants pre- and post-treatment with SGA medications 148
Figure 5.5: IL-17F levels in cell culture supernatants pre- and post-treatment with SGA medications 151
Figure 5.6: MAIT cell identification 153
Figure 5.7: Cytokine production by MAIT Cells 155

Figure 5.8: Cytokine production by MAIT cells stimulated with dopamine 156
Figure 5.9: CD4 T cell identification 158
Figure 5.10: Cytokine production by CD4 T Cells 160
Figure 5.11: Cytokine production by CD4 T Cells stimulated with dopamine .. 161
Figure 5.12: γδ T cell identification 163
Figure 5.13: Cytokine production by γδ T Cells 165
Figure 5.14: Cytokine production by γδ T Cells stimulated with dopamine 166

Acknowledgements

I would like to thank my supervisors Prof Louise Gallagher, Dr Jane McGrath and Dr Andrew Hogan. Louise, you have been an incredible mentor to me since you welcomed me into your research group as a Senior Registrar in 2015. Thank you for thinking of me when the opportunity for the study came about and for all your advice, knowledge, and input. Jane, I have looked up to you since I first worked with you while I was a Registrar on my first CAP placement, and you were the Senior Registrar. I like to think I am following in your footsteps! Thank you for all of your help, support and advice during this project, I couldn't have done it without you. Andy, it has been a pleasure to work with you. Thank you for your supervision and all the guidance over the last 3 years.

I would also like to thank Prof Donal O'Shea for his guidance and valuable insights during the project.

This project would not have been possible without the funding from the NCRC and I would like to sincerely thank the organisation for this.

Thank you to my colleagues in the Obesity Immunology research group who helped me in so many ways with training and support during the project. Thank you to Nicole Wood who trained me in on everything in the lab. She was so enthusiastic and really helped me settle into the lab work, I couldn't have done it without her. Also, Marta Pisarska, thank you for being on the other end of the phone to give me advice on setting up experiments and for all your help while I was doing the project and writing this thesis. Thanks to Eadaoin Hayes for all your help when working alongside me.

Thank you to my colleagues in the Autism Research group for all their support and insights during team meetings. Also, thank you to Nadia for keeping us all in line and for all your help and support since I have been working with the group. Thank you to everyone in the group for bringing the fun to my time in research. Thanks in particular to Sarah, Ciara and Claire. I will miss you all so much!

Thank you to everyone in the NCRC who were always there for a chat, usually over tea and cake! A particular thanks to Gemma, Kiva, Mevlut and Caroline who were always there to help when the Fortessa was acting up!

Thank you to all Linn Dara staff who contacted me with potential participants for the study. Thank you to the phlebotomy staff in Crumlin and Tallaght hospital for their help with collecting the blood samples.

I would like to extend my deepest gratitude to all the young people who participated in the study and to their parents. Without your help this study would not have been possible.

Thank you to everyone who I met during this project, who offered their help, guidance and support in many ways.

Thank you to my friends who were so supportive and understanding over the last few years. I am so lucky to know you all.

Thank you to my parents and brothers for your support and encouragement during my time in research and in everything that I do.

Thank you so much to my husband Brian for your support with my PhD and with everything else I have achieved since we met back when I was in second year of medical school. I most definitely would not have been able to do this without your support and encouragement and I will always be grateful to you for your belief in me. You encouraged me to take on this challenge and had to make sacrifices for it to happen. You always encouraged me to keep going when I felt I would never get there. Thank you for everything! Thank you to our two boys, Liam and Finn, who had to put up with me spending weekends working on this thesis for many months. Thank you for being supportive and your excitement and happiness for me when I finished was amazing. It was lovely to have a new addition to our family after I finished the project. Thank you to baby Áine for the company while I was working on the thesis corrections!

Publications arising from this thesis

Antipsychotic prescribing in GMS paediatric and young adult population in Ireland 2005-2015: repeated cross-sectional study

K Conlan, J McGrath, M Teeling, MJ MacAvin, K Bennett, L Gallagher

Published online in the *Irish Journal of Psychological Medicine, March 2021*.

Manuscripts to be submitted/in preparation

Second Generation Antipsychotics, weight gain and cardiometabolic complications. A narrative literature review

K Conlan, A.E. Hogan, D O'Shea, L Gallagher, J McGrath

To be submitted to the *Irish Journal of Psychological Medicine*

Mucosal Associated Invariant T-cells increase with weight gain

K Conlan, A.E. Hogan, D. O'Shea, L Gallagher, J McGrath

To be submitted to *Immunohorizons*

Second Generation Antipsychotics cause the development of an inflammatory phenotype: A 12 month longitudinal study

K Conlan, M Pisarska, N Wood, E Hayes, L Gallagher, J McGrath, D O'Shea, A.E. Hogan

To be submitted to *European Child and Adolescent Psychiatry*

Presentations National

Second-Generation Antipsychotics: weight gain and cardiometabolic complications – do different factors impact weight gain and cardiometabolic complications?

K Conlan, A.E. Hogan, D O'Shea, L Gallagher, J McGrath. *College of Psychiatrists of Ireland Winter Conference November 2022.* Poster Presentation

Antipsychotics cause the development of an inflammatory phenotype – A 12-month longitudinal study of children and adolescents prescribed Antipsychotic medications

K Conlan. *The RAMI Psychiatry Section and the Faculty of Academic Psychiatry of the College of Psychiatrists of Ireland HST Competition. December 2021.* Oral Presentation. Received commendation from the judges.

Is antipsychotic related weight gain in children and adolescents associated with inflammation?

K Conlan, A.E. Hogan, D O'Shea, L Gallagher, J McGrath. *College of Psychiatrists of Ireland Winter Conference November 2020 (Virtual).* Oral Presentation

Inflammatory markers in children who are prescribed antipsychotics – A 12 month follow up study

K Conlan, A.E. Hogan, D O'Shea, L Gallagher, J McGrath. *College of Psychiatrists of Ireland Winter Conference November 2019.* Poster Presentation

Inflammatory markers of antipsychotic weight gain and cardiometabolic dysfunction in youth mental health disorders

K Conlan, N Wood, A.E. Hogan, D O'Shea, L Gallagher, J McGrath. *College of Psychiatrists of Ireland Spring Conference April 2019.* Poster Presentation

Audit investigating adherence to Maudsley Prescribing Guidelines in a specialist Autism Spectrum Disorder service

K Conlan, L Gallagher, J McGrath. *College of Psychiatrists of Ireland Spring Conference April 2018.* Poster Presentation

Presentations International

Antipsychotics cause the development of an inflammatory phenotype – A 12-month longitudinal study of children and adolescents prescribed Antipsychotic medications

K Conlan. (Presented by Prof L Gallagher) *European College of Neuropsychopharmacology 34th Congress, Lisbon, October 2021.*

Antipsychotics cause the development of an inflammatory phenotype – A 12-month longitudinal study of children and adolescents prescribed Antipsychotic medications

K Conlan. *ECNP Workshop for Early Career Scientists in Europe. March 2021.* Oral Presentation

Chosen as a winner during this workshop and invited to present at the *European College of Neuropsychopharmacology 34th Congress, Lisbon, October 2021.*

Inflammatory markers of antipsychotic weight gain – a longitudinal study

K Conlan, A.E. Hogan, D O'Shea, L Gallagher, J McGrath. *European College of Neuropsychopharmacology 33rd Congress, Vienna September 2020.* Interactive Poster Presentation

Inflammatory markers of antipsychotic weight gain and cardiometabolic dysfunction in youth mental health disorders

K Conlan, E Hayes, A.E. Hogan, D O'Shea, L Gallagher, J McGrath. *Europaediatrics, Dublin, June 2019.* Poster Presentation

Pre-Registration Poster: Inflammatory markers of antipsychotic weight gain and cardiometabolic dysfunction in youth mental health disorders

K Conlan, N Wood, A.E. Hogan, D O'Shea, L Gallagher, J McGrath. *British Neuroscience Association April 2019.* Oral presentation and poster presentation

Statement of work

Dr Karen Conlan:

- Developed information leaflets and consent form for parents
- Developed age appropriate information leaflets and assent forms for participants
- Developed picture based information leaflet and assent form for participants with Intellectual Disability
- Obtained ethical approval from all Research Ethics Committees for all clinical sites involved in the study
- Developed clinical project design and protocols under the supervision of Prof Louise Gallagher and Prof Jane McGrath
- Developed laboratory project design and protocols under the supervision of Dr Andrew Hogan in the Obesity Immunology Research Group
- Was trained in all lab techniques in the study by members of the Obesity Immunology Research Group
- Recruited all participants to the study
- Followed up participants at each time point of study including clinical parameters
- Transported blood samples from paediatric hospitals to the NCRC lab
- Processed all blood samples: obtaining serum, isolation of PBMCs, cell culture and flow cytometry staining for all samples
- Performed in-vitro, ELISA and flow cytometry experiments described in thesis
- Analysed all the data presented in the thesis
- Wrote the thesis manuscript with amendments and alterations from supervisors

List of Abbreviations

α1	α adrenergic receptor
AAP	American Academy of Paediatrics
ADA	American Diabetes Association
ADHD	Attention Deficit Hyperactivity Disorder
ANOVA	Analysis of Variance
ASD	Autism Spectrum Disorder
BMI	Body Mass Index
BSA	Bovine Serum Albumin
CAMESA	Canadian Alliance for Monitoring Effectiveness and Safety of Antipsychotics
CDC	Centre for Disease Control and Prevention
CAMHS	Child and Adolescent Mental Health Services
CRP	C-reactive Protein
CRPMI	Complete Roswell Park Memorial Institute media
CVD	cardiovascular disease
DMSO	Dimethyl Sulfoxide
EGF	Epidermal Growth Factor
ELISA	Enzyme Linked Immunosorbent Assay
EOP	Early Onset Psychosis
EPSE	Extra Pyramidal Side Effect
FACS	Fluorescence Activated Cell Sorting
FBS	Fetal Bovine Serum
FDA	U.S. Food and Drug Administration
FGA	First Generation Antipsychotic

FMO	Fluorescence minus one
γδ T cell	Gamma Delta T cell
GLP-1	Glucagon Like Peptide-1
H1	Histamine receptor
HDL	High density lipoprotein
HEPES	4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid
HOMA-IR	Homeostasis model assessment for insulin resistance
HRP	Streptavidin horseradish peroxidase
5HT2	Serotonin receptor
IL-	Interleukin
IFN-γ	Interferon-gamma
iNKT cell	Invariant natural killer T-cell
IOTF	International Obesity Task Force
LDL	Low density lipoprotein
LPS	Lipopolysaccharide
M1	Macrophages classically activated
M2	Macrophages alternatively activated
MAIT cell	Mucosal associated invariant T cell
MHC	Major histocompatibility complex
NICE	National Institute for Health and Care Excellence
NK cell	Natural Killer cell
PAMP	Pathogen Associated Molecular Pattern
PBMC	Peripheral blood mononuclear cell
PBA	PBS supplemented with 10% (v/v) bovine serum albumin (BSA)

PBS	Phosphate buffered saline
PFA	Paraformaldehyde
PMA	Phorbol 12-myristate 13-acetate
PRR	Pattern Recognition Receptor
RPM	Revolutions Per Minute
sCD163	Soluble CD 163
SGA	Second Generation Antipsychotic
sIL-2R	Soluble Interleukin-2 receptor
SSRI	Selective Serotonin Reuptake Inhibitor
TCR	T cell receptor
TGF-β	Transforming Growth Factor beta
Th1	T helper 1
Th2	T helper 2
Th17	T helper 17
TMB	Tetramethylbenzidine
TNF-α	Tumour Necrosis Factor alpha
T1DM	Type 1 Diabetes Mellitus
T2DM	Type 2 Diabetes Mellitus
T4	Thyroid hormone
VEGF	Vascular Endothelial Growth Factor
WAT	White adipose tissue
WHO	World Health Organization

CHAPTER 1: Introduction

1.1 Obesity Overview

1.1.1 Obesity in children and adolescents

“Globesity” is the term used to describe the current global epidemic of obesity. In 2015 the World Health Organisation estimated that a staggering 41 million children under the age of 5 were overweight (1). Obesity is a complex disease that is currently a global health concern and a major challenge for health services. It poses a significant threat to physical and psychological wellbeing and is the most common preventable case of premature death.

Childhood obesity is causally implicated in serious and persistent negative health outcomes including adult obesity and a range of serious metabolic comorbidities and malignancies (2-7). Obesity has a complex multi-factorial aetiology that includes genetic predisposition, diet, sedentary behaviours, causal medical conditions, socio-economic factors and use of drugs that influence appetite (8, 9).

1.1.2 Defining obesity in children and adolescents

Anthropometric measures are used to calculate the Body Mass Index (BMI) and BMI percentile and BMI z-scores are used to assess and measure childhood obesity. BMI reference charts that have been developed by the World Health Organization (WHO) and Centre for Disease Control and Prevention (CDC) enable the identification of healthy weight, overweight and obesity in children and adolescents (10). Table 1.1 lists the proposed cut offs of childhood obesity. The definitions of childhood obesity depicted in the table show variances which may impact on research. The CDC, WHO and IOTF cut off points have been found to produce similar estimates of overall overweight prevalence but different estimates for obesity (11, 12).

Table 1.1: Cut-off points for childhood overweight and obesity

	Overweight	**Obesity**
Centre for Disease Control and Prevention (CDC)	≥85th-95th centile for BMI	>95th centile for BMI
World Health Organization (WHO)	BMI ≥ 1 SD above the WHO growth standard median	BMI ≥ 2 SDs above the WHO growth standard median
International Obesity Task Force (IOTF)	Age and sex appropriate BMI in childhood corresponds to cut off 25kg/m^2 in adults	Age and sex appropriate BMI in childhood corresponds to cut off 30kg/m^2 in adults

1.1.3 Obesity in Ireland

Obesity is a significant problem in Ireland. At present 2/3 of adults in Ireland are overweight or obese and the estimated costs of obesity in Ireland is €1.13 billion annually (13). For the first time in two centuries, there may be a decline in life expectancy due to the negative effects of obesity on longevity (14).

Almost 1 in 4 Irish children are currently either overweight or obese (15-17) and the WHO has predicted that Ireland will have the leading global rate of childhood obesity by 2030 (unpublished WHO data).

A study using data obtained from the Growing Up in Ireland study, a national longitudinal study of children in Ireland, examined the prevalence of obesity in a sample of 8,568 9-year old children (15). The study found that 19% of 9-year olds were overweight and 7% were obese, and of greater concern, that parents do not recognise when their child is overweight or obese and that children have less ability at judging weight status than their parents. 54% of parents of overweight children and 20% of parents of obese children reported that they judged that their child is about the right weight. In Ireland, the prevalence of childhood obesity has

doubled in the past two decades. This has resulted in the implementation of a National Task Force on Obesity, which was tasked with developing a strategy to stop the increase and reduce the prevalence of obesity in Ireland (18). The first report of the National Task Force stated, “the evidence is very clear that preventing children from becoming overweight and obese is the best approach for long-term sustainable change in Ireland’s population obesity profile” (18).

1.1.4 Complications of Obesity

Obesity is a driver of many co-morbidities that have short and long-term adverse health outcomes, as depicted in Figure 1.1. Children that are overweight or obese are likely to be overweight or obese as adults (5, 19). Obesity that occurs in childhood results in a longer duration of obesity and consequently a higher duration of morbidity compared to individuals who become obese as adults (5, 19). The complications associated with obesity include hypertension, dyslipidaemia, cardiovascular disease, insulin resistance and Type 2 Diabetes Mellitus (T2DM) (2, 20). People with consistently high BMI from childhood to adulthood have a higher risk of T2DM, hypertension, elevated low-density lipoprotein levels, reduced high-density lipoprotein levels, and carotid artery atherosclerosis when compared with individuals who were not overweight or obese as children or adults (2). Other complications include non-alcoholic fatty liver disease and obstructive sleep apnoea and an increased risk of autoimmune disorders such as rheumatoid arthritis (21-23). As the liver is important in regulating glucose and lipid metabolism, non-alcoholic fatty liver disease is associated with cardiometabolic complications such as insulin resistance, hyperglycaemia and dyslipidaemia (24). There are many studies that suggest that people who are obese are at an increased risk of developing malignancies including oesophageal adenocarcinoma and cancers of the pancreas, breast, colon, endometrium, kidney, and gallbladder (25, 26).

Obesity is associated with a chronic inflammatory response. This may contribute to the development of obesity related metabolic disorders including the metabolic syndrome and insulin resistance (27). The ‘metabolic syndrome’ refers to a cluster of risk factors that increase a person’s vulnerability to develop T2DM and

cardiovascular disease. The criteria include central adiposity accompanied by at least two other symptoms including high blood pressure, high triglyceride level, low High Density Lipoprotein (HDL) cholesterol level or high fasting glucose (28, 29).

In parallel with the negative impact from an increased burden of chronic physical illness, a systematic review found that there is an increase in the burden of mental illness including depression and anxiety as well as negative impacts on self-esteem (30-32).

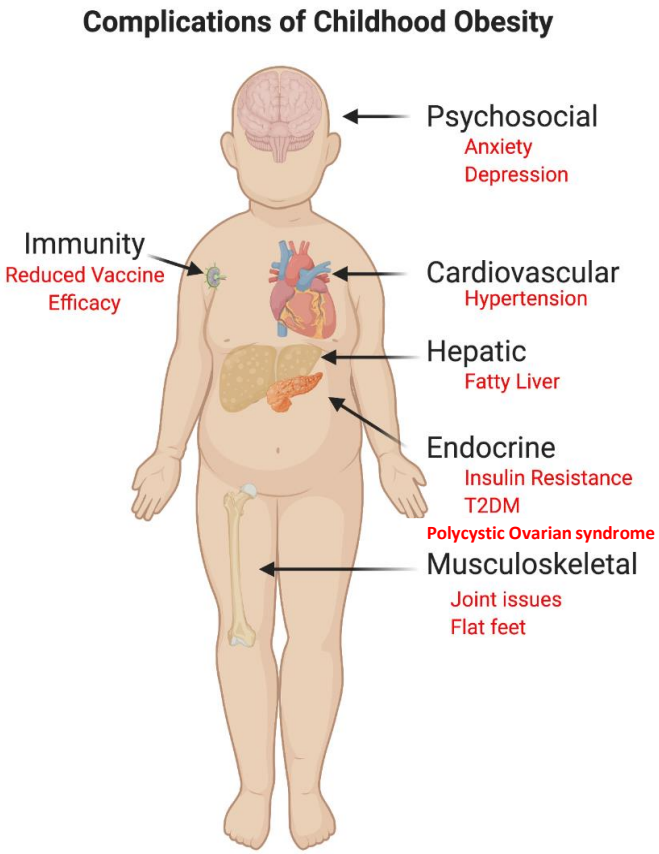


Figure 1.1: The short and long-term complications of childhood obesity

Representative image of the co-morbidities associated with obesity. (Image created in Biorender.com)

1.1.5 Management of childhood obesity

Due to the high global burden of obesity, there is a need for prevention strategies. However, the current evidence for effective economic policies specifically for obesity prevention is limited (33, 34). Treatment for obesity is indicated when prevention does not work (33). Diet and lifestyle interventions are the first line of treatment but, in general, compliance is poor (33). There are a growing number of pharmacological options. Metformin, an oral hypoglycaemic agent, has been shown to reduce BMI and improve the metabolic complications of obesity (35). Glucagon-like peptide-1 (GLP-1) is a gut peptide that is released after a meal which causes a lowering of blood glucose by suppressing glucagon release and increasing insulin sensitivity (36). The GLP-1 analogue, liraglutide, is used as a therapeutic treatment for the improvement of T2DM (37). It was recently approved by the FDA for use in children with T2DM (38). GLP-1 analogues have been shown to reduce inflammation by reducing the activation of inflammatory macrophages (36, 39). A recent randomized controlled trial in adults (n=1,961) assigned participants to 68 weeks of once weekly treatment with semaglutide (a GLP-1 analogue) or placebo, plus lifestyle intervention. The trial found that semaglutide caused a 14.9% reduction in body weight in the treatment group compared to a 2.4% reduction in body weight in the placebo group from baseline to the end of the study with an estimated treatment difference of -12.4 percentage points (40).

1.2 The immune system and inflammation in obesity

1.2.1 The immune system

The immune system refers to the different cells, chemicals, proteins and processes that protect the human body from danger and foreign invaders such as bacteria and viruses (41). The immune system is made up of two distinct but interlinked branches or lines of defence, the innate and adaptive immune systems. The innate immune response is the first line of defence against a pathogen. It is generally considered a non-specific line of defence that leads to a rapid response to tissue injury or infection (42). The innate immune system classically does not have any immunologic memory (41). Emerging evidence

however does support memory like responses by innate immune cells (43, 44). The main cells of the innate immune system are monocytes, dendritic cells, macrophages and natural killer (NK) cells (42, 45). These cells can target and kill foreign bodies (42). The cells of the innate immune system use pattern recognition receptors (PRRs) which allow innate immune cells to detect and respond to a range of pathogens by recognizing structures called pathogen associated molecular patterns (PAMPs), examples of which are lipopolysaccharide (LPS) which is found on the cell wall of gram negative bacteria, and RNA produced during viral infection (45, 46). Activation of the innate immune system leads to the rapid recruitment of immune cells to the site of infection or inflammation. This is mediated through the production of cytokines and chemokines, which are small proteins involved in cell to cell communication and activating cell responses to injury and infection (42, 47). The main inflammatory cytokines released during the initial stages of the innate immune response include Tumour Necrosis Factor-α (TNF-α), Interleukin-1 (IL-1) and Interleukin-6 (IL-6), which cause the recruitment of immune cells to the site of infection.

The second branch of the immune system is the adaptive immune system. Whilst the innate immune system controls the spread of the invading pathogen it also plays a role in activating the adaptive immune response by contributing to activation of antigen-specific cells (42, 45). Adaptive immune responses occur primarily due to antigen-specific receptors expressed on the surfaces of the cells of the adaptive immune system (47). The cells of the adaptive immune system are lymphocytes, of which there are two types: T cells and B cells. The main function of the adaptive immune response is to distinguish “non-self” from “self” antigens (41). T cells have highly specialised antigen receptors on their cell surface which allows them to recognize and respond to individual antigens (42, 48). Each T cell expresses a T-Cell Receptor (TCR) and can proliferate and differentiate depending on the signal it receives. Antigen presenting cells such as dendritic cells are required for T cells to recognize a specific antigen. Upon TCR stimulation, T cells can respond by proliferating, secreting cytokines and migrating to the site of infection (42). Adaptive T cells can take the form of two major subsets; cytotoxic cells (CD8+ cells), which are involved in the destruction

of cells or T-helper cells (CD4+ cells), which help to maximise the immune response (42). B cells are the second type of lymphocyte in the adaptive immune system. When they are activated, they can differentiate into either memory cells or antibody producing plasma cells (42, 49). Memory cells allow the body to produce a more rapid and specific response if a pathogen is encountered again in the future. Plasma cells produce antibodies which can recognize and eliminate specific pathogens. There are also T lymphocyte subsets which express both innate and adaptive properties, termed innate T cells, such as Mucosal Associated Invariant T Cells (MAIT cells), invariant Natural Killer T cells (iNKT cells) and Gamma Delta T cells (γδ T cells) (50). These innate T cells function as a link between the innate and adaptive immune system (49). These cells can become activated in an antigen dependent or independent manner, thus bridging the gap between the innate and adaptive immune system.

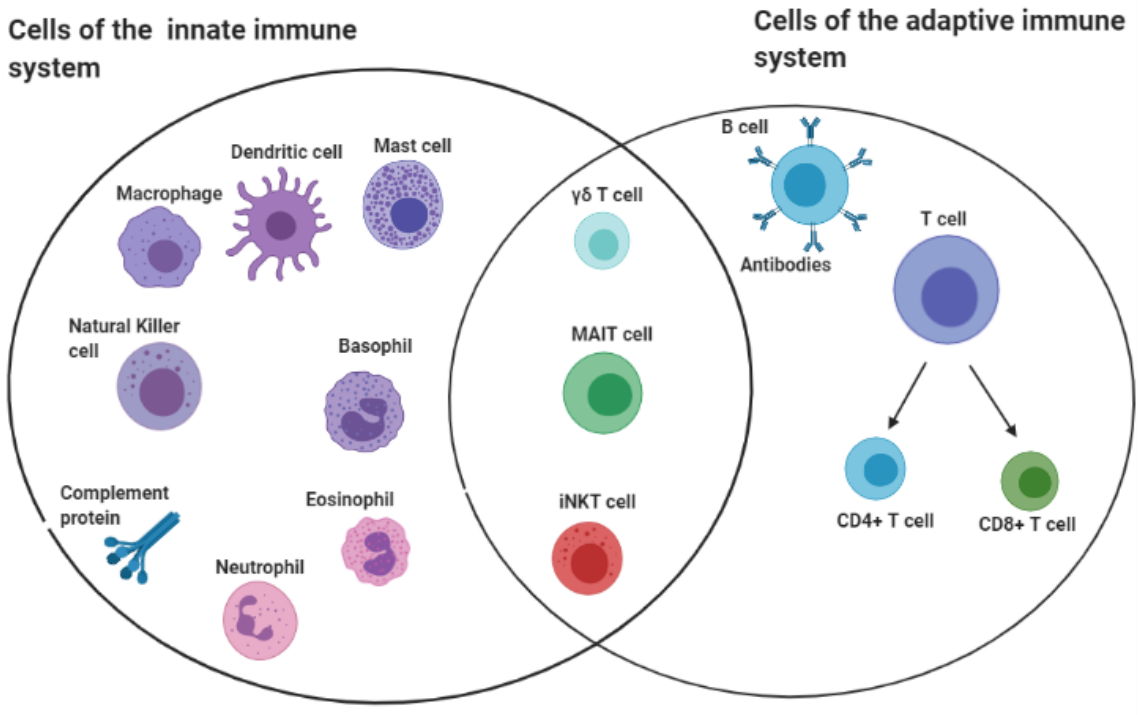


Figure 1.2: Cells of the innate and adaptive immune system

Cells of the immune system are split into innate and adaptive and cells that link both the innate and adaptive immune systems. Adapted from Dranoff 2004. (Image created in Biorender.com)

1.2.2 Inflammation

Inflammation is a fundamental physiological process in which the immune system responds to infection or tissue injury (9). It is a central component of innate immunity in which the body responds to infection or tissue injury which is triggered by interactions with PAMPS and cell surface receptors. This leads to the synthesis of immune mediators such as cytokines and chemokines which induce inflammation (42).

Acute inflammation typically subsides once the harmful stimuli have been eliminated (51, 52). Anti-inflammatory cytokines such as IL-10 and Transforming Growth Factor -β (TGF-β) are involved in the resolution of inflammation (51). Chronic inflammation arises when the inflammatory response fails to resolve and results in low-grade inflammation that is dysregulated (9). This form of inflammation is a driver for the development of many autoimmune disorders, cancers and metabolic diseases such as obesity, insulin resistance and T2DM (52, 53).

1.2.3 Obesity and inflammation. Chronic inflammation and immune cell dysregulation in childhood obesity

Obesity is associated with a state of chronic low-grade inflammation (54, 55). One mechanism for origin begins with adipocyte (adipose tissue cell) expansion caused by overnutrition. Enlarged adipocytes become necrotic due to oxygen and nutrients being unable to reach the inside of the cell (53). When adipocytes become necrotic the contents of the cell are released which includes free fatty acids and lipids (53). Free Fatty Acids have been shown to bind to Toll Like Receptor-4 on adipocytes and macrophages and initiate the pro-inflammatory response by inducing cytokine secretion in a variety of cell types (56). This leads to a recruitment of macrophages to the adipose tissue and production of additional mediators of inflammation (57, 58). In obesity, the adipose tissue is made up of “crown-like structures” which consist of macrophages surrounding dead and dying adipocytes (53).

Adipocytes produce a vast array of factors called adipokines, factors which regulate feeding, metabolism, insulin sensitivity, and immune reactions (59). The

interaction that occurs between adipocytes and macrophages stimulates the production of pro-inflammatory cytokines such as TNF-α and Interleukin 1-beta (IL-1β) (57). The production of these cytokines by macrophages impairs adipocyte function further, which contributes to the chronic inflammation and metabolic dysregulation (27, 55, 57). This unresolved inflammation in adipose tissue quickly becomes systemic.

Inflammation has a role in the pathophysiology of many obesity-related comorbidities as depicted in Figure 1.3. Pro-inflammatory profiles have been described in children, with reports of elevations in an acute marker of inflammation, C-reactive protein (CRP) in obese children as young as 3 years (19, 54).

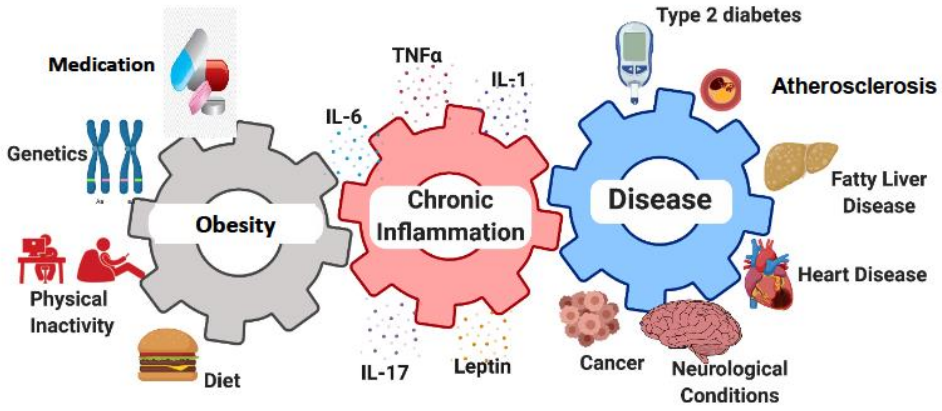


Figure 1.3: The relationship between obesity, chronic inflammation and disease

Obesity has a multi-factorial aetiology that results in a state of chronic inflammation. It is this inflammation that drives the complications associated with obesity. (Image created in Biorender.com)

The pro-inflammatory state in obesity has effects on macrophages and T cells. The macrophages that are present in adipose tissue are divided into two distinct subgroups – M1 and M2 – which are essentially pro- and anti-inflammatory (60). In healthy patients, macrophages are predominantly M2-type macrophages and produce anti-inflammatory cytokines whereas in patients with obesity, macrophages shift from an anti-inflammatory to a pro-inflammatory M1-type and produce pro-inflammatory cytokines (27, 54, 61). M1 macrophages secrete pro-inflammatory cytokines such as IL-1, IL-6 and TNF-α. These M1 macrophages are implicated in causing insulin resistance via their production of inflammatory mediators. Insulin resistance refers to a state when cells in the muscle, fat and liver no longer adequately respond to insulin and cannot easily take up glucose from the blood. M2 macrophages have been implicated in tissue repair and the reduction of inflammation (61, 62). Also, the loss of the beneficial anti-inflammatory effects of M2 macrophages may be implicated in contributing to metabolic complications of obesity (62), such as insulin resistance (27, 61). It has been shown that a reduction in body weight leads to a reversal of the ratio of M1 to M2 macrophages (59). Animal studies have shown that saturated fatty acids prime pro IL1β, an IL1β precursor, in macrophages and by doing this they prime IL1β production. These studies found that NLRP3 inflammasome impairs insulin sensitivity in obesity and demonstrated that lack of IL1β/NLRP3 in vivo protects against high fat diet induced insulin resistance (63, 64). This highlights that it is endogenous ligands activating the NLRP3 inflammasome that are particularly detrimental for obesity-induced insulin resistance (63).

Obesity also influences the T lymphocytes of the adaptive immune system that are present in the adipose tissue. T-helper 1 and T-helper-17 cells are pro-inflammatory and T-helper 2 cells are anti-inflammatory. Obesity changes the ratio of pro-inflammatory (T-helper 1 and T-helper 17 lymphocytes) and anti-inflammatory (T-helper 2 and regulatory T lymphocytes) towards a preponderance of Th1 lymphocytes (65). Th2-polarized cells contribute to the maintenance of adipose tissue function and insulin sensitivity by promoting anti-inflammatory activation of macrophages. Under conditions of obesity, the accumulation of Th1 cells in the adipose tissue generates pro-inflammatory signals which perpetuates the pro-inflammatory response that is associated with

insulin resistance. Obesity-induced changes in the balance of Th1- and Th2-type signals are likely to continue to influence macrophage recruitment and phenotype in adipose tissue generating a pathogenic (pro-inflammatory) rather than a protective (anti-inflammatory) environment (66). Animal studies have also suggested that genetics play a part in obesity, in particular single nucleotide polymorphisms in the fat mass and obesity associated (FTO) gene region may affect neuropeptide Y expression in the hypothalamus which impacts on feeding behaviour (67).

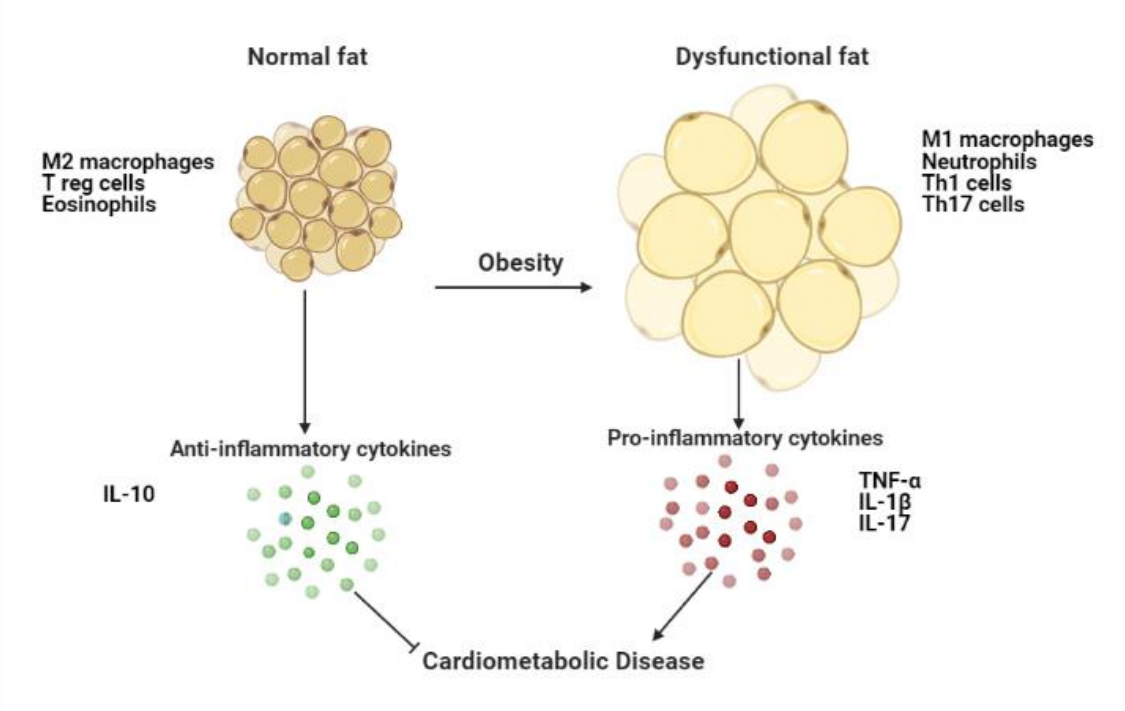


Figure 1.4: The changes in adipose tissue from lean to obese

Obese adipose tissue has an overrepresentation of M1 macrophages, neutrophils, Th1 cells and Th17 cells. Pro-inflammatory cytokines are increased, which include IL-1β, TNF-α and IL-17. (Image created in Biorender.com)

1.3 Second Generation Antipsychotics

1.3.1 Overview of Second Generation Antipsychotic medication

Second Generation Antipsychotics (SGAs) are medications that are used to treat symptoms in Autism Spectrum Disorder (ASD) and a variety of mental health conditions including Early Onset Psychosis (EOP), Bipolar Affective Disorder (BPAD), Tourette's syndrome and Conduct Disorder. All SGAs that are currently licensed show antagonistic binding to dopamine D2 receptors at therapeutic doses, which is critical for their therapeutic action (68). The most common indications for SGA medication use in children and adolescents are in treating irritability associated with ASD and psychotic symptoms in EOP.

ASD affects around 1% of the population and currently 1 in 68 children in the Irish school system have an ASD diagnosis (69, 70). Psychosis affects 3 in every 100 people and 11–18% of patients present with their first episode of psychosis before age 18 in what is known as early-onset psychosis (EOP) (71, 72).

There are guidelines for the use of SGA medications, and they differ between countries. In the USA, the FDA have licensed low-dose risperidone and aripiprazole for the treatment of irritability in autism spectrum disorders (ASD) and conduct disorder, for mixed or manic episodes in the context of bipolar disorder and Tourette's disorder (73).

There are currently no all-encompassing guidelines for SGA prescribing in the paediatric population in Ireland. In Ireland, licenses are more restrictive than the US, and the SGA aripiprazole is licensed only for the treatment of schizophrenia in children 15 years and older, and for acute manic episodes in children 13 years and older. Risperidone is licensed for the short-term symptomatic treatment of persistent aggression associated with conduct disorder or learning disability in children aged 5-18 (74). Licensing is restrictive due to lack of paediatric drug treatment trials. Clinicians often prescribe SGAs "off licence" when managing neurodevelopmental disorders to try to improve symptoms for the young person.

Prescribing rates for SGAs have increased in the last two decades; almost one-third of children visiting a psychiatrist in the US between 2005 and 2009 were prescribed an antipsychotic medication (73, 75, 76). The increased use of SGAs may be due to the perception that they have an improved safety profile as SGAs

have a reduced risk of Extrapyramidal Side Effects (EPSEs) compared with the earlier “First Generation Antipsychotics” (FGAs) (77). Studies report 8-18 fold increases in antipsychotic prescriptions for children in the US (73) and Canada (78) and data have indicated prescribing to children as young as two years (78). The most prescribed SGAs in children are risperidone, olanzapine, aripiprazole and quetiapine (79).

1.3.2 SGAs and weight gain

Despite the early perception and marketing of SGAs as medications with fewer side effects than FGAs, associated weight gain and long term cardiometabolic complications have now become a widespread concern (80, 81). SGA-related weight gain typically occurs within the first few months of treatment and is associated with abnormalities in lipid and metabolic profiles (76). Mean weight gain is significant, (4.4kg-8.5kg) and although it likely plateaus over time it remains elevated compared to same age peers (75, 76). Weight gain and metabolic side effects occur even after short-term use of low dose SGAs. A non-randomized trial of 505 young people between the age of 4 and 19, with a diagnosis of mood spectrum, schizophrenia spectrum or disruptive/aggressive behaviour spectrum disorders prescribed aripiprazole, olanzapine, risperidone or quetiapine for 12 weeks was conducted. 205 participants completed the study and 15 non-adherent patients served as the comparison group. In this study, 55-85% of the patients gained a significant (>7% of body weight) amount of weight with SGA treatment and 17.1% developed dyslipidaema (82). In a study of 36 children and adolescents with Early Onset Schizophrenia who were followed up at baseline and after 7 months of SGA treatment, one-third had increased cholesterol and triglycerides. Participants treated with olanzapine and quetiapine had a greater increase in serum triglycerides in this study (83). Another study examining the medical records of 95 child and adolescent psychiatric inpatients from age 5 to18, with early onset psychosis, found that 51% had elevated triglycerides and 48% had low HDL levels (79, 82-84).

SGA use in children also appears to be associated with more rapid weight gain than in adults (85, 86). A retrospective study comparing weight gain and other

metabolic changes between adolescents (n=179) and adults (n= 4,280) during olanzapine treatment found that 89.4% of the adolescents had weight gain of >7% compared to 55.4% of adult participants with a number needed to harm of 3 (87). Studies have shown that children and adolescents may be at an increased risk of weight gain with SGAs, possibly because of differences in how SGAs are metabolised. Another factor may be reduced top-down regulation of the hypothalamus due to the prefrontal cortex being immature in young people compared to adults leading to loss of appetite regulation (80, 88, 89).

Excess weight is associated with the development of insulin resistance and Type 2 Diabetes Mellitus (T2DM). Case-control studies of children prescribed SGAs have shown a three-fold increase in the risk of T2DM (86, 90). The strength of association between diabetes and SGAs is highest among children and adolescents, compared to other age groups (91). A retrospective cohort study compared 28,858 children and youth (age 6-24) who had recently commenced SGAs with 14,429 matched controls. These young people had a range of diagnoses including mood disorders, ADHD and conduct disorder and the study excluded those with diagnoses of schizophrenia and ASD. This study showed a three-fold increase in the risk of T2DM on antipsychotics (HR =2.49, 95% CI = 1.73- 5.32). The risk increased with cumulative dose during follow up and the risk remained elevated for up to 1 year after discontinuation of antipsychotic medication. When the cohort aged 6 to 17 was examined, patients on antipsychotics had more than a 3 fold increased risk of T2DM (HR 3.14 (CI 1.5-6.56)). As this study combined diagnoses in analysis, there is likely to be overgeneralisation when interpreting results. This study looked at the risk on all antipsychotic drugs rather than just SGAs, however, they found that the risk of T2DM was increased for use restricted to SGAs (HR 2.89, CI 1.64-5.1) and use of risperidone (HR 2.2, 95% CI 1.14-4.26) (90).

1.3.3 Factors influencing the risk of SGA-related weight gain

Key factors that appear to influence the risk of SGA-associated weight gain include age, sex, type of SGA, baseline BMI, hormone levels, and diagnosis (75, 88, 92-94). Youth, especially those who are antipsychotic naïve, are at a greater

risk of weight gain and metabolic effects than adult patients and younger children appear at greater risk (82, 88, 92, 94-97). Children often gain over 7% of their baseline weight within the first few months of treatment, as found in a 12 week non-randomized trial of 505 young people between the age of 4 and 19 who were prescribed SGAs [see 1.3.2] (96).

Weight gain is more strongly related to the type of SGA rather than the dose (98). A systematic review of adverse effects of SGAs in children and adolescents found that olanzapine and quetiapine carry the greatest risk, with aripiprazole associated with the lowest risk, although all confer some increase in risk (82, 92). Although the type of SGA is a more important determinant of risk of weight gain, there is also evidence to suggest the weight gain is dose-dependent for risperidone and aripiprazole in youth (75). Baseline BMI may affect risk of weight gain with SGAs. However, while some studies have shown that higher baseline BMI is associated with more rapid weight gain (93, 99), others have found that lower baseline BMI confers greater risk (94, 100). One study explored retrospective, cross sectional data of 65 adult patients on SGAs with psychotic symptoms. This study explored predictors of weight gain using explorative correlation and regression analyses. A high baseline BMI was found to be a predictor of weight gain (93). A study prospectively monitored 50 hospitalized adolescents treated with SGAs for the first 12 weeks of treatment. These participants had diagnoses of schizophrenia (n=46), schizoaffective disorder (n=2) and conduct disorder (n=2) (94). Various clinical risk factors were tested for an association with weight gain and low baseline BMI was found to be a risk factor. Another study of 99 adult patients with first episode schizophrenia and 51 healthy controls found that lower BMI at baseline and a diagnosis of undifferentiated schizophrenia were associated with antipsychotic-induced weight gain (100).

Baseline hormone levels also appear to impact weight gain differentially in the context of SGAs, although this has not been widely studied. Higher baseline levels of the hormones insulin and leptin have been associated with more weight gain on SGAs while higher baseline levels of free T4, ghrelin and adiponectin were associated with less weight gain (88). This was found in a one year longitudinal study of 127 children and adolescents on SGAs at 3, 6 and 12 months

compared to baseline (n=39 at 1 year assessment). This study included children with a range of diagnoses included schizophrenia spectrum disorders, bipolar disorder, tic disorders, disruptive behaviour disorders and depression (88).

Hyperglycaemia at baseline and earlier more rapid weight gain indicate increased risk of metabolic side-effects and it has been suggested that these factors could be exploited as biomarkers for increased morbidity (81, 89, 101). A retrospective chart review study of 456 adolescents between the ages of 13 and 18 examined early weight gain as a predictor for long term weight change (101). These adolescents had a range of diagnoses including schizophrenia, mood disorders, personality disorders, emotional disorders and substance misuse all of which were combined in the analysis. They found that weight gain of 4% in the first 3 months of treatment was associated with a greater risk of longer term weight gain and potential metabolic complications.

Children with mental illness and neurodevelopmental disorders such as ASD are also at increased risk for obesity, metabolic syndrome, T2DM, cardiovascular disease (CVD), coronary heart disease and cerebrovascular disease in comparison with the general population and independent of SGA prescribing (92, 98). Therefore, SGA prescribing further increases the risks in an already vulnerable group (92).

1.3.4 Implications of SGA-related weight gain

Multiple studies have described the negative consequences of SGA-related weight gain. Children with obesity are at increased risk of metabolic disorders such as T2DM, hypertension, hyperlipidaemia, insulin resistance, non-alcoholic fatty liver, and non-alcoholic steatohepatitis (2, 21). Data in psychiatric patients suggest that SGA use is associated with a five-fold increase in the prevalence of metabolic syndrome, a predictor of cardiovascular risk (76). Similarly, young people treated with SGAs are at increased risk for early atherosclerosis and cardiovascular disease (102, 103). SGAs increase the risk of T2DM two to three fold (91). A retrospective cohort study compared the incidence of T2DM in youth (6-24 years) started on SGAs (n=28,858), with diagnoses of mood disorders, ADHD and conduct disorders, with matched controls (n=14,429) and found that

the risk of diabetes in the SGA group was three times that of the control group [HR 3.03, 95% CI 1.73-5.32] (90). Olanzapine and clozapine are most strongly associated with a risk of T2DM, according to a systematic review of fifteen population-based studies on SGAs and metabolic side effects (104, 105).

In addition there are significant mental health consequences due to the negative impact of weight gain on self-esteem and body image (79, 91, 103, 106-108).

Typically, obesity research has focused on the impact of BMI and cardiometabolic abnormalities however rate of weight gain is also important. Patients with a higher weight gain in the first month have more weight gain over the first year (101). A study of 105 7-17 year olds treated with risperidone with rapid weight gain on this medication had a worse cardiometabolic profile and systemic inflammation compared with those who gained weight slowly (75). In this study, rate of weight change was found to be equally or more predictive for a range of cardiometabolic outcomes compared with the concurrently measured weight (or BMI z-score) (75).

Children, especially those who are antipsychotic naïve, are at substantially greater risk for rapid weight gain during their first six months on an SGA (82, 92). Pre-pubertal children run the greatest risk of rapid weight gain (82, 108). Consequently, children are at even greater risk of the negative inflammatory and cardiometabolic side effects of SGAs compared to adults (96, 109).

Overall, rapid weight gain in children and adolescents is a particularly concerning side effect of SGA medication that poses substantial health risks in later life.

1.3.5 Potential mechanisms for SGA-related weight gain

The mechanisms for SGA-related weight gain are not fully understood, however several factors are implicated. SGAs affect appetite and physical activity, and they have complex effects on adipokines, insulin-sensitive tissue, and the gut microbiome.

An increase in appetite in association with SGA-related weight gain is widely reported. This is thought to be due to the effect of SGAs on histamine receptors and alpha-1 adrenergic receptors (75, 110-114). SGAs are also thought to reduce resting energy expenditure - the number of calories required by the body during a non-active period (113, 115), which leads to weight gain. SGAs have pronounced sedative effects due to their effect on histamine receptors and this may also contribute to the reduction in resting energy expenditure (80, 111, 113, 115). SGAs affect glucose transport and may directly induce insulin resistance (116-118).

SGAs affect adipokines, signalling molecules that are produced by adipose tissue which are linked to glucose and lipid metabolism. The key adipokines are leptin and adiponectin. Leptin regulates body weight by signalling nutritional status to the hypothalamus, setting in train a cascade of neurotransmitter and neuropeptide release that regulate food intake and energy expenditure balance (53, 62). High leptin levels occur in the expanding adipose tissue associated with obesity. Elevated leptin has inhibitory effects on the hunger centre in the hypothalamus and is associated with a sensation of satiety and consequent reduced food intake (98). Chronically elevated leptin desensitizes hypothalamic neurons in the hunger centre, contributing to leptin resistance and culminating in weight gain (98, 119, 120). In addition, leptin increases synthesis of the pro-inflammatory cytokine TNF-α and is itself increased by TNF-α leading to a spiralling positive feedback loop (121).

Adiponectin is an adipokine that may suppress lipid accumulation and have anti-inflammatory effects on macrophages (53). Studies have found adiponectin levels inversely correlate with body weight and insulin levels (88, 111, 122).

SGAs are associated with an increase in leptin and a decrease in adiponectin in adult human studies (119, 122-125). The chronic exposure to increased levels of leptin contributes to leptin resistance, which in turn results in increased appetite and weight gain (98). Interestingly, leptin is disproportionally high in relation to adiposity in patients on antipsychotics, which raises the possibility that the leptin signalling mechanism may be disrupted by these medications. This would be

consistent with the increased appetite experienced by people taking antipsychotics despite significant weight gain (108).

The differential risk of weight gain that is associated with the different SGAs may be related to their variable receptor binding profiles. It has been suggested in animal studies that the balance of SGA binding to the histamine receptor, dopamine D2 receptor, alpha1A receptor and serotonin 2A and 2C (5HT2a and 5HT2c) receptors may play a role in the propensity to cause weight gain with histamine receptor antagonism being most correlated with weight gain and antagonism of alpha1A and 5HT2c also correlated with weight gain (80, 88, 114, 115, 120, 126-130).

The specific mechanism by which the SGA olanzapine leads to metabolic dysregulation has been investigated in healthy adults with no mental illness (114). This study had ten healthy adult participants who were assigned to olanzapine, aripiprazole or placebo for nine days under controlled inpatient conditions. A meal challenge was carried out before and after the olanzapine was taken and insulin sensitivity and glucose disposal were investigated. From this study, there appears to be two pathways. Firstly, olanzapine appears to induce postprandial insulin resistance and hyper-insulinaemia by direct effects on insulin-sensitive tissues. This is thought to be an early precipitant of olanzapine induced adiposity (114). Secondly, olanzapine elicits acute increases in postprandial GLP-1 and elevated glucagon concentrations. This is independent of weight gain, psychiatric disease, and food intake.

The gut microbiota is the bacterial ecosystem living in the human gut. It has been shown to play a role in normal weight gain and fat deposition; mice with no microbiota are resistant to diet-induced obesity (131). In animal models, SGA treatment has been shown to cause changes to mouse gut microbiota. These changes in gut microbiota were associated with suppressed energy expenditure and weight gain (131-133). This has also been studied in humans and a significant decrease in microbiome diversity has been reported in patients treated with SGAs (134-136). One study examined faecal samples from 117 adults with Bipolar Affective Disorder, of which 49 were treated with SGAs and 68 were not and they found a significant decrease in microbiome community diversity in the

participants treated with SGAs (134). Another study looked at drug naïve, normal weight first episode schizophrenia patients after 24 weeks of risperidone treatment. There were 41 patients who completed the 24 weeks of the study and 41 control participants. Multiple linear regression analyses showed that after controlling for potential confounders, changes in faecal Bifidobacterium species significantly correlated with the increase in weight and BMI after 24 weeks of treatment (135).

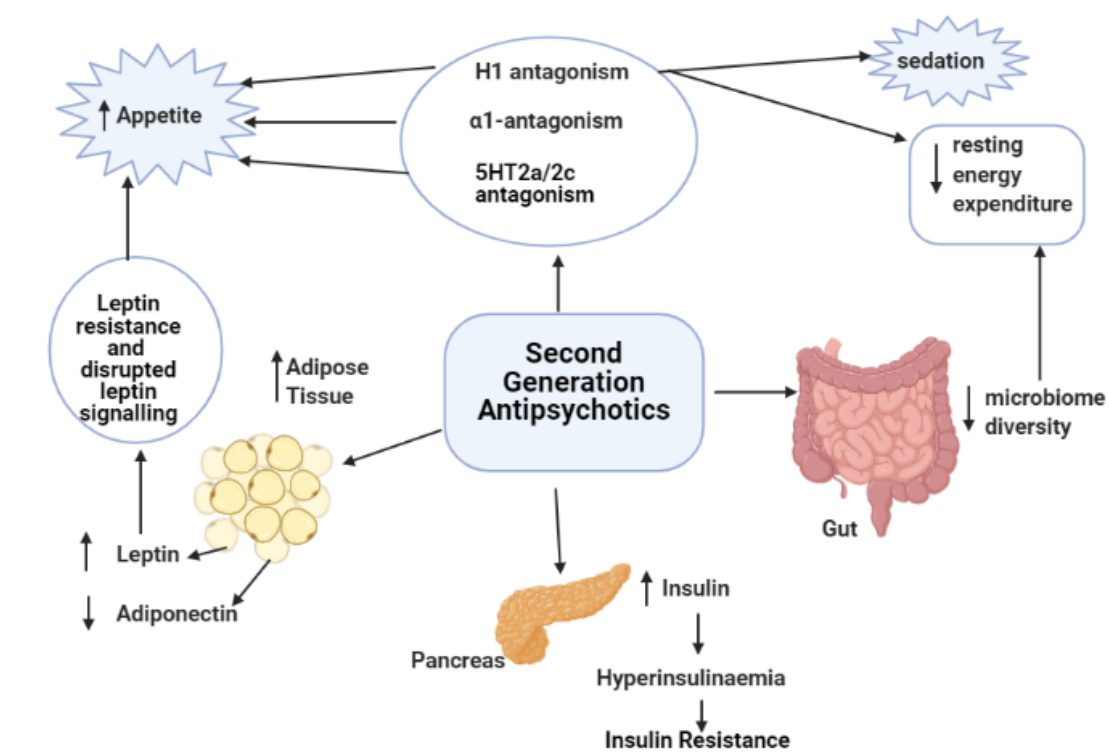


Figure 1.5: Mechanisms of SGA-related weight gain

SGAs cause an increase in leptin which results in disrupted leptin signalling causing increases appetite. H1, α1,5HT2a/2c antagonism increase appetite. H1 antagonism and reduced microbiome diversity cause sedation and reduces resting energy expenditure. SGAs cause an increase in insulin. All these mechanisms culminate in weight gain. (Image created in Biorender.com)

1.3.6 Other complications of SGAs

Some SGAs, e.g., risperidone, are associated with elevations in the hormone prolactin which has been associated with reductions in bone mineral density which increases the risk for osteopenia (137). Prolactin is a hormone that is released from the posterior pituitary gland, that causes breast tissue development and lactation in post-partum females. Both men and women have small amounts of prolactin in their blood. Excess prolactin can cause lactation in females who are not pregnant or breastfeeding as well as menstrual problems and infertility. In males it can cause erectile dysfunction. Excess prolactin is also associated with a reduction in bone mineral density and increased risk of osteoporosis (137). Secretion of prolactin by the pituitary gland is under inhibitory control via dopamine from the hypothalamus. As SGAs are dopamine antagonists, they can cause increases in prolactin.

1.4 SGAs and inflammation

1.4.1 Inflammation in psychosis and ASD

The main indication for the prescribing of SGAs in adults is for the treatment of psychotic symptoms, typically in patients with a diagnosis of schizophrenia. In children and adolescents, SGAs are most commonly prescribed to manage irritability in ASD. Interestingly, derangements of inflammatory markers have been reported in medication-naïve patients with both schizophrenia and ASD (138, 139).

In ASD, there are consistent reports of elevated proinflammatory cytokines such as IL-1β, TNF-α, and IL-6, and chemokines, such as monocyte chemoattractant protein-1 (MCP-1), in plasma, cerebrospinal fluid, and post-mortem brain tissue (140, 141). Similarly, studies of antipsychotic-naïve patients with First Episode Psychosis (FEP) have found increases in pro-inflammatory cytokines. Higher IL-6 (142-146), TNF-α and IL-1β have been reported at baseline in psychosis (143-151).

Miller et al, in a meta-analysis of 40 studies of cytokine alterations in schizophrenia, found that IL-12, Interferon-γ (IFN-γ) and TNF-α levels remained elevated in acute exacerbations of psychosis (143, 149). Some of these

cytokines, particularly IL- 6 and IL-1β have been so commonly reported that it has been suggested they may represent state markers for acute psychotic episodes (152). This suggests that dysregulations in the immune system may be implicated in the pathophysiology of schizophrenia and may explain some common risk pathways between schizophrenia and obesity. A recent study found that a group of adult patients with untreated schizophrenia (n=32) had significantly elevated MAIT cell and Th17 cell frequencies compared to controls (n=24) indicating a shift of balance towards a pro-inflammatory Th17 subtype (153). Raised C-reactive protein (CRP), a marker of acute inflammation, was shown to be associated with a diagnosis of schizophrenia in a meta-analysis looking at all cross-sectional studies that investigated CRP levels in patients with schizophrenia compared to controls (154). In a longitudinal cohort study of patients with FEP, it was reported that those with increased CRP levels also had high triglycerides. In this study, a multiple linear regression analysis found that the effects of inflammation on triglycerides specifically were independent from the effect of changes in weight (155). Fifty-three FEP patients were included in this longitudinal study. Weight, BMI, lipid profile and gluco-metabolic parameters were obtained at baseline and at three-month follow-up. Correlation analyses showed that those with increases in hsCRP over the three-month period also had increases in triglyceride levels ($r = 0.49$, $p = 0.02$).

Another study of 59 patients with non-affective psychotic disorders aged 18-70 and 22 controls yielded similar results (156). These results highlight the possible influence of inflammation that is associated with schizophrenia on the metabolic profile. It appears that patients with schizophrenia may develop dyslipidaemia and the metabolic syndrome independent of SGA medication (155, 156).

1.4.2 Th1 to Th2 shift theory in psychosis

There has been some research hypothesising that psychosis causes a shift in the immune system from a cell mediated response (T helper 1 associated) to a humoral mediated response (T helper 2 associated) (121, 157). T helper cells can mature into either T helper 1 (Th1) cells or T helper 2 (Th2) cells in response to specific types of cytokines. Th1 cells are involved in cell-mediated immunity against intracellular bacteria and viruses and autoimmune diseases such as

rheumatoid arthritis. Th2 cells are involved in humoral immunity against extracellular parasites and allergic reactions (157). The Th1 system promotes cell-mediated immune responses against intracellular pathogens, and the Th2 system helps B cell maturation and promotes humoral immune responses against extracellular pathogens. Th1 and Th2 cytokines antagonize each other in promoting their own type of response, by suppressing the other type of helper cell (157).

The data on cytokines in schizophrenia indicate a relatively reduced production of Th1 cytokines and a shift in production to Th2 cytokines (157). This highlights that psychosis may influence the immune system. The increased Th2 cytokine levels in schizophrenia appears to be downregulated by antipsychotic treatment (158). This was found in a study of 88 adult patients with FEP. Cytokine measurements were performed on day 0 and day 30. IL-4 (Th2 cytokine) concentrations were significantly lower after antipsychotic treatment (158).

1.4.3 SGAs as both anti-inflammatory and pro-inflammatory agents

There is very little known about the effect of SGAs on inflammation. Studies investigating cytokine levels in patients on SGA medication have reported very mixed results with some reporting an increase, some a decrease and some no change (124, 142, 158-161).

Anti-inflammatory effects of SGAs have been reported in a number of studies. Firstly, the chronic low-grade inflammation associated with psychotic episodes in schizophrenia is largely attenuated by SGA therapy (144, 162, 163). A systematic review on cytokine levels in schizophrenia before and after antipsychotic treatment found decreases in the pro-inflammatory cytokines IL-1β and TNF-α after treatment (142, 158, 159). Another systematic review of the acute effect of antipsychotics on cytokine levels found that IL-1β, IL-2 and IL-6 levels decrease after antipsychotic treatment**,** though TNFα, IL-1 and IFN-γ remain stable during antipsychotic treatment (152).

Secondly, in vitro studies have indicated that antipsychotics have anti-inflammatory effects. In one study, macrophages treated with risperidone or

clozapine and adipose tissue treated with olanzapine showed inhibition of pro-inflammatory cytokine production (164).

Finally, it has been hypothesised that the agranulocytosis associated with the SGA clozapine results from over activation of anti-inflammatory factors (124).

On the other hand, some studies have reported pro-inflammatory effects of SGAs. One study found an increase in pro-inflammatory gene transcription after adding antipsychotics to cultured human adipocytes. The authors of this study suggested that antipsychotics alter the gene expression patterns in adipocytes and prime them for a low-level inflammatory state (165). An in vitro study which treated macrophages with ziprasidone showed macrophage proliferation and pro-inflammatory cytokine production was increased, indicating a pro-inflammatory effect of the antipsychotic (166). A number of studies have reported increased levels of the pro-inflammatory cytokines, TNF-α, IL-1β and IL-17 in patients on SGA medication (124, 160, 161). A longitudinal study found that although inflammatory cytokines initially decreased following treatment with SGAs, by six months of treatment, these cytokines had increased to the pro-inflammatory level that they had been at baseline (148). This study of adult participants had a drug naïve first episode schizophrenia group (n=62) and a control group (n=60). Cytokines were measured at baseline and at five timepoints over six months. Risperidone initially caused an anti-inflammatory effect, but cytokines then increased to baseline levels over the six months, possibly due to weight gain. At baseline, levels of IL1β, IL6 and TNF-α were significantly higher in the schizophrenia group than the control group. Within the schizophrenia group, levels of these cytokines changed significantly during the six months of treatment. IL1β and IL6 decreased up to two months and then steadily increased and TNF-α increased at three and six months compared to levels at baseline.

1.5 Management of SGA related weight gain

1.5.1 Monitoring of children and adolescents who are prescribed SGAs

The recognition that SGA-related weight gain is associated with very significant long-term health implications has led to the development of guidelines for the monitoring of adults who are prescribed SGAs (167). For an adult population, the

British Association of Psychopharmacology (BAP) guidelines for the monitoring of adult patients prescribed SGAs are widely used (167, 168). These guidelines recommend assessing BMI, fasting glucose, lipids and blood pressure at 6 months, 12 months and then annually. The British Association of Psychopharmacology also recommend monitoring fasting glucose and blood pressure after 3 months and have developed guidelines for the management of weight gain and metabolic dysregulation on SGAs in adults (167).

For the paediatric population there are no formal guidelines, but several agencies have published recommendations on the monitoring and management of weight gain and metabolic complications associated with SGA use in children and adolescents. It is important to note these are only recommendations. Clinical guidelines are different in that they are systematically developed statements to assist practitioner decisions about appropriate healthcare for specific clinical circumstances. Clinicians are not legally obliged to follow guidelines in all cases, but they must be prepared to justify their decision and action if they depart from guidelines produced by a recognised body. This is not the case for recommendations.

The American Academy of Child and Adolescent Psychiatry (AAP), the Canadian Alliance for Monitoring Effectiveness and Safety of Antipsychotics in Children (CAMESA) and National Institute for Health and Care Excellence (NICE) organization have all published recommendations on SGA monitoring in children and adolescents (169-171).

For children and adolescents, recommendations differ on how often to record weight change, lipids, and glucose with some variations between NICE, AAP and CAMESA on the frequency of this monitoring (169-172). NICE guidance recommends weekly weight monitoring for the first 6 weeks, then 3 months and 6 months. Guidance from AAP does not give recommended timepoints for monitoring youth on SGAs. NICE recommends monitoring lipids and glucose at 3, 6 and 12 months. CAMESA do not give recommendations for the frequency of monitoring, instead they give recommendations on how to manage increases in weight, lipids, and glucose.

1.5.2 Prevention of SGA related weight gain

Strategies to prevent weight gain from occurring are important as it is extremely difficult to manage weight gain after it has occurred. Primary, secondary and tertiary weight-gain prevention strategies have been suggested (172). Primary prevention includes education and adherence to healthy lifestyle behaviours. Secondary prevention for overweight patients or those with mild metabolic abnormalities includes intensification of lifestyle measures, switching to a lower risk SGA or non-pharmacological weight loss treatment. Tertiary prevention measures are for patients who are obese or have hyperglycaemia or diabetes. These consist of weight reduction interventions and targeted treatments from appropriate specialists including an endocrinologist (172). Lifestyle interventions have been shown to be effective for prevention of weight gain in adult patients with first episode psychosis prescribed SGAs (173, 174). A randomized controlled trial comparing a behavioural intervention with routine care for SGA-associated weight gain in adults (n=61) found behavioural intervention to be more effective; almost 40% fewer patients had clinically significant weight gain in the behavioural intervention group (173). Lifestyle interventions have not been tested in a child population prescribed SGAs.

1.5.3 Management options for SGA-related weight gain

Secondary and tertiary prevention strategies include reducing dose (though this is rarely a practical option), switching to another antipsychotic with a lower metabolic profile, use of adjunctive aripiprazole, behavioural intervention, lifestyle intervention, and pharmacological treatment for weight loss (96).

One study demonstrated that weight management strategies can be effective. A randomized trial of a weight loss and lifestyle programme for adult patients on SGAs found that after 12 months the intervention group (n=104) had more weight loss and lower fasting glucose than control participants (n=96) (175). Intent to treat analysis found that intervention participants lost 4.4kg more than control participants from baseline to 6 months (95% CI -6.96kg, -1.78kg), and 2.6kg more than controls (95% CI -5.14kg, -0.07kg) from baseline to 12 months.

There are also pharmacological tertiary prevention measures. Glucophage (metformin) is an oral anti-hyperglycaemic medication that reduces hepatic glucose production, decreases absorption of glucose and leads to improved insulin sensitivity by increasing peripheral glucose uptake. Metformin improves insulin sensitivity and has been shown to reduce weight gain in children and adolescents with ASD on SGAs (176, 177). It is also effective in reducing weight in both children and adults who are overweight on SGAs (103, 178). There has been a recent clinical trial comparing metformin treatment, switching antipsychotics and continuing antipsychotics with lifestyle interventions for youth treated with SGAs. It found that adding metformin (n=47) or switching SGA medication to aripiprazole (n=30) resulted in a significantly decreased BMI z-score compared to continuing SGA treatment with lifestyle interventions (n=44) (109). The change in BMI z-score was significantly different between the three groups at weeks 12 and 24, with addition of metformin and switching antipsychotic both causing a greater reduction in BMI Z-score. Effect sizes were from 0.4 to 0.81, with no significant difference in BMI Z-score reduction between the addition of metformin group and switching medication group (109).

Other pharmacological options for the treatment of obesity are Glucagon-like peptide-1 (GLP-1) receptor agonists. Glucagon-like peptide 1 (GLP-1) is a gastrointestinal peptide hormone that is released by intestinal cells postprandially, that has been found to have anti-inflammatory effects in humans (39). GLP-1 receptor agonists are effective for weight reduction in obesity in the general population (167). In one study liraglutide, a GLP-1 receptor agonist, significantly improved glucose tolerance, and led to reductions in body weight, waist circumference, visceral fat, systolic blood pressure, and LDL cholesterol levels compared to placebo (179). GLP-1 receptor agonists have been found to improve glucose tolerance with SGAs in animal models (180, 181). A randomized controlled trial compared adult patients who were on SGAs and prescribed liraglutide (GLP-1 receptor agonists) for 16 weeks (n= 47) with patients who were on SGAs and prescribed a placebo (n=50) (179). This study showed that glucose tolerance (estimated treatment difference 2.1), body weight (estimated treatment difference -5.3), and cardiometabolic disturbance (estimated treatment difference -19.3) significantly improved on liraglutide compared to placebo (179).

These studies of GLP-1 agonists provide optimism as SGA associated weight gain is difficult to manage and it is rarely feasible to discontinue these medications.

1.6 Outstanding issues, aims and hypotheses of this thesis

1.6.1 Outstanding issues

SGAs are medications that are commonly used for the management of psychotic symptoms in EOP, and irritability associated with ASD in children and adolescents. SGAs are associated with rapid onset weight gain. Weight gain in general is associated with a chronic inflammatory state, and rapid weight gain is associated with a worse pro-inflammatory profile. There is a striking lack of studies investigating 1) the effects of SGAs on inflammatory markers, and 2) the association between these markers and SGA-associated weight gain.

1.6.2 Aims

This is the first study to prospectively investigate the effect of SGAs on weight gain and the immune system over time in youth; children and adolescents are followed up at three time points over a twelve-month period to investigate the impact of SGAs on cardiometabolic health, inflammatory markers, immunophenotyping and immune cell responses.

1. To determine how SGA medication impacts on cardiometabolic function in children and adolescents over a 12-month period.

2. To determine how SGA medication impacts on systemic inflammation and the frequency of expression of innate immune cells in children.

3. To determine how SGA medication impacts on immune cell responses in children.

1.6.3 Hypotheses

Hypotheses relating to each aim are outlined below:

1. That treatment with SGAs would lead to rapid weight gain which would be associated with changes in the metabolic profile over time including raised lipids, raised glucose and raised insulin.

2. That SGAs would cause an inflammatory state, similar to that observed in childhood obesity, and that this inflammatory state would be associated with metabolic dysregulation.

3. That SGA medication would cause immune cells to be dysregulated and produce pro-inflammatory cytokines.

CHAPTER 2: Materials and Methods

2.1 Ethical Approval

Ethical approval was obtained from the Medical Research Ethics Committees of Linn Dara Child and Adolescent Mental Health Services (CAMHS), Our Lady's Children's Hospital Crumlin, Tallaght University Hospital, Children's University Hospital Temple Street, Dublin North City Mental Health Services, St Patrick's Mental Health Services and St Michael's House. The study commenced in April 2018 after ethical approval was obtained.

2.2 Study Design

This was a longitudinal study over a 12-month period into the effects of treatment with Second Generation Antipsychotics (SGAs) in young people with neurodevelopmental disorders and mental health disorders. All parents of participants gave written informed consent prior to the participants partaking in the study.

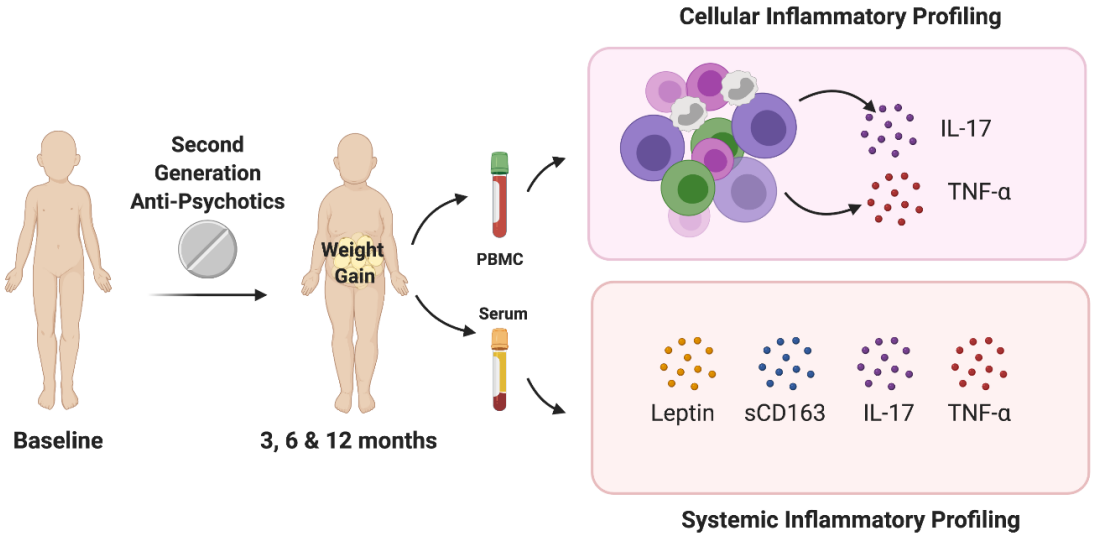


Figure 2.1: Study Overview

Participants who were about to commence SGA medication were recruited. Blood samples were taken at baseline. PBMCs were separated from whole blood and used to examine the cellular inflammatory profile. Serum was used to examine the systemic inflammatory profile. The participants were followed up at 3, 6 and 12 months and the above inflammatory profiling was repeated at each time point. Image created in Biorender.com

2.3 Participant Recruitment

2.3.1 SGA group participants

Patient recruitment was carried out in Linn Dara Child and Adolescent Mental Health Services, both inpatient and outpatient services and at Beechpark Autism Services. Sample size was calculated at 30-40 participants. However, recruitment was much more challenging than initially hoped for and 19 participants were recruited to the study.

Participants who were commenced on SGA medication by their treating clinician were invited to participate in the study. Inclusion criteria were: 1) age range 5-18 years 2) clinical indication for SGA treatment 3) SGA medication not yet commenced or within 2 weeks of commencement. Exclusion criteria were: 1) underlying hormone deficiency 2) known genetic disorder, 3) inflammatory condition 4) recent acute infection.

An age appropriate information leaflet was given to both the patient and their parents. All study queries were answered prior to signing consent forms. Research blood samples were only obtained while participants were having routine clinical bloods collected for SGA monitoring, so there was no additional phlebotomy burden for patients.

Each participant was given a unique identifier code. The identity of the participant was protected in a password-protected database on a computer in the Department of Psychiatry. In total 19 participants were recruited to the study and each participant had four visits at baseline, three months, six months, and twelve months. A total of ten participants completed the twelve months of follow up and attended for all four time points. A further two participants attended two time points and were followed up for three months. The other seven participants dropped out of the study after the first time point. Two of these participants did not commence antipsychotic treatment.

2.3.2 Control Participants

Healthy lean children attending hospital for either elective surgery or dynamic endocrine testing were recruited. These controls were recruited as control participants for another study on childhood obesity being carried out by a Clinical Research Fellow in Paediatrics in the Obesity Immunology Research Group. Only those lean children who were having intravenous cannulation for their own clinical care were approached. Consent was obtained for the samples being used in this study.

2.4 Participant interview

Each participant was interviewed using a standardized study case report form (see Appendix 6). Areas explored in the interview included past medical history (medical illnesses, medication use), current health (recent infections) and family history.

2.5 Clinical examination: weight, BMI, and metabolic parameters

Weight and height were measured at each time point to calculate BMI percentile. To account for children's natural growth, age and sex specific percentiles for BMI were determined.

2.6 Metabolic Profiling

Fasting blood samples were taken for total cholesterol, triglycerides, low-density lipoprotein (LDL) and high-density-lipoprotein (HDL). Samples for fasting glucose, fasting insulin, and HbA1c were collected. Lipids, insulin, and glucose levels were measured after a 12 hour fast.

Homeostasis model assessment for insulin resistance (HOMA-IR) is a method used to assess pancreatic β-cell function and insulin resistance. It is calculated using fasting glucose and fasting insulin concentrations (182, 183). HOMA-IR was calculated using the following equation: fasting plasma insulin (mlU/L) x

fasting glucose (mmol/L)/22.5. Scores usually range from 0 to 15, and higher scores indicate greater insulin resistance (184, 185)**.**

HbA1c is glycosylated haemoglobin used in the diagnosis of diabetes mellitus and the normal level is below 42 mmol/mol.

Blood samples were taken to measure prolactin levels at baseline and at 12 months. Normal reference ranges for prolactin are 0-424 mIU/L in males and 0-530 mIU/L in females.

2.7 Clinical analyses in study

To investigate factors that may impact on lipid profile, glucose regulation or prolactin levels we examined the impact of type of medication, rate of weight gain and baseline BMI on each of these.

1. We divided the participants into groups according to the type of SGA medication prescribed and then compared lipid profiles, glucose regulation, and prolactin levels between these groups.

2. We divided the participants into groups by those who gained weight in the first three months and those that did not gain weight and then compared lipid profiles, glucose regulation and prolactin levels between these groups.

3. A higher BMI at baseline has previously been shown to be a risk factor for greater weight gain on SGA medication (87). To investigate this, we separated the cohort into the participants that had a BMI >50th percentile at baseline and participants that had a BMI< 50th percentile at baseline and we looked at weight gain in the first three months on SGAs. We then compared lipid profiles, glucose regulation, and prolactin levels between these groups.

2.8 Blood Sampling for Immune Research Work

A total of 8-10mls of venous blood was collected in lithium heparinised vacutainers (*Becton Dickinson*) and 5-10mls in serum vacutainers (*Becton Dickinson*) when the participant was having routine clinical bloods obtained as per Maudsley Guidelines for SGA monitoring (186). All blood sample tubes used

were from the same company (*BD Vacutainer*). The samples were left at room temperature and transported by the investigator to the National Children's Research Centre laboratory within one hour of being taken.

2.8.1 Serum Preparation

Serum tubes containing venous blood from participants was centrifuged at 3000 rpm for 10 minutes. The serum layer formed at the top of the tube after centrifugation was aliquoted into volumes of 500µl using a pipette. These aliquots were labelled with the identifier code and were frozen at -80 degrees Celsius.

2.8.2 Peripheral Blood Mononuclear Cell Isolation

Peripheral Blood Mononuclear Cell (PBMC) isolation was carried out in sterile conditions using a laminar flow hood within two hours of the sample being taken from the participant. PBMCs were isolated using a density gradient centrifugation method with Lymphoprep (*Stemcell Technologies*) used as the density medium. Labelled 50ml centrifuge tubes were filled with 12.5mls of Lymphoprep solution. Venous blood samples were transferred into 50ml sterile tubes and diluted in a 2:1 ratio with Phosphate Buffered Saline (PBS) (*GIBCO Life Technologies*). A sterile transfer pipette was used to overlay the diluted blood on to the Lymphoprep, making sure to prevent the mixture of blood and the Lymphoprep solution. The mixture was centrifuged at 1800rpm for 20 minutes with the acceleration set at 3 and deceleration set at 1. This was to prevent mixing of the sample and damage to the cells. The buffy layer containing the PBMCs was harvested from the blood plasma:density medium interface using a sterile transfer pipette and placed in a new 50ml centrifugation tube. The cells were washed twice using 30mls of PBS and centrifuged at 1800rpm for 10 minutes with the acceleration set at 9 and the deceleration set at 9 to pellet the cells. Following the second centrifugation, the cells were resuspended in 5ml of fully supplemented sterile Roswell Park Memorial Institute (RPMI) 1640 media (*Sigma-Aldrich Co., USA*).

Roswell Park Memorial Institute (RPMI) 1640 media (*Sigma-Aldrich Co., USA*) was used and was supplemented with 10% FBS (*Sigma Aldrich Co., USA*), 2%

HEPES (*Gibco, Life Technologies Inc., USA*), 1% Penicillin Streptomycin (*Gibco, Life Technologies Inc., USA*), 0.8% Fungizone (*Gibco, Life Technologies Inc., USA*), Non-Essential Amino Acids (*Gibco, Life Technologies Inc., USA*) and Sodium Pyruvate (*Gibco, Life Technologies Inc., USA*) referred to as complete RPMI (CRPMI) in the following text.

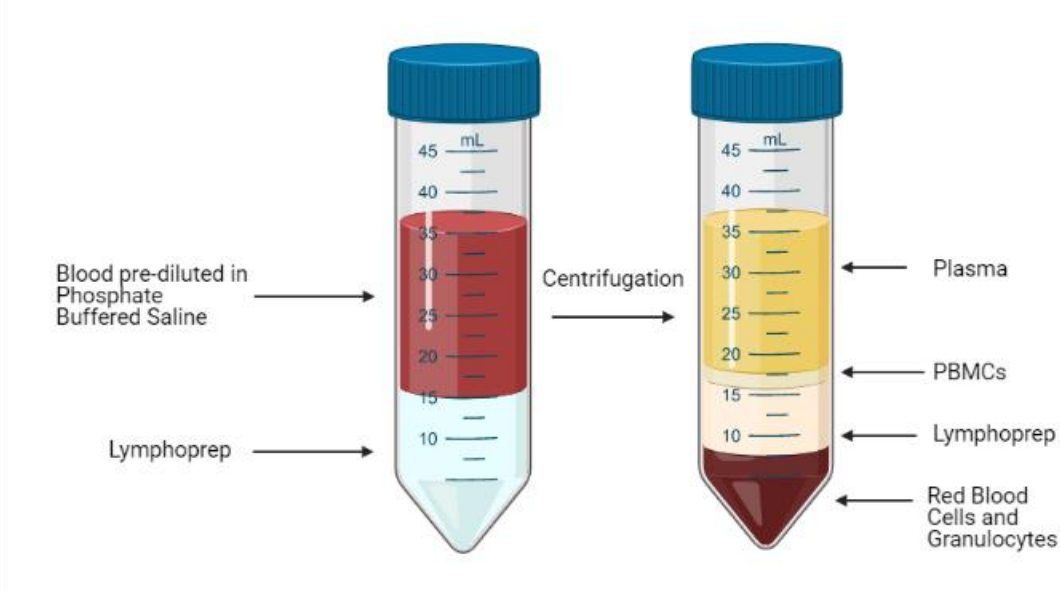


Figure 2.2: Peripheral Blood Mononuclear Cell Isolation

Representative image showing isolation of Peripheral Blood Mononuclear Cells. Image created in Biorender.com

2.8.3 Cell enumeration

A 1 in 20 dilution of cell suspension and Trypan Blue (*Sigma-Aldrich Co., USA*) was prepared with 10µl of cell suspension added to 190µl of Trypan Blue. A haemocytometer was used to allow for cell enumeration. 10µl containing cell suspension mixed with Trypan Blue was added to the haemocytometer chamber. The stained cells were counted by direct vision with a light microscope (*Olympus CKX41*). The total number of cells was calculated using the following formula:

$$Number\ of\ cells/ml = Cell\ number \times dilution\ factor \times 10^4$$

The PBMCs were resuspended in CRPMI to yield a final concentration of 1 x 10^6 cells per 500µl for further analysis. The cells were incubated at 37 degrees centigrade with 5% CO_2.

2.9 Activation of Immune Cells for Cytokine Production

Isolated PBMC (1 x 10^6 cells per well) were cultured in a 24 well plate (*Costar*) in cRPMI media alone or stimulated with TCR microbeads (25ng/ml)(T-cell activation/expansion kit, prepared as per manufacturer's instructions (*Miltenyi Biotec*), Lipopolysaccharide (100ng/ml), Cell stimulation cocktail consisting of phorbol 12-myristate 13-acetate (PMA) and ionomycin (stock at 500X, used at 1X) (*eBioscience*). Additional CRPMI media was added to each well to achieve a final volume of 1ml per well. The cells were incubated at 37 degrees centigrade with 5% CO_2 overnight.

2.10 Harvesting of supernatants after 24 hours

The cell culture supernatants were collected and transferred to a -80 degrees Celsius freezer in 500µl aliquots for analysis by ELISA at a later date.

2.11 Cryopreservation of peripheral blood mononuclear cells

Freezemix solution was prepared using 1ml of Dimethyl Sulfoxide, DMSO (*Sigma Aldrich Co., USA*) and 9mls of Foetal Bovine Serum (FBS) (*Miltenyi Biotec Inc., USA*). The PBMC suspension was centrifuged at 1800rpm for 7 minutes and freezemix was added to yield a final concentration of 5 x 10^6 cells per aliquot. The aliquots were labelled and placed on wet ice prior to transfer to the -80° freezer.

2.12 Thawing of frozen PBMC samples

One aliquot of each sample was taken from the -80°C freezer and placed on ice. 10ml of CRPMI media was placed into 15ml centrifuge tubes labelled with the sample names. The frozen sample tubes were swirled in a water bath for approximately one minute ensuring not to submerge the lid of the tube. When the samples were almost completely thawed, they were taken to the laminar flow hood. The 1ml cell suspension was pipetted from the sample tube to the 15ml centrifuge tube of CRPMI. Using some of the cell/CRPMI solution, the small sample tube was washed out to ensure all the PBMCs at the bottom of the tube were obtained. This was transferred back into the 15ml centrifuge tube. The tubes

were centrifuged at 1800rpm with acceleration set at 9 and deceleration set at 9 for seven minutes to pellet the cells. The tubes were removed from the centrifuge and the supernatant was poured off. 1ml of warm CRPMI media was added to the PBMCs. The PBMCs were placed in a 37°C incubator for >1 hour and allowed to rest before counting.

2.13 Setting up cell culture plate for intracellular and extracellular flow cytometry experiment

Isolated PBMC were plated on a 96 well plate (*Costar*) at a concentration of 500,000 PBMCs per 0.5ml. PBMCs were cultured in cRPMI media alone or stimulated with Cell stimulation cocktail consisting of phorbol 12-myristate 13-acetate (PMA) and ionomycin (stock at 500X, used at 1X) (*eBioscience*), dopamine hydrochloride (100µM concentration) (*Sigma-Alrich*) +/- PMA. All wells also had protein transport inhibitor, Cell Inhibitor Cocktail (*Invitrogen eBioscience*) cocktail added to keep the cytokines inside the cell (stock at 500X, used at 1X). The PBMCs were left in an incubator overnight.

2.14 Immunophenotyping by Flow Cytometry

2.14.1 Introduction to Flow Cytometry

Flow cytometry is used to analyse the physical and chemical characteristics of single cells suspended in a fluid as they pass through at least one laser. Cells are stained with fluorescent labelled antibodies specific for cell surface molecules or internal proteins. The fluorescent labels are then excited by the laser and emit light at wavelength specific to that fluorochrome. An optical-to-electronic coupling system is used to record the way in which the particle emits fluorescence and scatters incident light from the laser (187).

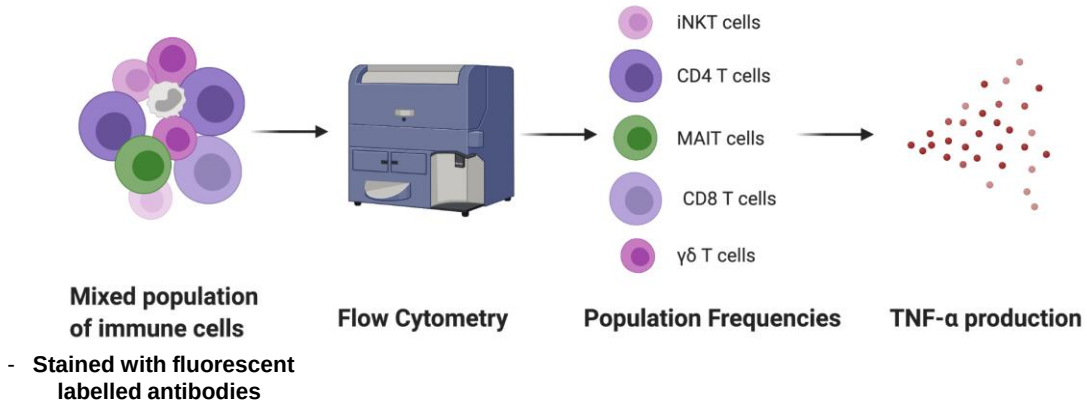


Figure 2.3: Overview of flow cytometry workflow

Image created in Biorender.com

Antibodies targeted against specific molecules were used to label the PBMCs in this project. They were purchased from Miltenyi-Biotec, unless otherwise stated (see table 2.1): FITC CD161 (Clone REA631), PE iNKT Tetramer (Clone 6B11, BD Bioscience); IL-17A (Clone REA1063), PerCP CD45RA (Clone REA562); CD14 (Clone REA599); CD56 (Clone REA196); CD8 (Clone REA734), Vα7.2 (Clone REA179), PEVio615 CD4 (Clone REA623), APC CD16 (Clone REA423), CD161 (Clone REA631); TNF (Clone REA656), APCVio770 CD8 (Clone REA734); TCR γ/δ (Clone REA591), VioBlue CD45RO (Clone REA611); VioGreen CD3 (Clone REA613).

Table 2.1: Flow cytometry antibodies used in this project

Target Molecule	Fluorochrome	Expression
CD45RA	PerCP	Present on naïve T cells, B cells and monocytes
CD8	APCy7	CD8+ T cells are cytotoxic T cells restricted by MHC-I

CD45RO	VioBlue	Present on T cell subsets, B cell subsets, monocytes and macrophages – isoforms of CD45 containing none of exons A, B or C
CD3	VioGreen	A T cell lineage specific marker – co-molecule to T cell receptors expressed at the cell surface, assists in signal transduction
iNKT Tetramer	PE	A subset of innate T cells that share properties of both T cells and NK cells
CD56	PerCP	Present on NK cells- a neural cell adhesion molecule (NECAM), adhesion molecule
CD8	PerCP	Present on Cytotoxic T cells – recognizes non-self peptide with MHC Class I molecule and kills cell
TCR Vα7.2	PECy7	Present on MAIT cells and conventional T cells – an invariant T receptor that characterizes MAIT cells – restricted by non-polymorphic class 1b MHC molecule - MR1
CD161	APC	Present on NK cells and T cells – important regulator of NK cell cytotoxicity function
CD14	PerCP	Acts as a co-receptor for the detection of bacterial lipopolysaccharide (LPS). Expressed by macrophages
CD16	APC	Required for Antibody-Dependent cell-mediated

		cytotoxicity processes carried out by monocytes
IL-17A	PE	A pro-inflammatory cytokine produced by activated T cells
CD4	PEVio615	Present on T-helper cells, - adhesion and signalling co-receptor in MHC Class II molecule T cell
TNF	APC	A cytokine involved in systemic inflammation and is one of the cytokines that makes up the acute phase reaction
Gamma Delta (γδ)	APCy7	T cells that have a distinct T cell receptor on their surface with one γ chain and one δ chain, bridge between innate and adaptive responses

2.14.2 Extracellular staining

Approximately 0.5 x 10^6 cells were transferred to labelled Fluorescence Activated Cell Sorting (FACS) tubes. The cells were centrifuged at 1800rpm for 10 minutes with acceleration set at 9 and deceleration set at 9. The supernatant was poured off and the cell pellets were resuspended in 1ml of FACS buffer (PBS supplemented with 1% FBS). The cells were centrifuged at 1800rpm for 7 minutes. The supernatant was poured off and disposed of and the cell pellets were kept. The cells underwent extracellular staining using recommended volumes of antibody (2-5µl). The cells were vortexed and incubated at 4°C in the dark for 30 minutes. 1ml of FACS buffer was added to each tube and the cells were spun down at 1800rpm for 7 minutes. The supernatant was poured off and the cells were fixed in 500µL of paraformaldehyde solution (*BD CellFix*). The cells were incubated at 4°C for 20 minutes in the dark. 1ml of FACS buffer was added to each tube and the cells were pelleted at 1800rpm for 7 minutes. The supernatant was poured off and the cell pellets were resuspended in 200µl of FACS buffer and kept at 4°C in the dark until the flow cytometry run. Unstained

and Fluorescence Minus One (FMO) controls were stained and acquired with each experiment. Data was acquired using a BD Fortessa flow cytometer in the National Children's Research Centre. Live cells were gated according to their forward and side scatter. Data was analysed using FlowJo Software (*Treestar*).

Table 2.2: Extracellular flow cytometry staining panel

This table shows the antibodies that were used to stain the cells and the corresponding colour channel the antibodies were conjugated to.

	FITC	**PE**	**PERCP**	**PECY 7**	**APC**	**APCY 7**	**VioBlue**	**VioGreen**
T cells	CD27		CD45RA			CD8	CD45RO	CD3
Monocytes	HLA-DR		CD14		CD16			
iNKT/NK	CD27	iNKT Tet	CD56				CD71	CD3
MAIT	CD27		CD8	Vα7.2	CD161		CD71	CD3

2.14.3 Fluorescence Minus One (FMO) Controls

FMO controls were prepared by using the recommended volumes of antibody in the panel, minus the FMO antibody e.g. the iNKT cell FMO was prepared by adding all the antibodies to the tube except for the iNKT Tetramer antibody. The absence of this one stain allows us to gate the negative space, and therefore any cells from stained samples that are in this gate are positive.

2.14.4 Intracellular staining

Intracellular staining was carried out to investigate cytokines being released. We stained for TNF-α and IL-17 and examined their production from CD4 T cells, MAIT cells and γδ T cells.

For intracellular cytokine staining, the use of a protein transport inhibitor, Cell Inhibitor Cocktail (*eBioscience*) was required. This was used to block protein transport and retain the cytokines inside the cell. The protein transport inhibitor was added to the cell culture plate with the stimulations. As described above, cells were stained with extracellular antibodies. The cells were then washed with FACS buffer and centrifuged at 1800rpm for 7 minutes with acceleration set at 9 and deceleration set at 9. The cells were fixed and permeabilized using 300µl of Fixation Permeabilization Solution 1X (*BD Biosciences*) for 30 minutes at 4°C. This was followed with a wash with 1X PermWash Buffer (*BD Biosciences*). It was then possible for the cells to undergo intracellular staining, using the recommended volumes of antibody (2-5µl). Intracellular staining mix was prepared in PermWash Buffer (see Table 2.3 with panel below). The cells were vortexed and incubated for 40 minutes at 4°C. Following staining, the cells were washed with FACS buffer and resuspended in 300µl of FACS buffer for flow cytometry analysis. Live cells were gated according to their forward and side scatter. Data was acquired on an Attune FACS flow cytometer in NUI Maynooth and analysed using FloJo software.

Table 2.3: Intracellular flow cytometry staining panel

FITC	PE	PEVio615	PECy7	APC	APCy7	VioBlue	VioGreen
CD161	IL-17A	CD4	Vα7.2	TNFα	γδ	CD138	CD3

2.14.5 Compensation staining

In multiparameter flow cytometric analysis, compensation for spectral overlap between the lasers is necessary. We used bead compensation methods to do this. 15µl of positive REA/Mouse beads and 15µl of negative REA/Mouse beads were added to the FACS tubes for each channel (8 tubes). 1µl of one antibody from each panel was added to the tubes. After 30mins 400µl of FACS buffer was added to the tubes. The tubes were left to incubate at 4°C in the dark overnight.

2.14.6 Flow Cytometry Analysis

Cell populations were analysed using FlowJo software (*Treestar*). The cells were gated electronically by their granularity and density and analysed for markers of interest. Results are expressed as a percentage of the lymphogate or percentage of parent populations and determined using FMO. The populations analysed were T cells, MAIT cells, NK cells, iNKT cells and monocytes. Production of TNF-α and IL-17 by CD4 T cells, MAIT cells and γδ T cells was analysed in the intracellular experiment.

2.15 Enzyme Linked Immunosorbent Assay (ELISA)

2.15.1 Introduction to ELISA

Enzyme-linked immunosorbent assay (ELISA) measures protein levels. This is a quantitative assay, which allows total levels of a specific protein to be determined. ELISA is a plate-based assay in which an antigen is immobilized to a solid surface and then complexed with an antibody that is linked to an enzyme (see figure 2.2). Detection is completed by assessing the conjugated enzyme activity via incubation with a Tetramethylbenzidine (TMB) substrate to produce a chromogen, a substance which can be readily converted into a dye or other coloured compound, which can be measured using a plate reader (188).

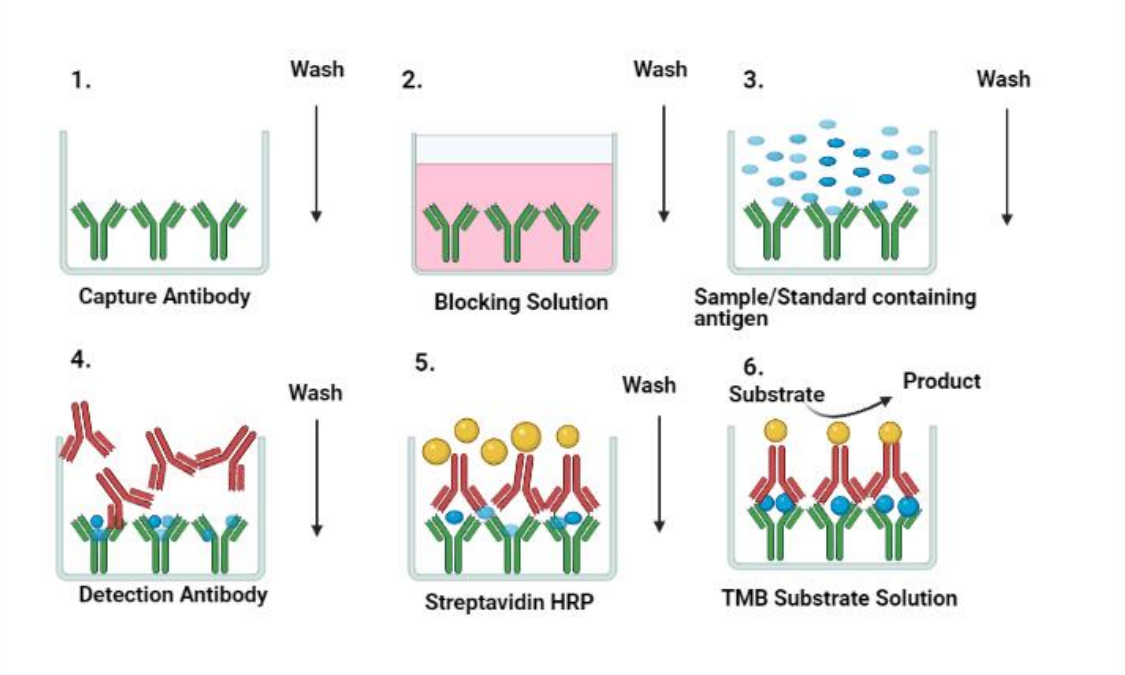


Figure 2.4: Overview of ELISA Assay

Diagrammatic representation of the mechanism of sandwich ELISA. Image created in Biorender.com

2.15.2 Leptin and Soluble CD163 Quantification by Enzyme Linked Immunosorbent Assay (ELISA) in serum

All samples analysed by ELISA technique were carried out according to specific manufacturer's instructions.

Quantification of adipokine/protein production was detected using DuoSet ELISA kits (*R&D BioSystems*).

A 96 well Nunc Maxisorp TM (*Thermoscientific, NY, USA*) plate was coated with 50µl of specific capture antibody (human soluble CD163, leptin) diluted in PBS according to the Certificate of Analysis that came with each specific kit. The plate was sealed with parafilm and incubated on a shaker plate at room temperature overnight.

The following morning the liquid was poured off, the plate was washed three times with wash buffer (PBS supplemented with 0.05% Tween-20) and dried. To block nonspecific binding, 150µl of blocking solution (PBS supplemented with 1% w/v

Bovine Serum Albumin (BSA)) was added to each well. The plate was left to incubate at room temperature on a shaker plate for one hour.

The liquid was poured off and the plate was washed three times with wash buffer as previously. The samples and standards were prepared. Serial dilutions of the specific standard were prepared. The samples were diluted as follows: Leptin 1:50, soluble CD163 1:50. 50µl of samples and standards were added to each well in triplicate or duplicate. The plate was covered and left to incubate for two hours at room temperature on a shaker plate.

The liquid was poured off and the plate was washed three times. 50µl of diluted detection antibody (prepared according to instructions with each specific kit) was added to each well. The plate was left to incubate at room temperature for two hours on a shaker plate.

The liquid was poured off and the plate was washed three times. 50µl of Streptavidin HRP diluted in 1% BSA (40-fold dilution) was added to each well. The plate was left to incubate at room temperature for 20 minutes covered in tinfoil on a shaker plate.

The liquid was poured off and the plate was washed 3 times. 50µl of substrate solution (TMB Substrate, *Sigma-Aldrich, UK*) was added to each well. The substrate solution was prepared by mixing equal volumes of TMB-substrate A and TMB-substrate B. The plate was left to incubate in the dark until a colour change to blue occurred. The reaction was arrested after 20 minutes by adding 25µl/well of 1M H_2SO_4. The optical density of each well was determined within 30 minutes using a microplate reader with 450 nm excitation wavelength. The adipokine/protein concentration of each sample was determined by comparison to the standard curve of known cytokine concentration. The equation $y=mx+c$ was used to generate the standard curve to determine the concentration of adipokine/protein.

2.15.3 IL-10, IL-17A, IL-17F and TNF-α Quantification in Cell Culture Supernatants by Enzyme Linked Immunosorbent Assay (ELISA)

Quantification of cytokine production was detected using DuoSet ELISA kits (*R&D BioSystems*). Isolated PBMCs were cultured in a 24 well plate in CRPMI media, or with LPS, or with TCR beads, or with Cell Stimulation Cocktail (PMA) for 18 hours. Supernatants were harvested and used to measure cytokine production. Cell culture supernatants were kept in the freezer (-80°C) until ELISAs were carried out. The protocol was carried out according to manufacturer's guidelines.

Supernatants stimulated with LPS were diluted 1:5 due to oversaturation.

Supernatants stimulated with TCR beads and used to measure IL17F were diluted 1:5 due to oversaturation.

The ELISA method outlined in 2.15.2 was then used.

2.15.4 TNF-α and IL-17 Quantification in serum by High Sensitivity Enzyme Linked Immunosorbent Assay (ELISA)

High sensitivity kits were used to measure TNF-α and IL-17 in serum because these kits can detect lower levels of the cytokine than standard kits. All samples analysed by High Sensitivity ELISA technique were carried out according to specific manufacturer's instructions.

All reagents used in High Sensitivity ELISA were the reagents that came with the specific kits and were used according to the specific instructions provided. Reagents were prepared and 50µl of assay diluent was added to each well in the pre-coated plate supplied in the kit.

50 µl of serum samples or standards were loaded onto the plate and it was left to incubate at room temperature for 2 hours on a shaker plate.

The plate was washed 3 times with the wash buffer provided in the kit and dried. 200µl of the specific detection antibody provided in the kit was added to each well and the plate was left to incubate at room temperature for 1 hour on a shaker plate.

The plate was washed as previously. 200µl of Streptavidin Polymer-HRP (1X) was added to each well. The plate was protected from the light and left to incubate for 30 minutes at room temperature on a shaker plate.

The plate was washed again as previously. 200µl of substrate solution (provided in kit) was added to each well. The plate was left to incubate in the dark for 30 minutes at room temperature. The reaction was arrested by adding 50µl of stop solution (provided in the kit). The optical density of each well was determined within 30 minutes using a microplate reader. The cytokine concentration of each sample was determined by comparison to the standard curve of known cytokine concentration.

2.16 Method for investigating the effect of dopamine and risperidone on the production of TNF-α and IL17F by PBMCs

PBMCs were thawed using the method described in section 2.10 above. Thawed PBMCs were placed in an incubator for one hour and allowed to rest.

Isolated PBMC were plated on a 96 well plate (*Costar*) at a concentration of 500,000 PBMCs per 0.5ml. PBMCs were cultured in cRPMI media and stimulated with TCR microbeads (25ng/ml)(T cell activation/expansion kit, prepared as per manufacturer's instructions (*Miltenyi Biotec*), +/- dopamine hydrochloride (100µM concentration) (*Sigma-Alrich*), +/- risperidone (1µg/ml).

The plate was left in an incubator overnight. The following morning the supernatants were harvested and transferred to the freezer (-80°C) until analysis of cytokines by ELISA.

2.17 Methods for the in-vitro experiments

In-vitro experiments using a monocyte cell line were carried out to investigate the effect dopamine and risperidone had on the production of TNF-α. THP-1 cells were plated on a 24-well cell culture plate. To polarize the cells to inflammatory M1 macrophages, PMA was added at a concentration of 100nM and the THP-1 cells were plated at a concentration of 1×10^6 cells/ml.

The THP-1 cells were plated on a 24 well plate in cRPMI media alone or stimulated with dopamine hydrochloride (100µM concentration), risperidone (1µg/ml), dopamine hydrochloride (100µM concentration) plus risperidone (1µg/ml) +/- Lipopolysaccharide (100ng/ml). Previous dose and time studies carried out in the Obesity Immunology Group had highlighted the dose and times used in this experiment. The supernatants were harvested and TNF-α concentration was examined by ELISA.

2.18 Statistical Analysis

Statistical analysis was completed using Graph Pad Prism 9 Software (USA). Data is expressed as Standard Error of the Mean. We tested the data for normality and determined the differences between the participants at each time-point using Analysis of Variance (ANOVA) and Friedman where appropriate, with Bonferroni correction where appropriate. P values were expressed with significance set at < 0.05.

Table 2.4: Summary of Media for Cultured Cells

Medium Name	Description	Supplier
Media for PBMC Preparation	RPMI (500ml)	Invitrogen-Gibco
	10% v/v Foetal Bovine Serum	Invitrogen-Gibco
	5% v/v HEPES Solution	Invitrogen-Gibco
	1% v/v Penicillin Streptomycin	Invitrogen-Gibco
	1% v/v Fungizone Antimycotic	Invitrogen-Gibco
	Non-essential Amino Acids	Invitrogen-Gibco
	Sodium Pyruvate	Invitrogen-Gibco

Table 2.5: ELISA Kits

ELISA Kit Name	Supplier	Catalogue Number
Human CD163	R & D Systems	DY1607-05
Human Leptin	R & D Systems	DY398-05
Quantikine HS ELISA Human TNF-α	R & D Systems	HSTA00E
Quantikine HS ELISA Human IL17	R & D Systems	HS170
Human TNF-α	R & D Systems	DY210-05
Human IL-10	R & D Systems	DY217B-05
Human IL-17	R & D Systems	DY317-05
Human IL-17F	R & D Systems	DY1335B-05

Table 2.6: Antibodies used for Flow Cytometry

Medium Name	Antibody	Clone	Company	Catalogue No.
FITC				
	CD161	REA631	Miltenyi Biotec	130-113-598
PE				
	iNKT Tetramer	6B11	BD Bioscience	552825
	IL-17A	REA1063	Miltenyi Biotec	130-118-243
PEVio615				
	CD4	REA623	Miltenyi Biotec	130-113-226
PERCP				

	CD45RA	REA562	Miltenyi Biotec	130-113-368
	CD14	REA599	Miltenyi Biotec	130-110-520
	CD56	REA196	Miltenyi Biotec	130-114-551
	CD8	REA734	Miltenyi Biotec	130-110-682
PECy7				
	Vα7.2	REA179	Miltenyi Biotec	130-100-206
APC				
	CD16	REA423	Miltenyi Biotec	130-113-389
	CD161	REA631	Miltenyi Biotec	130-113-595
	TNF	REA656	Miltenyi Biotec	130-120-063
APCy7				
	CD8	REA734	Miltenyi Biotec	130-110-681
	TCR γ/δ	REA591	Miltenyi Biotec	130-113-509
VioBlue				
	CD45RO	REA611	Miltenyi Biotec	130-119-620
VioGreen				
	CD3	REA613	Miltenyi Biotec	130-113-142

CHAPTER 3: Clinical and metabolic characteristics of the SGA cohort at baseline and during follow up over a 12-month period

3.1 Introduction

3.1.1 Global increase in SGA prescribing

Several studies in Europe, Canada, Australia, New Zealand, and South America have reported an increase in SGA prescribing over the past two decades (189-191). A multinational study examining antipsychotic prescribing trends in the UK, USA, Denmark, Germany, and the Netherlands between 2005 and 2012 reported an increase in prescribing rates of SGAs across all age ranges between 2005 and 2012 in the UK, Denmark, Germany, and the Netherlands (190). In this study SGA prescribing reduced in the USA over the time period, however these changes occurred in the context of higher rates of SGA prescribing in the USA at the start of the period investigated (190). A study in France found SGA prescribing is increasing but the overall rate of antipsychotic prescribing remained stable between 2006 and 2013 due to a decline in the prescription of typical (First Generation) antipsychotics (192). In Germany, a study of nationwide health data incorporating approximately 30% of all children indicated that SGA prescribing increased between 2004-2012 from 2.3/1000 to 3.1/1000. Overall, there was a levelling off of total prescriptions of all antipsychotics and no change in the incidence of new prescriptions, thus the increase is likely explained by longer term prescribing in individuals commenced on SGAs (193). In British Columbia, Canada, prescribing rates for SGAs were reported to have increased from 1.66/1000 in 1996/97 to 6.37/1000 in 2010/11, particularly in males aged 6-12 years (from 2.3/1000 to 8.6/1000), males 13-18 years (from 2.8/1000 to 10.7/1000), and females aged 13-18 years (from 2.8 to 10.7/1000) (78).

There has also been an increasing prevalence of co-prescribing of other medications used to treat mental health disorders. A French study noted a decline in prescribing rates of SGAs in the male age group 21-24 (192). Interestingly, this reduction in SGA prescribing was paralleled by an increase in the prescribing rate of other medications. This may indicate that SGAs were prescribed less often for disorders that they may have been used for previously, such as Attention Deficit Hyperactivity Disorder (ADHD). This trend in reduction of SGA use and higher use of other medications has also been seen in other countries such as the USA due to more judicious use among youth with disorders such as ADHD which have less indication for SGA use (194).

3.1.2 SGA prescribing in Ireland

In Ireland, aripiprazole is licensed for the treatment of schizophrenia in children 15 years and older, and for acute manic episodes in children 13 years and older. Risperidone is licensed for the short-term symptomatic treatment of persistent aggression associated with conduct disorder or learning disability in children aged 5-18 (74). Antipsychotic licensing is however quite limited compared to other countries as they are not licensed for the management of irritability in ASD even though they are commonly prescribed for this indication. Two SGAs, risperidone and aripiprazole, were licenced by the FDA in the US for treatment of irritability in Autism Spectrum Disorder (ASD) in 2006 and 2009. A recent US study showed that over 5% of children with ASD aged 2-11 years and >17% of those aged 12-17 years were prescribed at least one SGA (195). They have not been approved in European countries; however, it is accepted best practice to prescribe SGAs for the management of extreme aggression or irritability in the context of ASD.

In a study investigating SGA prescribing patterns in Ireland, we analysed prescription data from the Irish General Medical Services Database (196). This database contains prescription information on around a third of young people in Ireland who are in receipt of state-funded health care. While the trends of prescribing did not significantly change from 2005-2015 due to significant population growth, the absolute numbers of young people prescribed antipsychotic medication has increased significantly in Ireland. Of concern, the study showed that antipsychotic medications are prescribed for very young children (< four years) in Ireland. Although diagnostic data were not available, these young children are more likely to have ASD, since antipsychotic medications are frequently used in this group to manage behaviours that challenge that do not respond to behavioural interventions.

Overall, the results of this study on SGA prescribing rates indicated that there was no significant increase in the rates of prescription of SGA medication in children, adolescents and young adults in Ireland between 2005 and 2015. In 2010 in particular there was a significant decline, which contrasts with the findings of the majority of previous studies in the US, Canada and Europe. Our study found that there was a decline in the prescribing of SGAs over time for males

aged 16-24 and a significant decline in the yearly rate of prescribing in all age groups in females. There are a three key reasons why SGA prescribing may not have increased in Ireland over the time period of the study. Firstly, shortly before the beginning of the study period, in 2004, the American Diabetes Association published a position statement on antipsychotic drugs, obesity and diabetes (197). This statement stated that there is considerable evidence that SGAs cause rapid weight gain in the first few months that may not reach a plateau even after one year of treatment. Clinicians who became aware of this statement may have started prescribing more judiciously. Secondly, there was increasing consensus that SGAs should be a treatment of last resort after behavioural treatments have been tried and failed (198). This may be explained in part by the statement of concern about SGAs from the American Diabetes Association, which may have led to a reluctance in physician prescribing due to an increased knowledge about the short-term effects of SGAs, in particular the risk of significant weight gain and metabolic complications. Thirdly, the decline could potentially be due to an increase in the prescribing of psychostimulants for ADHD and antidepressants, both used as medications in disorders where SGAs may previously have been prescribed. However, the lack of information about the clinical indication for the SGA prescription makes it difficult to comment on the reasons for the decline over time in prescribing. Even though prescribing rates reduced overall, it is important to note that one group in this study – those aged 12-15 years - had an increase in prescribing rates. There are a few factors which may have impacted this. This may reflect the increase in prevalence of mental health disorders in this age group. The license for use of risperidone in conduct disorder was introduced during this time which also may have led to increased prescribing rates in this group. In July 2009, risperidone was licensed in Ireland for the short-term symptomatic treatment, up to 6 weeks, of persistent aggression in conduct disorder. We also found that a small but significant proportion (8.9%) of those receiving SGAs received repeat prescriptions for longer than one year. This is concerning due to the significant side-effects associated with antipsychotic use. It is possible that these are youth with emerging severe and enduring mental health disorders. However, SGA prescribing in children is typically for non-psychotic conditions such as disruptive behaviour (199).

3.1.3 An audit of SGA prescribing in a specialist ASD service

Given the concerns about SGA prescribing in Ireland and internationally particularly in a population with ASD, an audit of SGA prescribing was completed in a regional specialist ASD service in Dublin/Kildare/Wicklow. Beechpark autism services is a multi-disciplinary autism specialist service for children and adolescents aged up to 18 years with a clinical diagnosis of autism and mild ID or normal cognitive functioning. Children attending the service receive multi-disciplinary supports in the form of psychosocial interventions that are tailored to autism. A consultation psychiatric service is provided to assess and manage comorbid psychiatric presentations or to assess the need for medication in children with behaviours of concern who have not responded to behavioural interventions. In this service, all children are assessed by members of the autism specialist multi-disciplinary team (MDT) and all non-pharmacological options to manage behaviours that challenge are trialled before reverting to medication. Young people in whom non-pharmacological options fail to lead to improvement in behaviours that challenge are referred to psychiatry for consideration for SGA medication.

This audit indicated that 25% of children referred to psychiatry were prescribed SGAs. The most prescribed medication was risperidone (79%), followed by aripiprazole (16%) and then olanzapine (5%). Where data were available, 36% of children prescribed SGAs had a BMI>25 and 37% had lipid abnormalities, namely elevated triglycerides, LDL and total cholesterol, and reduced HDL levels (Figure 3.1; unpublished data).

Typically, parents of patients are informed regarding the risks of SGAs and educated regarding dietary management and exercise. Patients are monitored regularly for side effects but ameliorating these side-effects is still challenging. We do not currently have these data for our regional Child and Adolescent Mental Health Services (CAMHS), where SGAs are commonly used to treat young people presenting with major mental health disorders such as Early Onset Psychosis, but it is likely that many of these patients have experienced weight gain and lipid abnormalities on SGAs. Worryingly, disability services (including services for children and adolescents with ASD) in Ireland have recently been reconfigured as part of the national policy for disability services, Progressing

Disability Services (PDS). Children with ASD are now referred to CAMHS psychiatry for pharmacological management of behaviours that challenge. However, CAMHS Multi-Disciplinary Teams are non-specialised and do not engage in non-pharmacological work for children with ASD which is provided by network disability teams or primary care for autism interventions. Unfortunately, due to resourcing and long waiting lists in disability services, many of the children attending CAMHS will no longer receive ASD-specific interventions. This is likely to contribute to a deterioration in their behavioural presentations and secondary psychiatric comorbidities leading to further increases in prescribing rates for SGAs in children with ASD attending CAMHS.

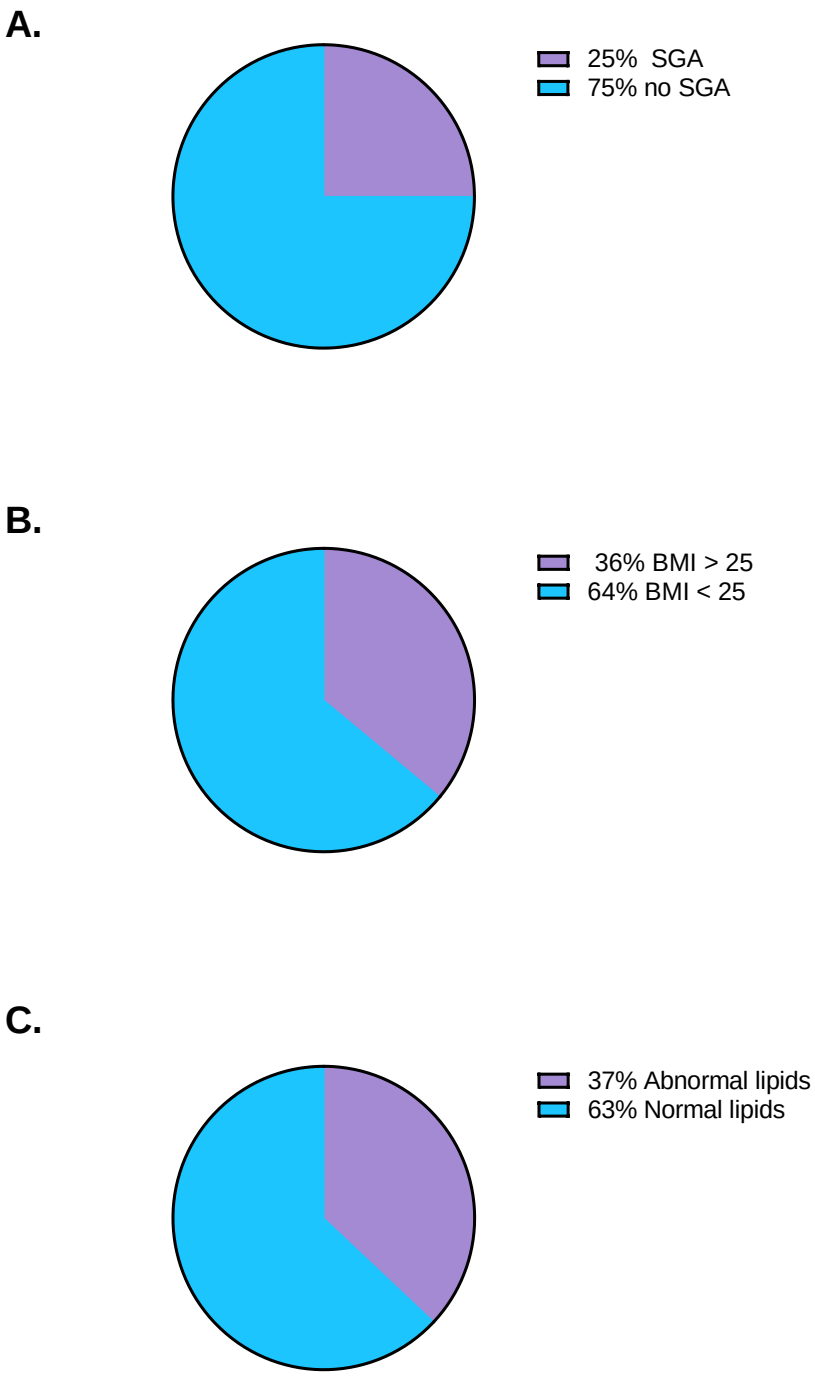


Figure 3.1: Pilot data from a specialist ASD in Dublin/Kildare/Wicklow demonstrating overweight and lipid abnormalities in children with ASD who were on SGA medication

(A) Pie chart showing % of patients prescribed SGA medication in the service **(B)** Pie chart showing % of patients on SGAs who were overweight **(C)** Pie chart showing % of patients on SGAs with lipid abnormalities

3.1.4 Clinical implications of SGA medication

There are health and safety concerns associated with the use of SGA medications, particularly cardiometabolic side-effects that can predispose to chronic disease in adulthood. Children and adolescents are more sensitive to the side-effects of SGAs (76). Cardiometabolic risks are considerably increased in youth exposed to SGAs for the first time; weight gain and disturbances in lipid and metabolic parameters are reported (76). The impact of overweight and adverse metabolic profiles on morbidity and mortality is well documented (200), although the long-term impact of exposure to SGAs in paediatric populations has not been studied. As a consequence of these and other side effects it is recommended that SGAs are used judiciously and with close monitoring in the paediatric population (201).

Additionally, some SGAs, e.g., risperidone, are associated with elevations in the hormone prolactin which has been associated with reductions in bone mineral density which increases the risk for osteopenia (137) (see 1.3.6). Excess prolactin can cause lactation in females who are not pregnant or breastfeeding as well as menstrual problems and infertility. In males it can cause erectile dysfunction. Excess prolactin is also associated with a reduction in bone mineral density and increased risk of osteoporosis (137). Secretion of prolactin by the pituitary gland is under inhibitory control via dopamine from the hypothalamus. As SGAs are dopamine antagonists, they can cause increases in prolactin.

3.2 Specific aims of this chapter

1. To describe the cohort of participants in the study.
2. To describe the cardiometabolic risk factors in the cohort at baseline and assess their change after 3, 6, and 12 months of follow up on SGA medication.
3. To examine if factors such as baseline BMI, type of SGA prescribed, and rapid weight gain had an influence on lipid profile and glucose regulation.
4. To describe prolactin levels in the cohort at baseline and assess their change at 3, 6, and 12 months on SGA medication.

3.3 Results

3.3.1 Study Cohort

As this was a multi-site study, participant inclusion into the study was in accordance with guidelines from the relevant research ethics committees (REC). These included the RECs in Linn Dara Child and Adolescent Mental Health Services, Our Lady's Children's Hospital Crumlin, Tallaght University Hospital, Children's University Hospital at Temple Street, St. Patrick's Mental Health Services, North Dublin City and County Child and Adolescent Mental Health Services, and St. Michael's House. However, despite involvement of many sites, the participants that were recruited to the study were all recruited from Linn Dara Child and Adolescent Mental Health Services and Beechpark Autism Services.

Children and adolescents attending Linn Dara Child and Adolescent Mental Health Services, both inpatient and out-patient, as well as Beechpark Autism Services who were about to commence treatment with SGA medication were recruited. The Linn Dara child and adolescent psychiatry service covers South Dublin, West Wicklow and Kildare. Children aged 6-16 years were categorised according to the International Obesity Task Force and the Centre for Disease Control and Prevention (CDC) cut-offs for lean, overweight, and obese as depicted in Table 1.1. Each participant who met study inclusion criteria (described in Section 2.3.1) had an assessment (See Appendix 6) which included anthropometric data (weight, height, and BMI percentile). Phlebotomy sampling was carried out in the fasting state.

To characterise the cardiometabolic risk factors of the SGA cohort at baseline, data on blood glucose, insulin resistance and lipid profile were collected and analysed. The participants were followed up at 3 months, 6 months, and 12 months and data on BMI percentile, blood glucose, insulin resistance, and lipid profile were collected and analysed.

3.3.2 Study Cohort: Participant Demographics

There were 19 participants recruited to the study. Ten participants completed the full study and data was collected at all four time points of the study period. Two participants completed three months of follow up. Seven participants left the study after the first time-point at baseline. This occurred when SGA medication was not commenced as planned, or when the SGA was discontinued early due to side effects, e.g., seizures. Figure 3.1 below depicts the number of participants recruited to the study, the points that some participants left the study and the numbers included in the final analysis. Table 3.1 below depicts the age range and gender of participants recruited to the study. Table 3.2 depicts the number of follow up visits completed by participants. Table 3.3 shows the age range, gender, and mean BMI percentile of the control group.

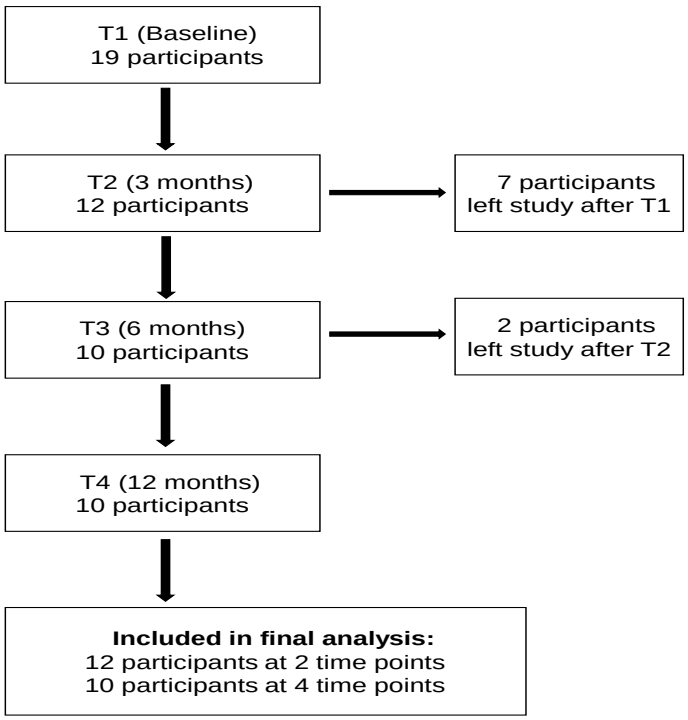


Figure 3.2: Flow chart of study participants

Flow diagram depicting the number of participants recruited to the study, the points where participants left the study and the numbers included in the final analysis

Table 3.1: Age range and gender of participants recruited to the study (N = 19)

Age range	7-10 years	11-14 years	15-18 years
Number of males	2 (10.5%)	8 (42.1%)	3 (15.8%)
Number of females	0 (0%)	3 (15.8%)	3 (15.8%)

Table 3.2: Table depicting number of follow up visits completed by participants

	T1 Baseline	T2 3 months	T3 6 months	T4 12 months
No of females	6 (31.58%)	3 (15.7%)	2 (10.5%)	2 (10.5%)
No of males	13 (68.42%)	9 (47.37%)	8 (42.1%)	8 (42.1%)

Table 3.3: Table depicting age range, gender, and mean BMI of control group

	Age range	Number of males	Number of females	Mean BMI percentile
Control group	9-15	10	6	37th

3.3.3 Study cohort: SGAs prescribed

Risperidone was the most commonly prescribed SGA in this cohort of participants and irritability/agitation in ASD was the most common reason for prescription of an SGA in this cohort. Table 3.4 below shows the medications prescribed in this cohort. Table 3.5 shows the indications for commencing the SGA medication.

Table 3.4: SGA medication prescribed

Medication	Number of participants	% of cohort
Risperidone	6	31.6 %
Aripiprazole	4	21 %
Olanzapine	3	15.8 %
Quetiapine	3	15.8 %
Switch between SGAs during follow up (Risperidone to Aripiprazole)	3	15.8 %
Did not commence SGA	2	10.5 %

Table 3.5: Reason for commencing SGA medication

Reason for prescribing	Number of participants	% of Cohort
Irritability/agitation in ASD	12	63.2 %
Psychosis/ query prodromal	5	26.3%
Tics	1	5.3 %
Emotionally Unstable Personality Disorder	1	5.3 %

3.3.4 Weight gain during treatment with SGAs

Overall, SGAs caused weight gain within the first few months of commencing treatment (Figure 3.3). The following results are for the participants that completed the twelve months of follow up (n=10).

At baseline, BMI percentile ranged from the 12th percentile to the 87th percentile with a mean at the 55th percentile. At three months, BMI percentile ranged from the 53rd percentile to the 97th percentile with a mean at the 74th percentile. At six months, BMI percentile ranged from the 41st percentile to the 99th percentile with a mean at the 78th percentile. At twelve months, BMI percentile ranged from the 44th to the 100th percentile with a mean at the 75th percentile.

At baseline, 30% of these participants had a baseline BMI above the 80th percentile and by six months, 70% of the participants had a BMI percentile above the 80th percentile. Most of the weight gain occurred in the first three months, with BMI percentile plateauing and reducing by twelve months. Overall, however, BMI percentile remained higher than baseline levels. In the following figure (Figure 3.3), data are presented on 12 participants at baseline and 3 months and then 10 participants at all four time points. This is because 10 participants completed the full 12-month follow up and a further two participants completed follow up to three months.

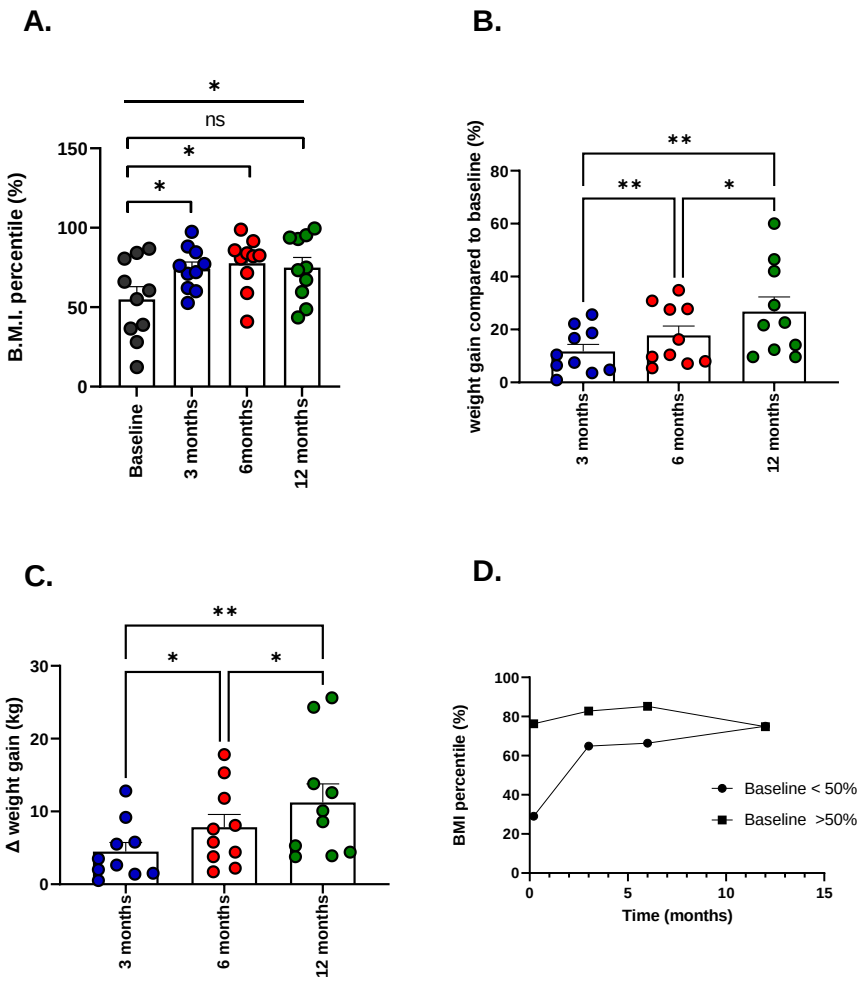


Figure 3.3: BMI percentile pre- and post-treatment with SGA medications

(A) Scatter plot showing BMI percentile at baseline, 3 months, 6 months and 12 months (n=10) **(B)** Scatter plot showing % weight gain at 3 months, 6 months and 12 months compared to baseline weight **(C)** Scatter plot showing the change in weight between baseline and 3 months, 6 months and 12 months **(D)** Mean BMI percentile at baseline, 3 months, 6 months and 12 months (n=10), the participants have been split into two groups according to baseline BMI percentile (n =4 <50th/n=6 >50th) this graph shows the trajectory of weight between the two groups. Statistical analysis performed using repeated measures ANOVA with multiple comparisons, ns – not significant, * = p<0.05, ** = p<0.01

3.3.5 Lower BMI percentile at baseline resulted in more weight gain

Participants with a lower BMI percentile at baseline gained significantly more weight when compared to participants with a higher BMI percentile at baseline (Figure 3.4A) (see Methods section 2.8). We also looked at the differences in weight gain according to the SGA prescribed (risperidone or aripiprazole). There was no difference in weight gain between the two medications (Figure 3.4B). The data show that lower baseline BMI percentile resulted in more weight gain and could potentially be a risk factor for rapid weight gain on SGA medication.

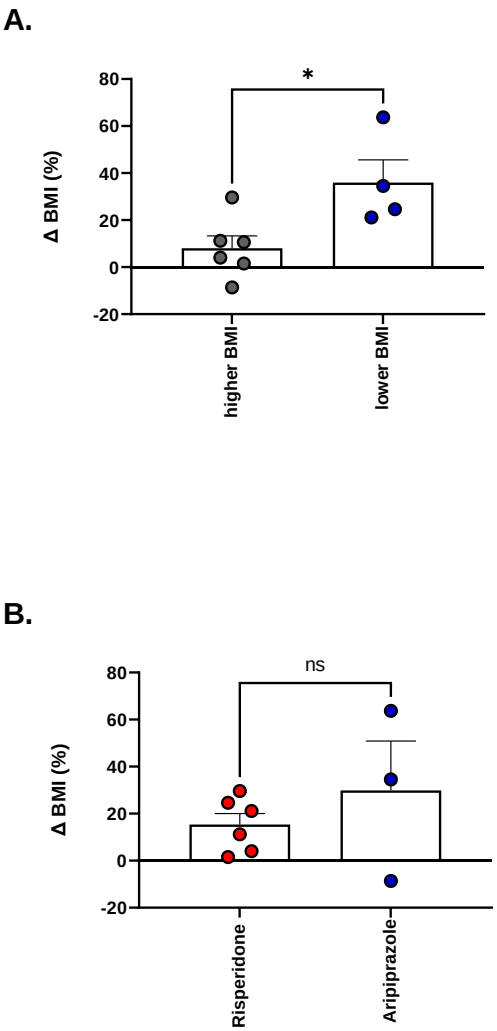


Figure 3.4: The effect of baseline BMI and medication type on weight gain

Scatter plots showing: **(A)** The change in BMI percentile between baseline and 3 months, the participants have been split into two groups: those with a baseline BMI percentile > 50th and those with a baseline BMI percentile <50th **(C)** The change in BMI percentile between baseline and 3 months, the participants have been split into two groups according to medication prescribed (risperidone or aripiprazole). Statistical analysis performed using Mann Whitney test, ns – not significant, ** = $p<0.01$

3.3.6 Lipid profile during treatment with SGAs

Overall, treatment with SGA medication did not have a significant impact on the lipid profiles of the participants in the study, as can be seen in Figure 3.5.

There was no difference in total cholesterol levels (Figure 3.5A), triglyceride levels (Figure 3.5B), LDL-cholesterol levels (Figure 3.5C), or HDL-cholesterol levels (Figure 3.5D) between the baseline time point and the follow up time-points on SGAs. The data show that SGAs did not have a significant impact on lipid profile.

Even though there were no significant increases in lipid profile overall, some of the participants had abnormal lipid profiles both at baseline and on SGAs. Regarding total cholesterol, at baseline 40% of participants (n=4) had a total cholesterol level that was higher than the normal range (>4.5 mg/dL). By the twelve-month time point this had increased to 60% of participants. For triglycerides, 10% of participants (n=1) had raised triglycerides at baseline (> 1.4 mg/dL). At the three-month time-point, this increased to 30% of participants and this reduced back down to 10% at six months. In relation to LDL-cholesterol, 30% of participants had high LDL-cholesterol at baseline (>3 mg/dL). This reduced to 20% after three months. For HDL-cholesterol, 40% of participants (n=4) had HDL-cholesterol levels that were below the normal range (<1.5 mg/dL). This increased to 80% of participants by the end of the study (Figure 3.5D).

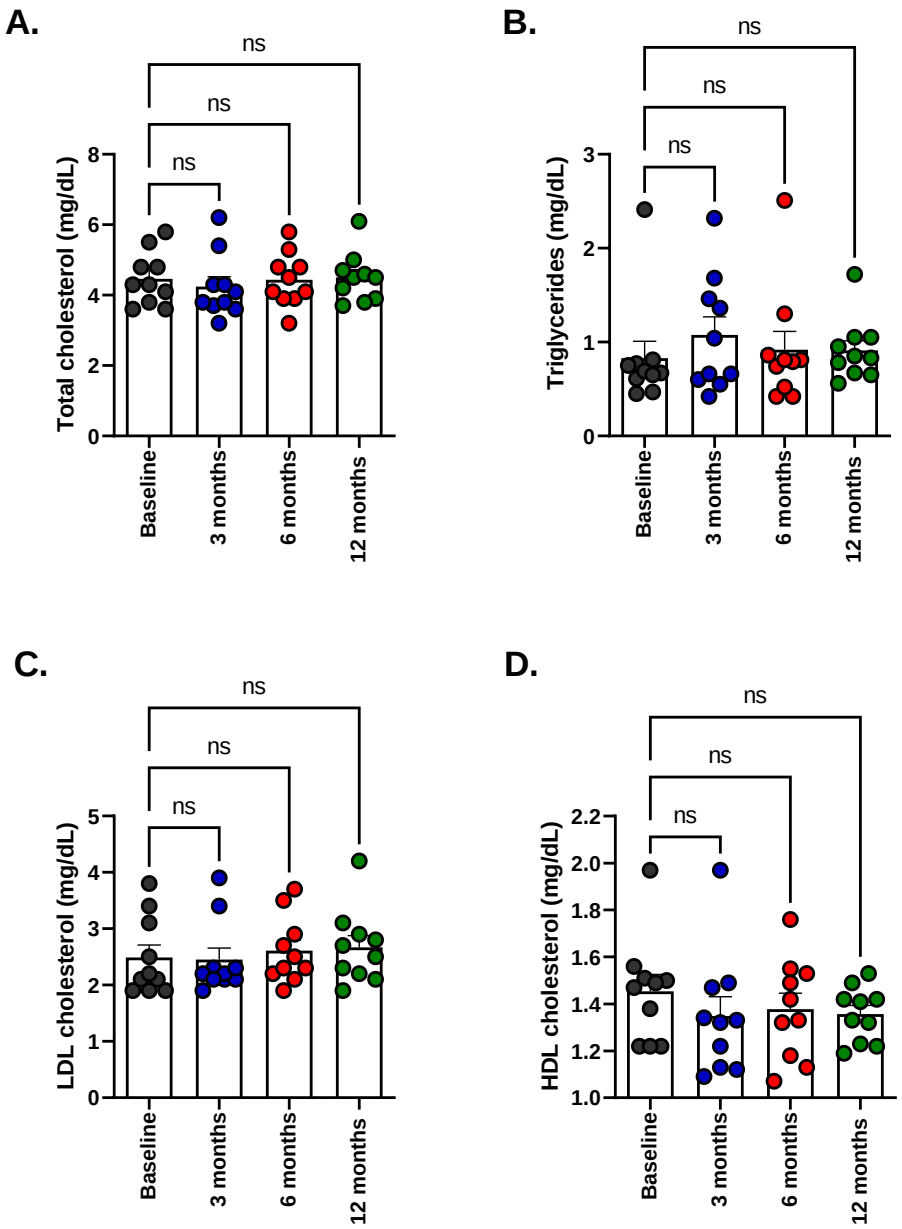


Figure 3.5: Lipid profiles pre- and post-treatment with SGA medications

Scatter plots showing: **(A)** Total cholesterol at baseline, 3 months, 6 months, and 12 months on SGAs (n=10) **(B)** Triglycerides at baseline, 3 months, 6 months, and 12 months on SGAs (n=10) **(C)** LDL-cholesterol at baseline, 3 months, 6 months, and 12 months on SGAs (n=10) **(D)** HDL-cholesterol at baseline, 3 months, 6 months, and 12 months on SGAs (n=10) Statistical analysis performed using repeated measures ANOVA with multiple comparisons and Friedman test with multiple comparisons (LDL and triglycerides), ns – not significant

3.3.7 Glucose regulation at baseline and on SGAs

Overall, treatment with SGA medication did not have a significant impact on fasting glucose, fasting insulin, HOMA-IR or HbA1c, as can be seen in Figure 3.6.

The normal reference range for fasting glucose is less than 5.6 mmol/L. Levels from 5.6 to 6.9 mmol/L indicate impaired fasting glucose and is considered pre-diabetes. At baseline, fasting glucose ranged from 4.3 mmol/L to 5.5 mmol/L with a mean of 5.1 mmol/L. At three months, fasting glucose ranged from 4.6 mmol/L to 5.9 mmol/L with a mean of 5.1 mmol/L. At six months, fasting glucose ranged from 4.4 mmol/L to 5.9 mmol/L with a mean of 5.1 mmol/L. At twelve months, fasting glucose ranged from 4.5 mmol/L to 6.4 mmol/L with a mean of 5.1 mmol/L. While at baseline no participants had an elevated blood glucose, it can be seen from the range of values that by three months there were participants with increased fasting glucose, and this continued to increase up to the end of the study period (Figure 3.6A).

The normal reference range for fasting insulin is <25mlU/L. At baseline, fasting insulin ranged from 9mlU/l to 27mlU/L with a mean of 19.5mlU/L. At three months, fasting insulin ranged from 21mlU/L to 109mlU/L with a mean of 50mlU/L. At six months, fasting insulin ranged from 5.8mlU/L to 105mlU/L with a mean of 39.8mlU/L. At twelve months, fasting insulin ranged from 5.7mlU/L to 88mlU/L with a mean of 44mlU/L (Figure 3.6B).

At baseline, HOMA-IR scores ranged from 1.96 to 6.4 with a mean of 4.8. On SGA medication, HOMA-IR scores ranged from 3.4 to 20.3 with a mean of 8.7 (Figure 3.6C).

Overall, there was no difference in HbA1c level pre-SGA medication and on SGA medication. At baseline, HbA1c ranged from 31mmol/mol to 38mmol/mol with a mean of 35.9mmol/mol. On SGAs, HbA1c ranged from 28mmol/mol to 39mmol/mol with a mean of 34.4mmol/mol (Fig 3.6D)

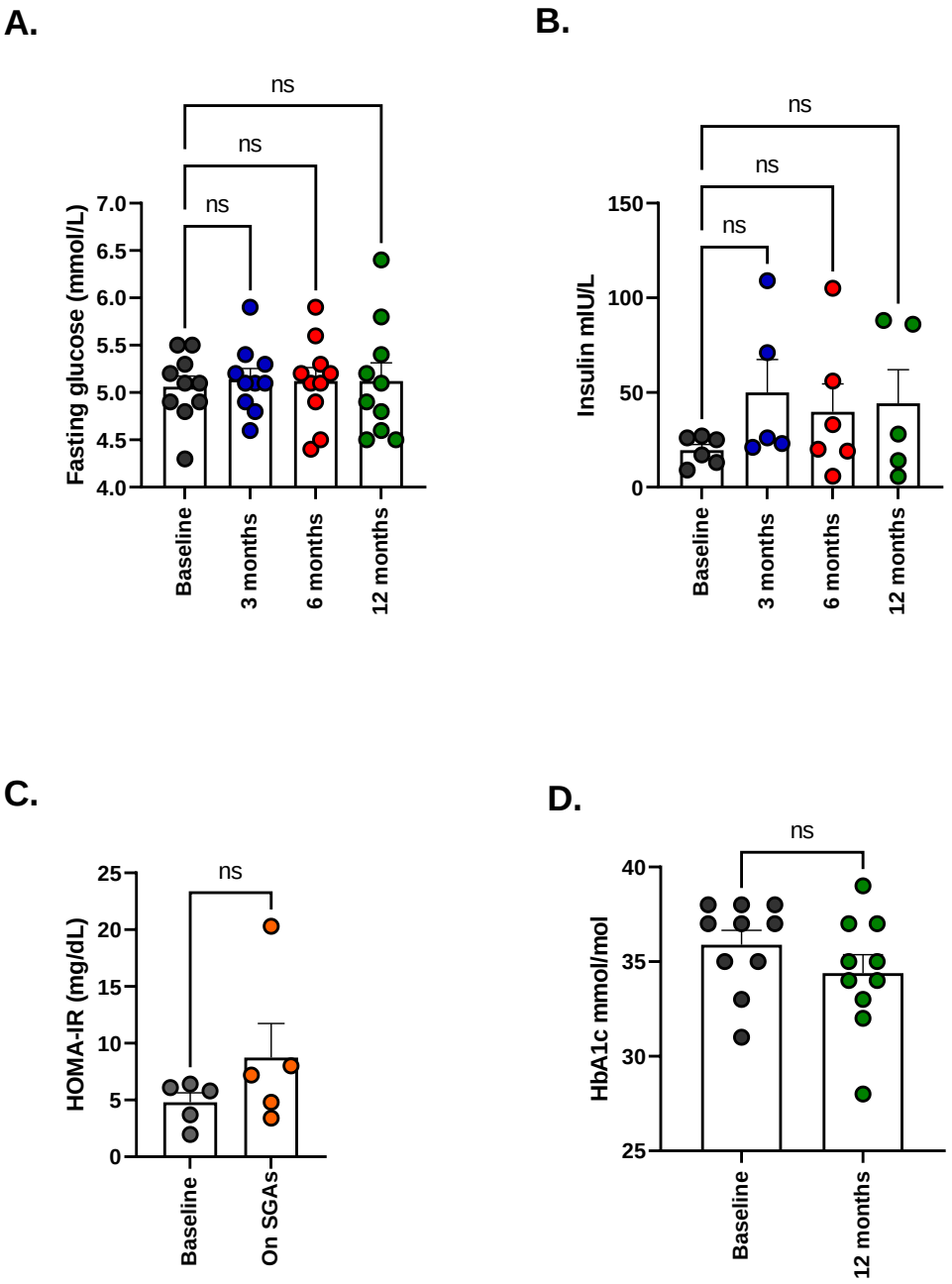


Figure 3.6: Glucose regulation pre- and post-treatment with SGA medications

Scatter plots showing: **(A)** Fasting glucose at baseline, 3 months, 6 months, and 12 months on SGAs (n=10) **(B)** Fasting insulin at baseline, 3 months, 6 months, and 12 months on SGAs (n=10) **(C)** HOMA-IR at baseline and on SGAs (n=5) **(D)** HbA1c at baseline and on SGAs (n=10). Statistical analysis performed using repeated measures ANOVA with multiple comparisons, Friedman test with multiple comparisons and paired t-test, ns – not significant

3.3.8 The influence of medication type, weight gain and BMI at baseline on changes in lipid profile and glucose regulation

The type of medication prescribed, risperidone or aripiprazole, had no significant effect on change in total cholesterol, LDL-cholesterol, HDL-cholesterol, or triglycerides (Figure 3.7 A-D). Medication type also had no significant effect on fasting glucose and HbA1c (Figure 3.8 A-B).

The cohort was split into two groups, those who gained weight in the first three months (>5 BMI percentile) and those that did not gain weight (see Methods section 2.7). The participants who gained weight did not have increased lipids compared to the participants who did not gain weight (Figure 3.9 A-D). The impact of weight gain on fasting glucose and HbA1c was investigated by comparing glucose and HbA1c levels in the group who gained weight with the group who did not gain weight. The participants who gained weight did not have increased fasting glucose or HbA1c compared to the participants who did not gain weight (Figure 3.10 A-B).

We compared the change in lipid profile between baseline and three months in participants with a low BMI percentile at baseline (<50%) and participants with a baseline BMI percentile that was higher than the 50th percentile. There were no differences in the change in total cholesterol, LDL-cholesterol, HDL-cholesterol or triglyceride levels on SGAs (Figure 3.11 A-D). We also compared fasting glucose and HbA1c according to the BMI percentile at baseline (see Methods section 2.7). There were no differences in fasting glucose or HbA1c levels on SGAs between participants with a higher baseline BMI percentile and those with a lower baseline BMI percentile (Figure 3.12 A-B).

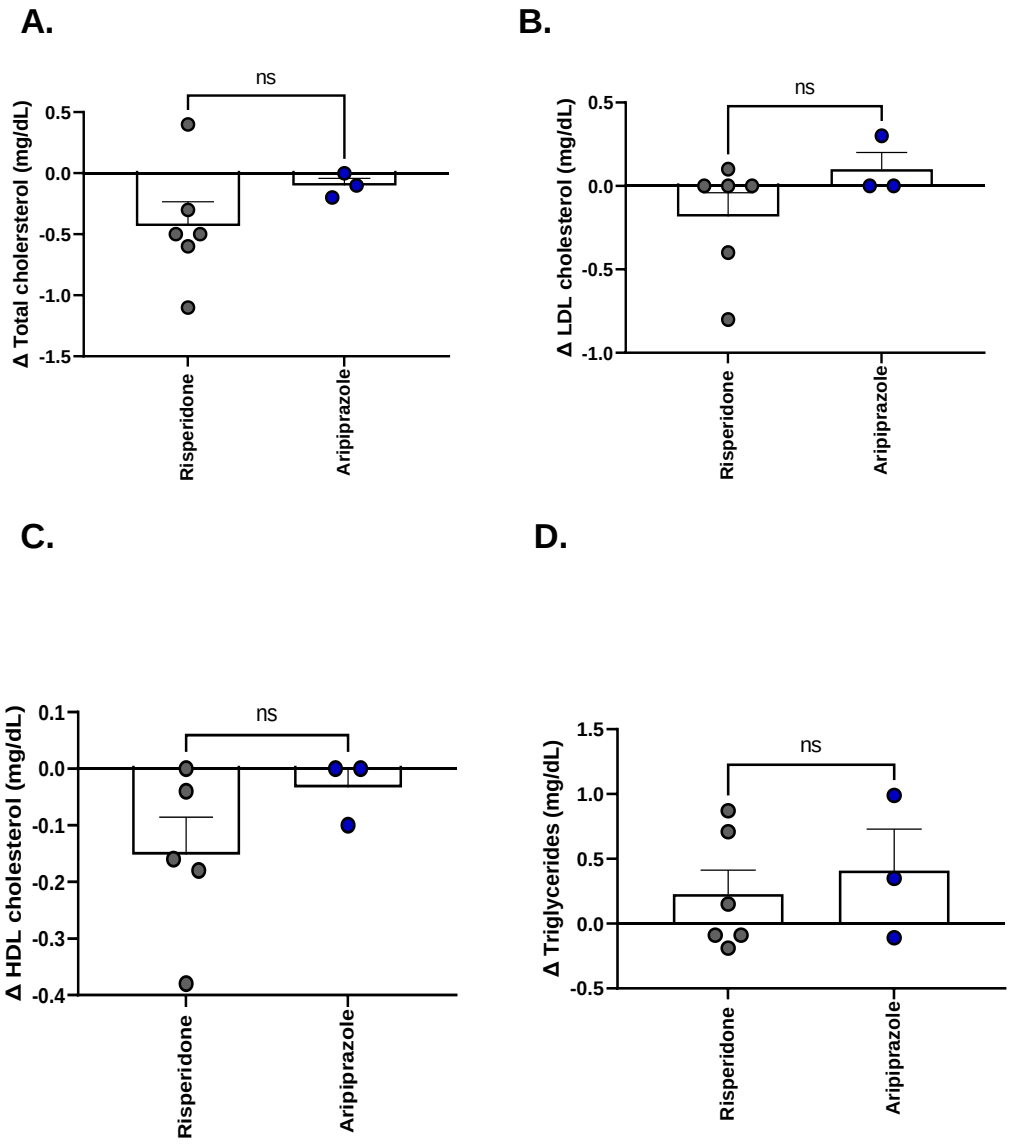


Figure 3.7: The effect of medication type on lipid profile

Scatter plots showing: **(A)** the change in total cholesterol between baseline and 3 months, the participants have been split into two groups according to medication prescribed (risperidone or aripiprazole). **(B)** the change in LDL-cholesterol between baseline and 3 months, the participants have been split into two groups according to medication prescribed (risperidone or aripiprazole). **(C)** the change in HDL-cholesterol between baseline and 3 months, the participants have been split into two groups according to medication prescribed (risperidone or aripiprazole). **(D)** the change in triglycerides between baseline and 3 months, the participants have been split into two groups according to medication prescribed (risperidone or aripiprazole). Statistical analysis performed using Mann Whitney test, ns – not significant

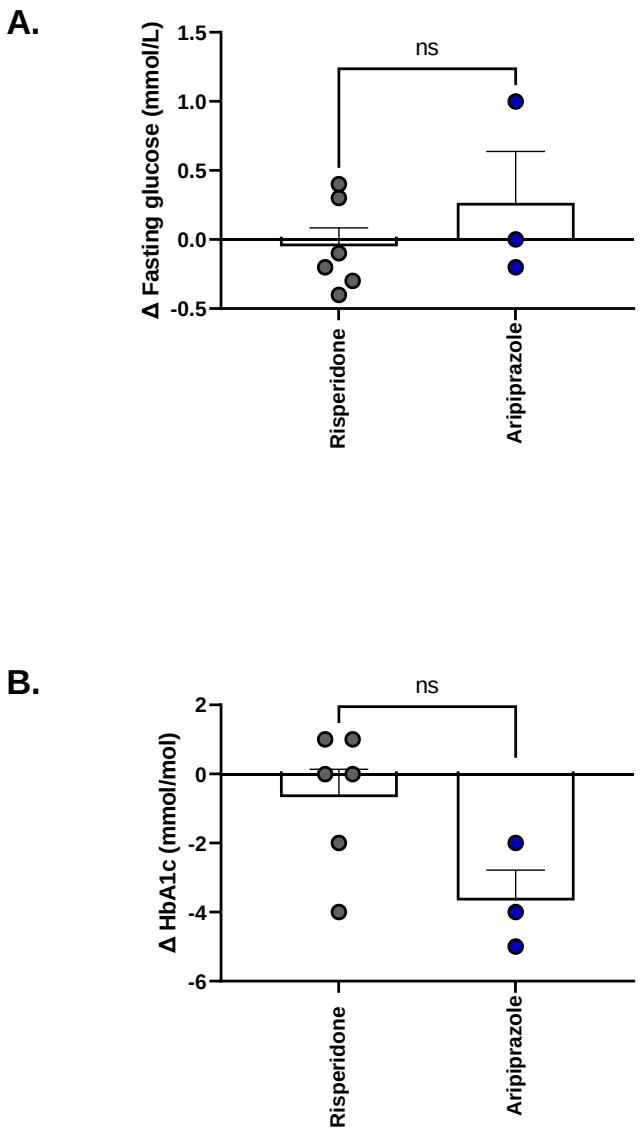


Figure 3.8: The effect of medication type on glucose regulation

Scatter plots showing: **(A)** the change in fasting glucose between baseline and 3 months, the participants have been split into two groups according to medication prescribed (risperidone or aripiprazole) **(B)** the change in HbA1c between baseline and 3 months, the participants have been split into two groups according to medication prescribed (risperidone or aripiprazole). Statistical analysis performed using Mann Whitney test, ns – not significant

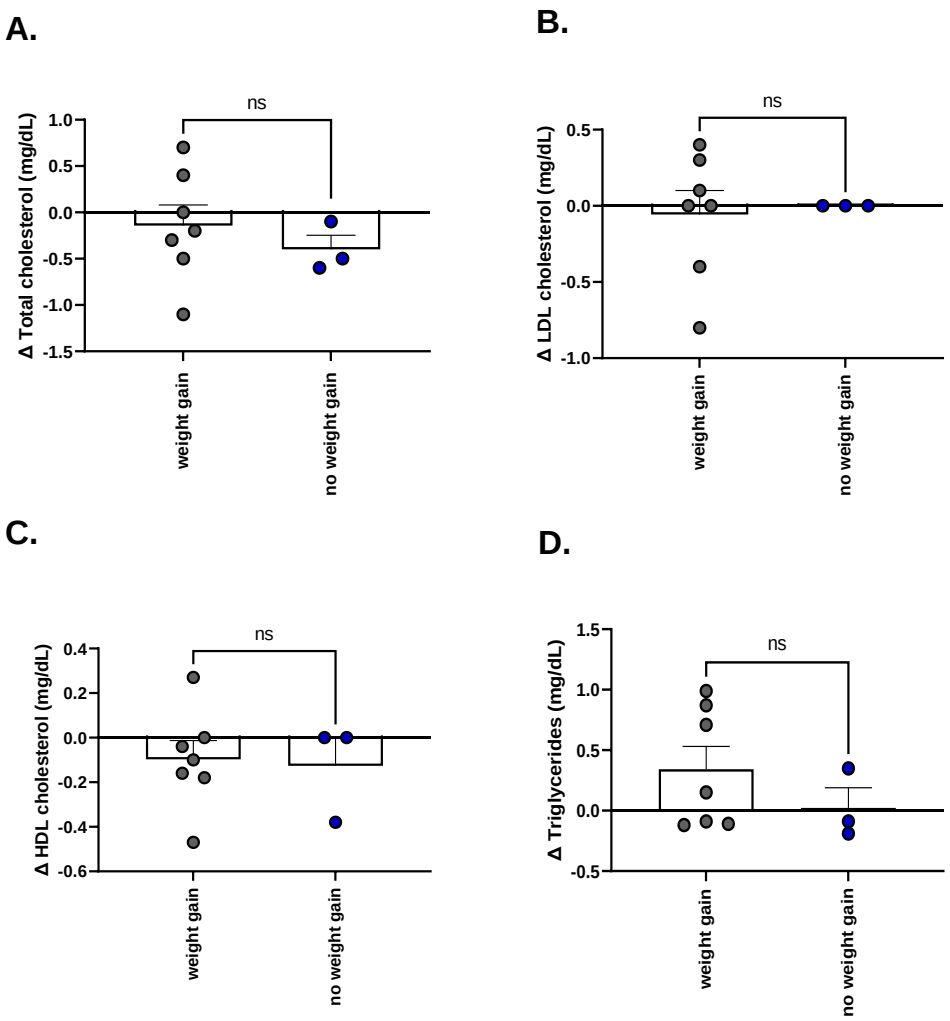
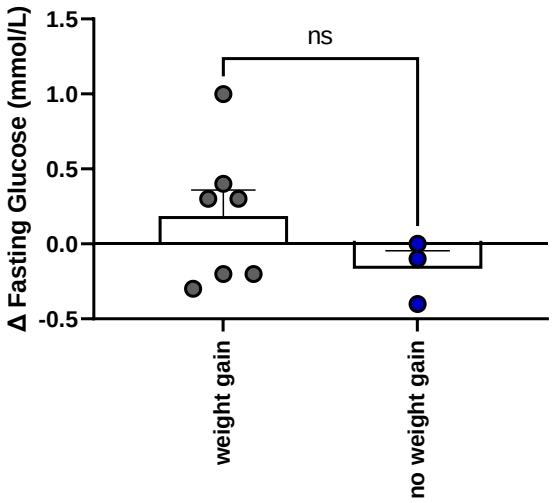


Figure 3.9: The effect of weight gain on lipid profile

Scatter plots showing: **(A)** the change in total cholesterol between baseline and 3 months, the participants have been split into two groups according to amount of weight gain **(B)** the change in LDL-cholesterol between baseline and 3 months, the participants have been split into two groups according to amount of weight gain **(C)** the change in HDL-cholesterol between baseline and 3 months, the participants have been split into two groups according to amount of weight gain **(D)** the change in triglycerides between baseline and 3 months, the participants have been split into two groups according to amount of weight gain. Statistical analysis performed using Mann Whitney test, ns – not significant

A.



B.

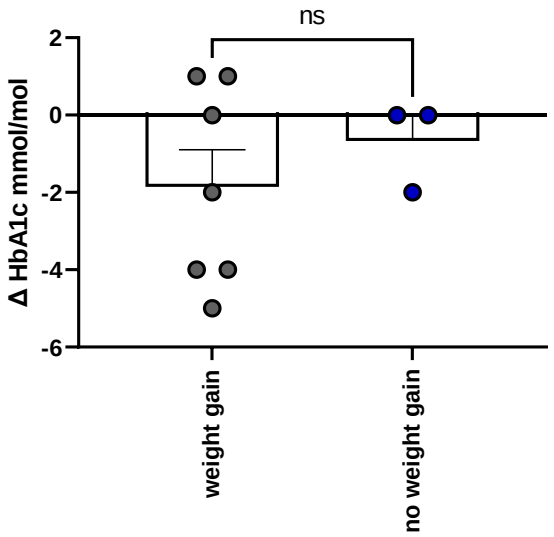


Figure 3.10: The effect of weight gain on glucose regulation

Scatter plots showing: **(A)** the change in fasting glucose between baseline and 3 months, the participants have been split into two groups according to amount of weight gain **(B)** the change in HbA1c between baseline and 3 months, the participants have been split into two groups according to amount of weight gain. Statistical analysis performed using Mann-Whitney test, ns – not significant

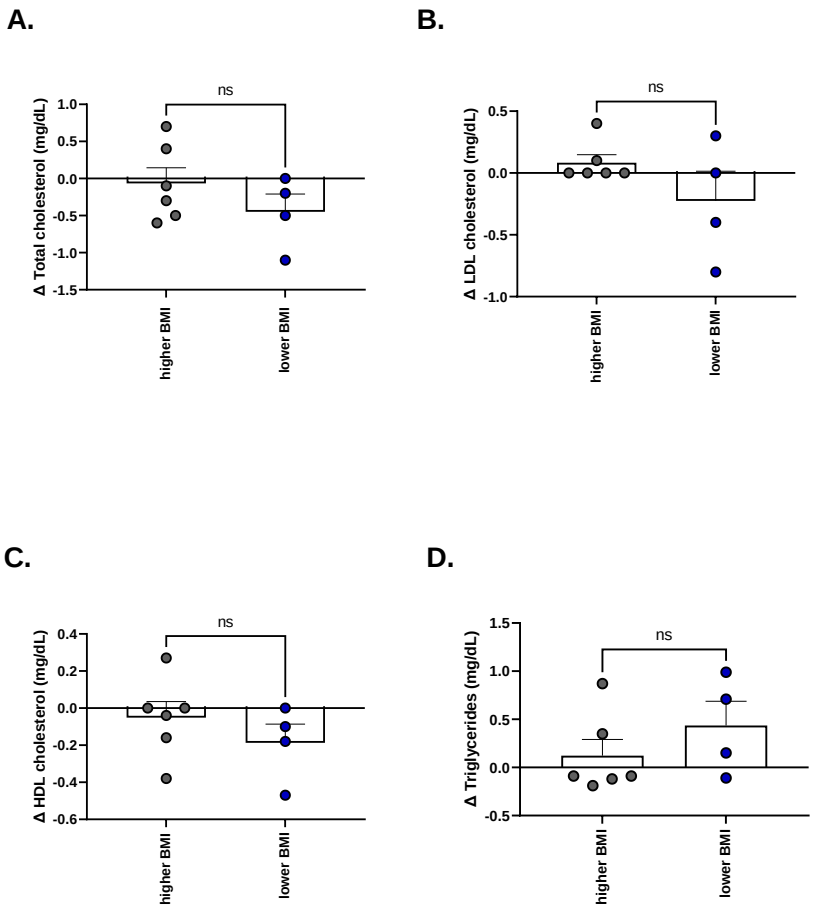
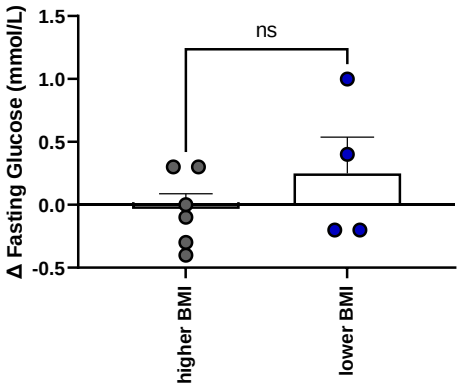


Figure 3.11: The effect of BMI at baseline on lipid profile

Scatter plots showing: **(A)** the change in total cholesterol between baseline and 3 months, the participants have been split into two groups according to BMI percentile at baseline **(B)** the change in LDL-cholesterol between baseline and 3 months, the participants have been split into two groups according to BMI percentile at baseline **(C)** the change in HDL-cholesterol between baseline and 3 months, the participants have been split into two groups according to BMI percentile at baseline **(D)** the change in Triglycerides between baseline and 3 months, the participants have been split into two groups according to BMI percentile at baseline. Statistical analysis performed using Mann-Whitney test, ns – not significant, * - $p < 0.05$

A.



B.

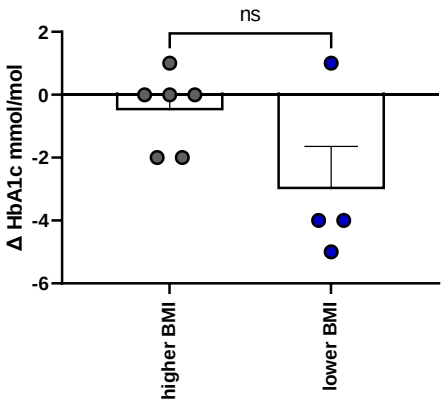


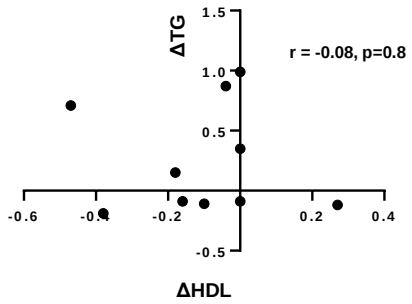
Figure 3.12: The effect of BMI at baseline on glucose regulation

Scatter plots showing: **(A)** the change in fasting glucose between baseline and 3 months, the participants have been split into two groups according to BMI percentile at baseline **(B)** the change in HbA1c between baseline and 12 months, the participants have been split into two groups according to BMI percentile at baseline. Statistical analysis performed using Mann-Whitney test, ns – not significant

3.3.9 Correlation between ΔHDL-cholesterol/ΔTriglycerides and ΔHDL-cholesterol/Δweight gain

We performed correlation analysis to investigate for correlation between the difference in HDL cholesterol (ΔHDL) between baseline and 3 months and the difference in triglycerides (ΔTG) between baseline and 3 months. We also investigated for correlation between the difference in HDL cholesterol (ΔHDL) between baseline and 3 months and the difference in weight (Δweight) between baseline and 3 months. There was no significant correlation between the difference in HDL cholesterol (ΔHDL) between baseline and 3 months and the difference in triglycerides (ΔTG) between baseline and 3 months (Figure 3.13A). There was no significant correlation between the difference in HDL cholesterol (ΔHDL) between baseline and 3 months and the difference in weight (Δweight) between baseline and 3 months (Figure 3.13B).

A.



B.

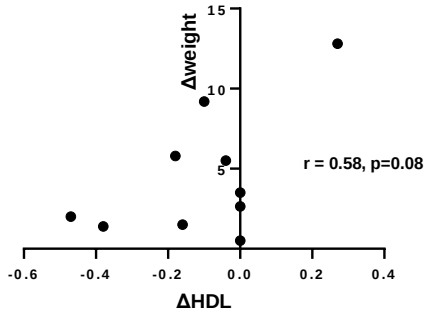


Figure 3.13: Correlation between ΔHDL-cholesterol/Triglycerides and ΔHDL-cholesterol /Δweight

Correlation plot of the change in HDL-cholesterol with **(A)** change in triglycerides and **(B)** change in weight. Statistical analysis performed using Pearson's correlation coefficient.

3.3.10 Correlation between ΔBMI/ΔHDL-cholesterol, ΔBMI/ΔLDL-cholesterol, ΔBMI/Δtriglycerides and ΔBMI/Δfasting glucose at 3 months, 6 months and 12 months

We performed correlation analysis to investigate for correlation between the difference in BMI percentile between baseline and 3 months, 6 months and 12 months and the difference between HDL-cholesterol, LDL-cholesterol, triglycerides and fasting glucose between baseline and 3 months, 6 months and 12 months.

There was no correlation between ΔBMI percentile and ΔHDL-cholesterol at 3 months and 6 months. There was a significant correlation between ΔBMI and ΔHDL-cholesterol at 12 months (Pearson's correlation coefficient 0.64, p=0.04) (Figure 3.14).

There was no correlation between ΔBMI percentile and ΔLDL-cholesterol at 3 months, 6 months and 12 months (Figure 3.15).

There was no correlation between ΔBMI percentile and Δtriglycerides at 3 months, 6 months and 12 months (Figure 3.16).

There was a significant correlation between ΔBMI and Δfasting glucose at 3 months (Pearson's correlation coefficient 0.698, p=0.02). There was no correlation between ΔBMI percentile and Δfasting glucose at 6 months and 12 months (Figure 3.17).

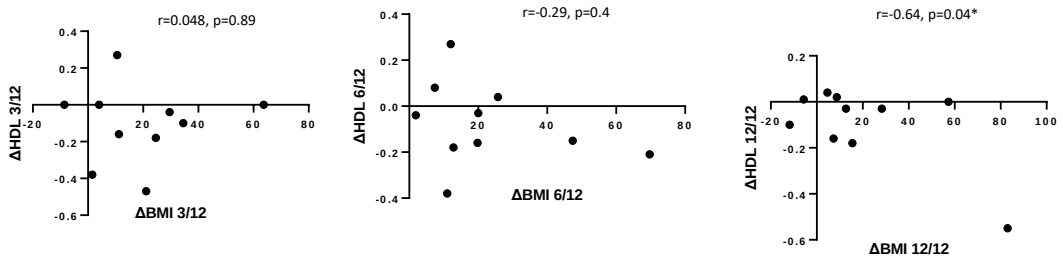


Figure 3.14: Correlation between ΔHDL and ΔBMI at 3 months, 6 months and 12 months

Correlation plot of the change in HDL-cholesterol with change in BMI percentile at 3 months, 6 months and 12 months. Statistical analysis performed using Pearson's correlation coefficient. * - p< 0.05

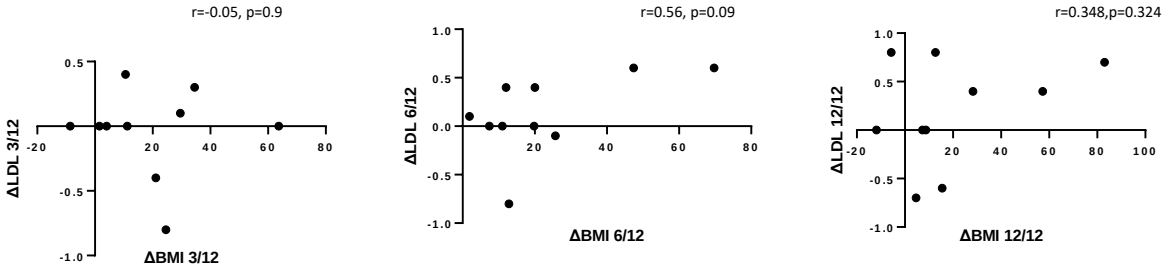


Figure 3.15: Correlation between ΔLDL and ΔBMI at 3 months, 6 months and 12 months

Correlation plot of the change in LDL-cholesterol with change in BMI percentile at 3 months, 6 months and 12 months. Statistical analysis performed using Pearson's correlation coefficient.

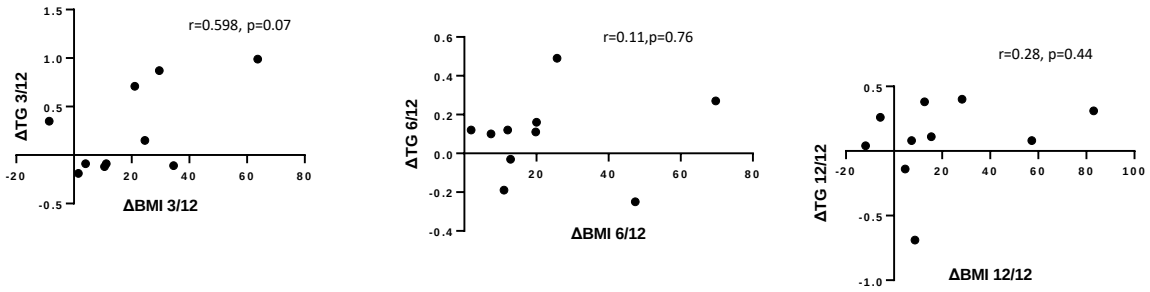


Figure 3.16: Correlation between ΔTriglycerides and ΔBMI at 3 months, 6 months and 12 months

Correlation plot of the change in triglycerides with change in BMI percentile at 3 months, 6 months and 12 months. Statistical analysis performed using Pearson's correlation coefficient.

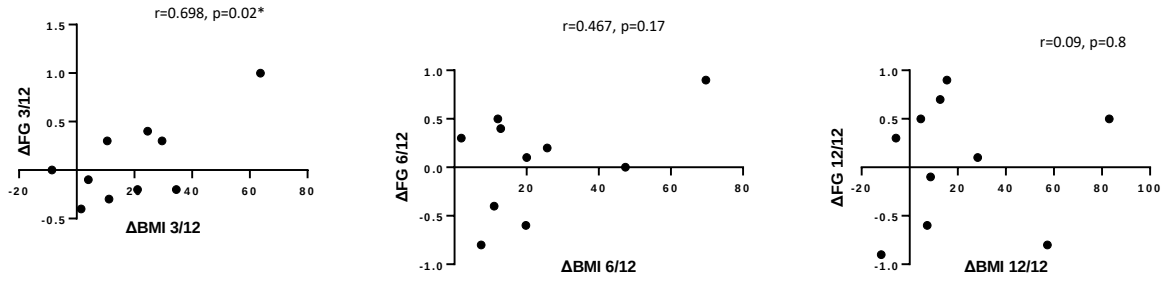


Figure 3.17: Correlation between Δfasting glucose and ΔBMI at 3 months, 6 months and 12 months

Correlation plot of the change in fasting glucose with change in BMI percentile at 3 months, 6 months and 12 months. Statistical analysis performed using Pearson's correlation coefficient. * - p< 0.05

3.3.11 Effect of SGAs on prolactin

SGAs caused a significant increase in prolactin levels compared to baseline, as can be seen in figure 3.18. Normal reference ranges for prolactin are 0-424mIU/L in males and 0-530mIU/L in females. At baseline, prolactin levels ranged from 133mlU/L to 220mlU/L with a mean of 164mlU/L. On SGAs, prolactin levels ranged from 20mlU/L to 1,759mlU/L with a mean of 598mlU/L. In 40% of the participants, there was a significant increase in prolactin. SGAs caused an increase in prolactin in this cohort (Figure 3.18A)

Medication type had the biggest influence on prolactin levels (Figure 3.18B). Prolactin increased significantly in the participants who were prescribed risperidone. The participants prescribed aripiprazole did not experience an increase in prolactin levels. As expected, weight gain and higher BMI percentile at baseline did not influence prolactin levels on SGAs (Figure 3.84C-D) (see Methods section 2.8).

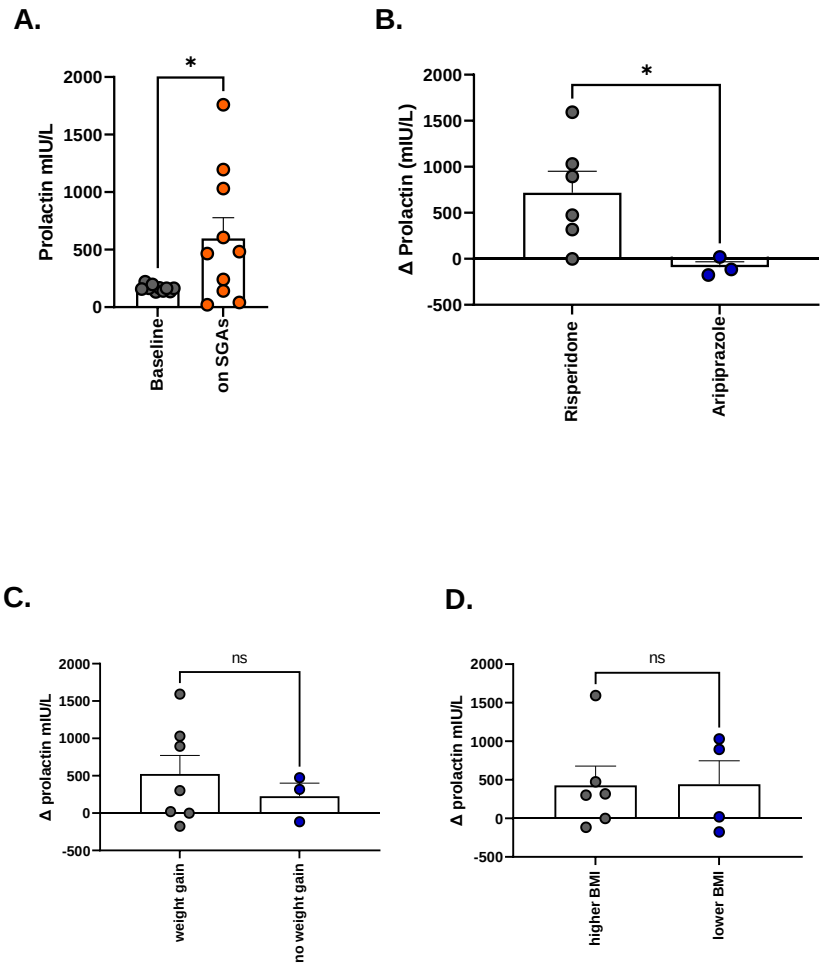


Figure 3.18: Prolactin levels pre- and post-treatment with SGA medications

Scatter plot showing: **(A)** Prolactin levels at baseline and on SGAs (n=10) **(B)** the change in Prolactin levels between baseline and 3 months in participants who were prescribed risperidone and aripiprazole **(C)** the change in Prolactin levels between baseline and 3 months in participants who gained weight in the first 3 months and participants who did not **(D)** the change in Prolactin levels between baseline and 3 months in participants who had a higher BMI at baseline (>50th centile) and a lower BMI at baseline (<50th centile), Statistical analysis performed using Mann-Whitney test, ns = non-significant, * = $p < 0.05$

3.4 Discussion

It is well established that SGAs cause weight gain and children and adolescents are at greater risk of rapid weight gain on SGAs than adults (82, 92, 94). In this study, we found that weight gain on SGAs occurred early on, within the first three months of treatment. BMI percentile plateaued and reduced by twelve months, but it remained higher than baseline levels. This is in keeping with the literature, which shows that while weight gain plateaus, it does not fall to baseline levels and remains elevated compared to same age peers (75, 76).

Baseline BMI has been investigated as a predictor of antipsychotic induced weight gain in a number of studies, but the results are mixed. We found that participants with a lower BMI at baseline gained more weight on SGAs than participants with a higher BMI at baseline.

Another study examining predictors of antipsychotic induced weight gain in medication naïve patients with first episode psychosis (n=99), compared to healthy controls (n=51) also found that a lower BMI at baseline was associated with antipsychotic-induced weight gain (100). A study conducted with adolescents looking at predictors of weight gain associated with olanzapine and risperidone (n=42) also found that low baseline BMI was positively correlated with weight gain (94).

Other studies have found that higher baseline BMI is a risk factor for the cardiometabolic side effects of SGAs (93, 97, 99), however we did not find this in our study. Gebhardt et al identified that high BMI prior to commencing SGA medication was a significant predictor of weight gain with SGAs. This study was done retrospectively (n=65). The authors discussed that there were methodological limitations that meant that data on premorbid and pre-antipsychotic assessed retrospectively and by face-to-face interview and might not be fully sufficient for exact statistical calculations.

Another study examined risk factors and patterns of weight gain in youth on SGAs (99). This study prospectively followed up children on SGAs (n=54) for a six-month period and they also retrospectively, over a five-year period, collected data for children and adolescents that had been prescribed SGAs (n=90). Overall, in the retrospective study, they found that a higher baseline BMI z-score was

associated with greater weight gain. They found that a lower baseline BMI during the first 15 weeks of treatment resulted in a faster increase in weight which was what we also found in our study. When they combined the variables in the final analysis, they concluded that higher baseline BMI was a predictor of greater weight gain on SGAs.

Due to discrepancies in the literature about the effect of baseline BMI on weight gain with SGAs, there is a need for future well designed prospective studies to investigate this. We found that participants with a lower baseline BMI gained more weight. This is concerning as at present clinicians are likely to be much less concerned about weight gain in children who are at a lower baseline BMI when starting SGA medication. However, our study, along with Sadiccha et al and Ratzoni et al have revealed a genuine increase in risk of rapid weight gain in this lower BMI population, and clinical education is urgently needed (94, 100).

Despite the significant increase in BMI percentile found in this study, it did not result in increases in mean values of total cholesterol, LDL-cholesterol or triglycerides or decreased HDL cholesterol. Overall, SGA treatment did not cause a significant increase in fasting glucose, fasting insulin, HOMA-IR or HbA1c in the first 12 months in this study group. In childhood obesity, florid metabolic disease is usually absent and there is a time delay before the metabolic disease develops. However, in both childhood and adult obesity, a similar pro-inflammatory phenotype is observed which leads to the later development of metabolic disease. Early presence of this inflammatory phenotype is therefore likely to lead to much earlier onset of metabolic disease for children treated with SGAs. The following chapters of this thesis investigate the emergence of a pro-inflammatory phenotype in our study population.

LDL-cholesterol is “bad cholesterol”. High levels of LDL-cholesterol increase the risk of myocardial infarction and stroke. HDL-cholesterol is “good cholesterol”, it absorbs cholesterol and carries it back to the liver. Higher HDL-cholesterol can lower the risk of myocardial infarction and stroke (202). In this study, 40% of participants (n=4) had HDL-cholesterol levels that were below the normal range (<1.5 mg/dL) at baseline, and this increased to 80% of participants by the end of the study. It is concerning that 8 out of the 10 participants had HDL-cholesterol

levels that were below the normal cut off. Low HDL-cholesterol is a diagnostic criterion of the metabolic syndrome and signifies an increased risk of cardiovascular disease. This highlights that SGAs have a negative impact on cardiovascular and metabolic health in a relatively short time-frame and in a cohort that are a young age. In terms of metabolic risk factors, the results of this study differed from the audit completed in the regional specialist ASD service in Dublin, Kildare and Wicklow where we found that there were more overt metabolic complications (increased LDL and triglycerides as well as low HDL) in the audit population. This may reflect the negative trajectory of metabolic complications over a longer time-frame. Many of the young people attending this service had been on SGAs for more than one year and in many cases, they would have been on SGAs for many years.

In this study, there were similar concerning results in relation to glucose regulation, evidenced by changes in fasting glucose levels, fasting insulin levels, and overall insulin resistance. At baseline, all the participants in the study had fasting glucose levels within the normal range (< 5.6 mmol/l). At three months, 10% of participants (n=1) had an increased fasting glucose level and this increased to 20% of participants at the six and twelve-month follow up points. Even though these are low numbers, the trend is concerning.

In obese children, hyperinsulinemia is an early marker for cardiovascular and metabolic disease risk and compensates for insulin resistance in the early stages (203). In this study, at baseline 20% of participants (n=2) had fasting insulin levels that were just above the normal range (>25mIU/l). These participants were not overweight at baseline. Therefore, even prior to commencing SGAs, some of these children were already at increased risk of metabolic disease. At three, six and twelve months, 30% of participants had fasting insulin levels that were above the normal range. The HOMA-IR score, a measure of insulin resistance ranging from 0 – 15 was measured in 50% of children in this study. It was not possible to calculate HOMA-IR in all participants at all time points as fasting insulin was the last sample taken. This was so that the clinical blood samples that were requested by the prescribing clinician could be obtained as well as the research blood samples (2-3 extra tubes). Therefore, the insulin levels were not obtained in some cases. All participants had a HOMA-IR in the normal range at baseline.

After commencing SGAs, one participant developed a high HOMA-IR. The numbers in this study are small, however it is concerning that such a significant metabolic change occurred over the time-frame of the study as this indicates that children on SGAs are at increased risk of Type 2 Diabetes Mellitus (T2DM).

In Ireland, the population prevalence of T2DM in adults aged 50 and over is 8.4%. T2DM is associated with microvascular complications affecting small blood vessels e.g., in the kidney and eyes, and macrovascular complications affecting larger vessels and leading to cardiovascular disease. In this Irish population studied, the prevalence of microvascular complications was 26% and the presence of macrovascular complications was 15.1% (204). The prevalence of T2DM in the paediatric and young adult population in Ireland has not been studied, however a small cross-sectional survey of adolescents estimated the prevalence of T2DM in children under 16 years as 1.2 per 100,000 (205). Longer duration of T2DM leads to an increased risk of micro and macrovascular complications, therefore children with T2DM are at much greater risk of these complications in earlier adulthood (206). T2DM in adolescence has been found to be a strong predictor of premature death in adulthood (207). Currently, almost 25% of Irish children are overweight or obese (15). If this increasing trend of childhood overweight and obesity continues, the prevalence of T2DM in the paediatric and young adult population in Ireland may increase leading to a significant morbidity for these patients due to the complications associated with T2DM including cardiovascular disease, cerebrovascular disease, and renal disease at a younger age. This would cause a significant burden for health services. As SGA medication use increases the risk of T2DM, there is a need for careful monitoring of lipids, glucose, and insulin levels in children and adolescents who are prescribed SGAs and appropriate management of any abnormalities that arise.

We examined the change in a number of metabolic parameters in the first three months on SGAs, therefore some of the values are below zero which indicates a decrease between baseline and three months.

The influence of the type of SGA (risperidone or aripiprazole) on weight gain, lipid profile, glucose regulation, and prolactin levels were investigated in the study.

Medication type did not influence weight gain, lipid profile, or glucose regulation but it did affect prolactin levels. Previous studies have shown that aripiprazole is associated with a lower risk for weight gain, however we did not find that to be the case in this study (82, 92).

The type of medication prescribed, risperidone or aripiprazole, had no differential effect on fasting glucose or HbA1c. The data show that weight gain did not impact the lipid profile or glucose regulation of this cohort in the time period of the study. Baseline BMI did not impact the changes in total cholesterol, LDL-cholesterol, HDL-cholesterol, triglycerides or glucose regulation in this cohort in the time period of the study. The study followed these participants up for twelve months so it is possible that a longer follow up period would find differences in LDL cholesterol and glucose regulation. This study had a low sample size with ten participants completing the full study, therefore it may not have been possible to detect an effect due to the low numbers.

As the participants were followed up for one year after commencing SGAs, we were able to use each participant's baseline as their own control. To do this we measured the change in variables at 3 months, 6 months and 12 months on SGAs. In some cases, the values of the variables decreased on SGAs which resulted in minus values for the change in variables. We performed correlation analysis and examined for correlation between the change in BMI percentile and HDL-cholesterol, LDL-cholesterol, triglycerides and fasting glucose at 3 months, 6 months and 12 months. We found a significant correlation between the change in BMI percentile and the change in HDL cholesterol at 12 months only. We also found a significant correlation between the change in BMI percentile and fasting glucose at three months only. The greatest increase in BMI percentile happened in the first three months which may be a possibility for the correlation with change in fasting glucose.

The finding of significantly increased prolactin levels associated with risperidone in this study was quite striking. The mechanism for antipsychotic induced hyperprolactinaemia is well understood. Secretion of prolactin is controlled by dopaminergic inhibition from the anterior pituitary gland. Antipsychotics cause dopamine blockade in the tubero-infundibular tract of the hypothalamus, which

results in reversal of the dopaminergic inhibition of prolactin in the anterior pituitary and a corresponding increase in prolactin release (208). The participants on risperidone had higher prolactin levels on SGA treatment than participants on aripiprazole. This is not surprising as aripiprazole is a partial dopamine agonist and is often used as an adjunctive treatment when prolactin levels are raised on SGAs. This is because it does not tend to cause high prolactin due to the partial dopamine agonism (209, 210). While it was not surprising that risperidone caused greater prolactin levels, SGA associated hyperprolactinaemia has not been well studied in children. This is surprising as hyperprolactinaemia in children may lead to osteoporosis and osteoporotic fractures, as has been described in people with schizophrenia on antipsychotics and in adults with intellectual disability on antipsychotics in the IDS TILDA study (137, 211, 212). Osteoporosis results from direct and indirect effects of high prolactin. Firstly, the increased prolactin directly affects osteoblasts resulting in a reduction in proliferation of these cells. This may lead to reduced bone formation and osteoporosis. Secondly, increased levels of prolactin also inhibit release of GnRH. This leads to a reduction in FSH and LH which reduces levels of oestrogen and testosterone. This reduction leads to an increase in bone resorption and osteoporosis as a reduction in these hormones increases the number of osteoclasts and decreases the number of osteoblasts resulting in overall bone resorption (211). Given the risks and implications for long-term bone health, it is important that prolactin levels are monitored in children and adolescents who are prescribed SGA medication. A systematic review of guidelines for prolactin monitoring has highlighted the discrepancy regarding prolactin monitoring among the guidelines (213). Most of the guidelines recommend monitoring prolactin at three months and then annually. However, the risks of hyperprolactinaemia are not highlighted in the guidelines and are poorly understood by many clinicians. This often leads to poor monitoring of prolactin and suboptimal management of hyperprolactinaemia in patients on SGAs.

In this study, we found that risperidone was the most commonly prescribed SGA. This is similar to the findings from the audit of antipsychotic prescribing in an Irish specialist ASD service. This is also similar to overall SGA prescribing in Ireland,

Europe and Canada. In the USA, risperidone and aripiprazole are both licensed for the treatment of irritability in ASD (73). They are not licensed for this particular indication in Ireland, but SGAs are widely accepted as an appropriate medication to use in these patients and over half of the participants in this study were prescribed SGAs for the treatment of irritability in ASD.

In recent years the awareness of weight and cardiometabolic side-effects has improved significantly in adult patients with psychosis. Studies have reported quite shocking findings of premature mortality in both schizophrenia (average of 25 years earlier death) and ASD (average of 14 years earlier death), frequently related to cardiovascular causes (214, 215). A Swedish national cohort study of over 6 million Swedish adults, including 8,277 with schizophrenia followed up for 7 years, found that participants with schizophrenia had premature mortality and they found the leading causes were ischaemic heart disease and cancer (216).

The recognition that SGA-related weight gain is associated with very significant long-term health implications has led to the development of guidelines for monitoring of adults who are prescribed SGAs (167). Clinical guidelines are systematically developed statements to assist practitioner decisions about appropriate healthcare for specific clinical circumstances. Clinicians are not legally obliged to follow guidelines in all cases, but they must be prepared to justify their decision and action if they depart from guidelines produced by a recognized body e.g., National Institute for Health and Care Excellence (NICE) guideline. A similar awareness of weight and cardiometabolic side-effects on SGAs is only slowly emerging in relation to children and all patients with ASD. The current study replicates the finding that children are at even higher risk of cardiometabolic side effects of SGAs compared to adults (82, 86, 92, 94).

There has been a global increase in SGA use in youth, a population who appear to be more vulnerable to SGA-related weight gain than adults yet despite this to date there is a paucity of formal guidelines for SGA prescribing or monitoring in youth. However, evidenced based recommendations have been published for monitoring of children and adolescents who are prescribed SGAs. These have been published by the National Institute for Health and Care Excellence (NICE)

in the UK, the American Diabetes Association (ADA), the American Academy of Child and Adolescent Psychiatry (AACAP) and the Canadian Alliance for Monitoring Effectiveness and Safety of Antipsychotics in Children (CAMESA) (169-171). Recommendations differ on how often to record weight change with variations between NICE, AACAP and CAMESA on the frequency of this monitoring which can cause confusion for clinicians (169-172).

Due to the number of young people prescribed SGAs and other psychotropic medications, there is a clear need for coherent all-encompassing prescribing guidelines in Ireland for physicians to follow. These are needed for clinicians who are prescribing SGAs in youth to ensure that recognition and management of metabolic side effects occurs in a timely manner.

There is also a need for clinicians who prescribe SGAs to review existing recommendations and carefully consider the clinical indication, monitoring requirements, implications of co-prescribing, and duration of treatment. Initiatives promoting evidence-based prescribing and monitoring practices regarding SGA treatment should be implemented to reduce the risks of cardiometabolic side effects in children and adolescents.

SGA use in children is associated with early and rapid weight gain, which is linked to metabolic complications including low HDL cholesterol and insulin resistance from a very early stage in treatment. Lower BMI at baseline may be an important clinical predictor of more rapid weight gain but further research is needed in this area. There are several initiatives that are required to improve safety in SGA prescribing in Ireland. Formal guidelines on prescription and monitoring of SGAs in children are urgently required. Databases recording Irish prescribing need to improve to ensure an accurate picture of SGA prescription rates.

CHAPTER 4: Investigating the effect of SGAs on immunophenotype

4.1 Introduction

4.1.1 Obesity and inflammation

Obesity is associated with a state of chronic low-grade inflammation which results in dysregulation of innate and adaptive immune cell responses (54, 55). Inflammation and immune dysregulation drive the pathogenesis of metabolic diseases such as insulin resistance, Type 2 Diabetes Mellitus (T2DM), and the metabolic syndrome (217, 218). It has been shown that children with obesity also have immune cell dysregulation prior to the onset of metabolic disease (54). Pro-inflammatory profiles in obese children are associated with the dysregulation of multiple immune cell populations including macrophages, T cells and Natural Killer (NK) cells, resulting in the systemic production of inflammatory cytokines, small glycoproteins that mediate signal communications among various immune and neuronal cells during the immune response (54, 219-221). Cytokine production occurs as a response to stress signals from hypertrophic adipocytes (57, 222, 223) (see section 1.2.3).

Adipose tissue is now recognised as an organ responsible for metabolic homeostasis. It is an active endocrine organ, which produces a vast array of adipokines, a collective term for factors that are released by adipose tissue (53). Adipokines regulate feeding, metabolism, insulin sensitivity, immune reactions and includes several hormones that regulate appetite, lipid, and glucose metabolism such as leptin and adiponectin, as well as growth factors (vascular endothelial growth factor), pro- and anti-inflammatory cytokines, and complement proteins (55). The obesogenic environment within adipose tissue results in elevated levels of systemic pro-inflammatory cytokines including TNF-α, IL-1β, IL-6 and IFN–γ (54, 57, 224). These can impair normal homeostatic processes contributing further to the cycle of inflammation and metabolic dysregulation (27, 57). Inflammation has a role in the pathophysiology of many obesity-related comorbidities such as insulin resistance and T2DM (62).

4.1.2 SGA medication and inflammation

While the early weight gain associated with SGA medication may lead to an increase in inflammation, it is also possible that the SGA itself influences inflammatory processes.

Research into the mechanism of action of SGAs in the treatment of psychosis suggests that they are anti-inflammatory. Several studies have shown that psychosis is associated with a pro-inflammatory state (121, 147, 149, 154) and indeed this increased inflammation is hypothesised to be central to the pathophysiology of psychosis (143, 149, 157). Driven by this theory that psychosis is underpinned by increased inflammation, the effects of antipsychotics on inflammatory processes have been investigated. Several studies have suggested that SGAs may exert anti-inflammatory effects (121, 142, 152, 163). Anti-inflammatory effects of risperidone specifically have been demonstrated in cultured macrophages (150, 225-228).

The situation is not clear however, as antipsychotic use in psychosis has also been associated with an increase in pro-inflammatory cytokines including TNF-α, IL-1β, IL-8, IL-17 and IL-23 (138, 146, 148, 160, 161, 165, 229). Dopamine has also been found to influence the immune system by inhibiting inflammatory macrophages in isolation (230, 231). SGAs are antagonists at dopamine receptors therefore this causes a reduction in the natural inhibition of inflammatory macrophages that occurs with dopamine.

To summarise, on one hand, SGAs lead to weight gain which is associated with an increase in inflammation, and they have also been associated with an increase in pro-inflammatory cytokines. On the other hand, SGAs appear to also have anti-inflammatory properties, which may underpin their clinicial effectiveness in the treatment of psychosis. There is clearly a complex interplay of factors and further research in this area is needed.

4.1.3 Innate immune cell populations that are important in obesity: monocytes, NK cells

The sections below describe the individual immune cell populations that will be examined in this chapter to provide a broad overview of the effect SGAs have on the immune system over time.

4.1.3.1 Monocytes

Monocytes are a circulating innate immune cell population that constitute approximately 10% of peripheral leukocytes. Upon activation, monocytes can develop a wide range of effector functions, ranging from the production of inflammatory mediators to tissue regulation (232). Monocytes can also act as the precursors for tissue macrophages and dendritic cells (53). Under pathological conditions such as the low-grade chronic inflammation that occurs with obesity, monocytes become highly inflammatory, and migrate to sites of inflammation such as adipose tissue. Migrating monocytes are associated with an increase in the number of pro-inflammatory macrophages in adipose tissue. Adipose tissue macrophages differentiate into a pro-inflammatory state in obesity (M1) and surround dying adipocytes, organized into “crown like structures” (53, 60, 233) (See Figure 1.4). These pro-inflammatory macrophages secrete pro-inflammatory cytokines such as TNF-α which contribute to the development of insulin resistance (53, 233).

4.1.3.2 Natural Killer Cells

Natural Killer (NK) cells are a population of innate immune cells that constitute about 10% of circulating lymphocytes, but are also found in liver, adipose tissue, and bone marrow (234). NK cells are potent anti-viral and anti-cancer effector cells, capable of the killing of infected cells and transformed cells such as cancer cells via perforin or granzyme through the induction of apoptosis (234). NK cells can also secrete cytokines and chemokines, such as IFN-γ that have an influence on the subsequent immune response of the host. In addition to their role in host protection, more recently NK cells have been found to play a role in homeostatic maintenance of many tissues including adipose tissue (222). Several studies

have demonstrated obesity associated defects in NK cells, with both reduced frequencies and effector function (235, 236). Similar findings were also shown in childhood obesity, with NK cells less able to carry out their basic functions (221). Dim NK cells are cytolytic and comprise more than 90% of NK cells, whereas bright NK cells are immunoregulatory but do show cytokine production (221).

4.1.4 Adaptive immune cell populations of relevance in obesity: B cells and T cells

4.1.4.1 B cells

B cells are formed in the bone marrow and they contain a unique antigen binding receptor on their cell membrane, which allows them to recognize antigens directly without the need for antigen presenting cells (42). When activated by foreign antigens, B cells can differentiate into plasma cells which secrete antibodies, or they can differentiate into memory cells (42). Memory cells can quickly produce antibodies upon re-exposure to an antigen (42).

4.1.4.2 T cells

T cells develop in the thymus gland (48). There are different types of T lymphocytes including cytotoxic T lymphocytes (CD8+ lymphocytes), T Helper Cells (CD4+ lymphocytes), Regulatory T cells and Th17 cells. Cytotoxic T lymphocytes (CD8+) kill target cells in a contact-dependent mechanism (48). T Helper CD4+ lymphocytes help to maintain the balance in the immune system between protecting against pathogens and cancer cells and having self-tolerance (47). Regulatory T cells are important for immunological tolerance and loss of these cells can result in the emergence of autoimmune disease and allergy (237). Th17 cells produce pro-inflammatory cytokines including IL-17A and IL-17F. Obesity is associated with a predisposition to a Th17 cell bias with an increase in the production of pro-inflammatory IL-17 (55, 238, 239).

4.1.5 Other immune cell populations: MAIT cells, iNKT cells

4.1.5.1 MAIT cells

Mucosal Associated Invariant T (MAIT) cells are a subset of unconventional T cells which bridge the innate and adaptive arms of the immune system by having properties associated with both. MAIT cells are very abundant in peripheral blood (2-8% of T cells), and have been reported in the gut, liver, skin, and adipose tissue. They play an important role in immunosurveillance and defence against microbial infection (240-242).

Like iNKT cells, MAIT cells have attributes of both the innate and adaptive immune system (241). MAIT cells are absent in germ-free mice, implying that the gut microbiota is essential for the development and expansion of MAIT cells (243). MAIT cells produce cytokines on activation. MAIT cells produce Th1 type cytokines such as IFN-γ and TNF-α and Th17 type cytokines such as IL-17A (241). MAIT cells also have a cytotoxic function and lyse bacterially infected cells using molecules such as granzymes and perforin (241). They frequently decrease in the blood in response to disease, the potential mechanism being activation-induced cell death (240, 242-244).

MAIT cells have been implicated in the development of several metabolic diseases including obesity, Non-Alcoholic Fatty Liver Disease (NAFLD) and T2DM (241). Altered MAIT cell frequencies have been shown in children and adults with obesity, with reduced MAIT cell frequencies in adults with obesity and higher MAIT cell frequencies in children with obesity compared to their lean counterparts (219). As children with obesity progress into adulthood, their MAIT cell frequency declines (219, 241). Increased MAIT cell IL-17 production has been shown in children with obesity (219). The altered frequency of MAIT cells is associated with increased fasting insulin and insulin resistance, which contributes to the development of T2DM (241, 243).

MAIT cells isolated from patients with obesity produce significantly higher levels of IL-17 and significantly reduced levels of IFN-γ compared to healthy controls (219, 241, 245). This IL-17 phenotype may be contributing to the chronic inflammation that occurs in obesity and insulin resistance (219, 241).

4.1.5.2 Invariant Natural Killer T cells

Invariant Natural Killer T (iNKT) cells are another subset of unconventional or innate T cells and are important regulators of the immune response (50, 246). Even though they are T cells, they have less specificity and become active more rapidly than adaptive immune cells. They are rare in the periphery but abundant in both the liver and white adipose tissue (WAT) and they are involved in modulating WAT immunity in lean individuals and individuals with obesity (246, 247). iNKT cells produce anti-inflammatory cytokines, IL-10 and IL-4 and regulate the function of M2 macrophages to reduce adipose tissue inflammation (50). In obesity, the number of iNKT cells decrease in parallel with the increase in adipose tissue inflammation. The corresponding reduction in anti-inflammatory cytokines contributes to the pro-inflammatory environment in obesity (248, 249). Animal studies have shown that mice lacking iNKTs show increased weight gain and larger adipocytes. Mice lacking iNKTs also show insulin resistance on a high fat diet. This was shown to be associated with increased infiltration of macrophages into AT (248).

4.1.6 Cytokines in the context of obesity

Chronic elevations of pro-inflammatory cytokines may damage host tissue and are implicated in several autoimmune disorders such as rheumatoid arthritis (250). Some of the cytokines implicated in obesity are discussed below.

4.1.6.1 TNF-α

TNF-α is a pro-inflammatory cytokine produced primarily by macrophages neutrophils and/or dendritic cells and has both local and systemic effects, including stimulation of immune cells locally or in the tissues distant from its site of production. It is implicated in the development of obesity-related diseases including insulin resistance (58, 251, 252). Mechanistic animal studies have indicated a role for TNF- α in insulin resistance and T2DM associated with obesity (253).

4.1.6.2 IL-1

Interleukins are a group of cytokines that are secreted by white blood cells and are involved in systemic inflammation and immune system modulation (251). IL-1β is a pro-inflammatory cytokine. It is mainly secreted by cells of the myeloid lineage including macrophages, monocytes, and dendritic cells and mediates many immunological reactions, tissue injury, and inflammation (251). The secretion of IL-1β leads to an increase in TNF-α production which boosts the inflammatory response (254). Several studies have shown that IL- 1β has an important role in in the pathogenesis of obesity, insulin resistance, and T2DM (251, 252, 254). In adipose tissue, adipocyte insulin signalling is impaired by IL-1β which leads to insulin resistance (254). NLPR3 is present in several tissues including adipose tissues and when activated it becomes NLRP3 inflammasome (255). Activation of the NLRP3 inflammasome in AT macrophages results in the secretion of IL-1β which is associated with T2DM (255). The role played by NLRP3 inflammasome in obesity induced insulin resistance is supported by studies showing that genetic ablation of NLRP3 prevents the obesity induced inflammasome activation in AT and protects against high fat diet induced insulin resistance (64, 255).

4.1.6.3 IL-17

IL-17 is an inflammatory cytokine secreted by Th17 cells, MAIT cells, and gamma delta (γδ) T cells (256, 257). IL-17 is a pro-inflammatory cytokine and has been implicated in various autoimmune and chronic inflammatory diseases linked to obesity (241, 257, 258). IL-17 has been implicated in the metabolic syndrome and T2DM because it induces adipocyte insulin resistance (257, 259).

4.1.7 Other signalling molecules of relevance in obesity

4.1.7.1 Soluble CD163

CD163 is a haptoglobin-haemoglobin receptor that is affected by macrophage activation (260). This is due to the extracellular part of the CD163 molecule being

shed into the blood upon macrophage activation as soluble CD163 (260). Therefore, when macrophages are activated and in a pro-inflammatory state (M1), there is an increase in levels of soluble CD163 (54). Soluble CD163 is strongly associated with insulin resistance (54) and has been identified as a strong risk marker for the development of T2DM (261). Parkner et al showed that soluble CD163 is associated with insulin resistance independently of TNF-α and other pro-inflammatory markers. They also found that soluble CD163 was a predictor of homeostasis model assessment for insulin resistance (HOMA-IR), glucose, insulin, triglycerides, and HDL-cholesterol (261). Overall, this indicates that soluble CD163 is a biomarker for insulin resistance.

4.1.7.2 Leptin

Leptin is a hormone that plays a key role in regulating appetite and body weight and it is mainly secreted by adipocytes (53). Leptin regulates body weight as it signals nutritional status to the hypothalamus. The hypothalamus then produces neurotransmitters and neuropeptides that regulate the food intake and energy expenditure balance (62). Obesity is associated with an increased level of leptin in the expanding adipose tissue (53) and increased levels lead to leptin resistance (98, 119, 120) (see section 1.3.5). The production of circulating leptin is related to adiposity and peripheral administration of leptin reduces appetite and feeding in rats (262).

4.2 Specific aims for this chapter

The aims of this chapter were to gain a broad overview of the effect of SGAs on systemic inflammation and immune cells over time.

Specifically, the aims were to:

1. Characterise the systemic inflammatory response to SGA treatment in the cohort of participants and investigate for the presence of an inflammatory state.
2. Characterise the immunophenotype in response to SGA treatment and investigate for dysregulation in immune cells with SGA treatment compared to baseline.
3. Investigate the baseline immune profile in the SGA group compared to a group of healthy controls. We were interested in the inflammatory phenotype of this cohort prior to SGA treatment, and we were interested in investigating if this group differed from a healthy control group in terms of baseline inflammatory profile. See Table 3.3 (Chapter 3) for information on control participants.

4.3 Results

4.3.1 Systemic inflammatory cytokine and adipokine profiling: Leptin Quantification

Leptin is an adipokine that is secreted by adipose tissue. As adipose tissue expands, the production of circulating leptin also increases (52). Leptin has been shown to increase on SGA medication (112, 116). We first investigated if the SGA cohort had increased leptin levels at baseline compared to the control group. Leptin was measured by ELISA (*R&D Systems*). At baseline, the SGA cohort had higher levels of leptin compared to the healthy control group (Figure 4.1A). We next investigated if leptin levels were changed after SGA treatment. Leptin levels were significantly elevated 3 months after commencing SGA treatment (Figure 4.1B). In the SGA group that were followed up for 12 months (n=10), leptin levels followed an increasing trend up to 12 months, in line with the BMI percentile changes, however this was not statistically significant (Figure 4.1C). Finally, we compared the change in leptin levels (Δ leptin) between baseline and three months of the participants that gained weight (>5 BMI percentile) in the first three months of the study (Group 1) to the participants that did not gain weight in the first three months (Group 2) (n=12). We found no significant differences in Δ leptin levels between Group 1 and Group 2 (Figure 4.1D). There was no correlation between the change in leptin levels from baseline to 6 months and the change in weight from baseline to 6 months (Figure 4.2A). There was a significant correlation between the change in leptin from baseline to 6 months and the change in BMI percentile from baseline to 6 months (Spearman's Correlation 0.7, p=0.03) (Figure 4.2B).

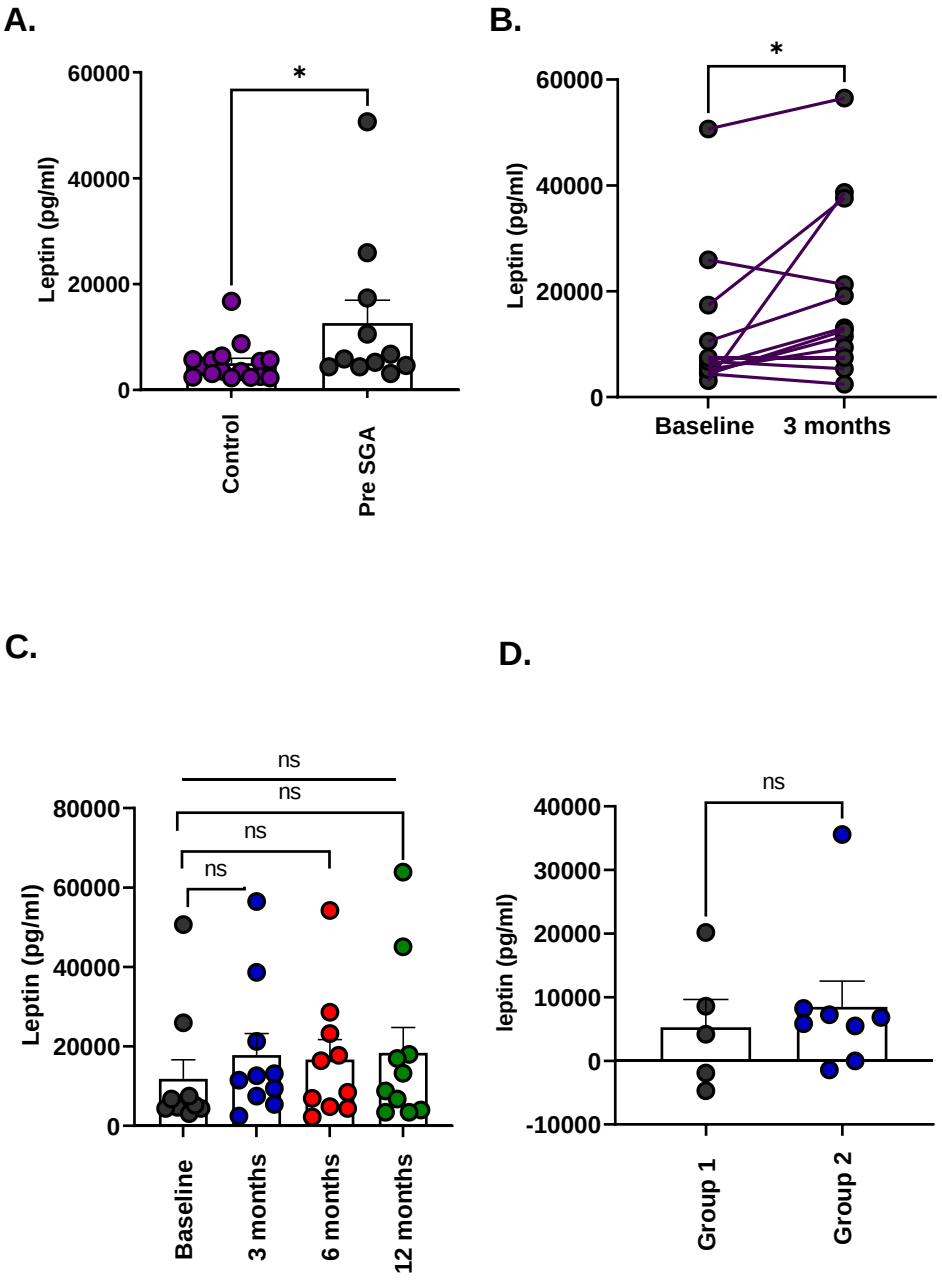
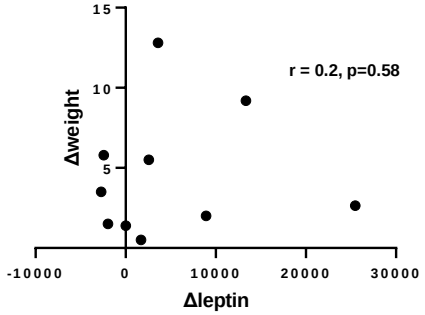


Figure 4.1: Leptin levels pre- and post-treatment with SGA medications

Scatter plots showing: **(A)** Leptin levels in the serum of healthy controls and the participants in the SGA cohort at baseline prior to commencing treatment. **(B)** Leptin levels in the serum of participants in the SGA cohort at baseline and 3 months after commencing SGA treatment (n = 12). **(C)** Leptin levels in the serum of the SGA cohort at baseline and at 3, 6, and 12 months after commencing SGA treatment (n=10). **(D)** The change in Leptin levels in the SGA cohort between baseline and 3 months after commencing SGA treatment (Δ leptin). The patients who did not gain weight (Group 1) on SGA treatment are separated from the rest of the cohort who did gain weight (Group 2). Statistical analysis performed using Wilcoxon matched-pairs signed rank test and Mann-Whitney test, ns – not significant, * = $p<0.05$

A.



B.

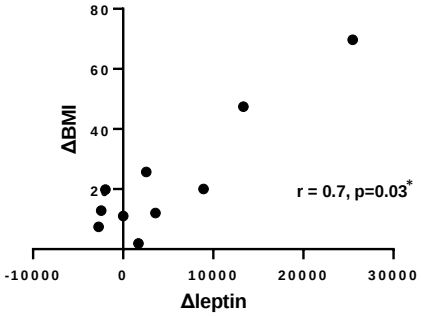


Figure 4.2: Correlation between Δleptin and Δweight/ΔBMI

Correlation plot of Δleptin between baseline and 6 months with **(A)** Δweight, **(B)** ΔBMI percentile. Correlation analysis performed using Spearman's correlation coefficient, * = $p<0.05$

4.3.2 Systemic inflammatory cytokine and adipokine profiling: Soluble CD163 Quantification

After establishing that leptin increased systemically on SGA medication, we investigated the effect SGAs had on soluble CD163 levels in serum. CD163 was measured by ELISA (*R&D Systems*). There was no difference in soluble CD163 levels between the healthy control group and the SGA group at baseline (Figure 4.3A).

We next investigated if soluble CD163 levels were changed after SGA treatment. Overall, there were no differences in soluble CD163 levels on SGA medication (Figure 4.3B). The data show that treatment with SGAs did not have an impact on soluble CD163 production. In a similar analysis to that described for leptin in the previous section, we compared the change in soluble CD163 levels (Δ sCD163) of the participants that gained weight (>5 BMI percentile) in the first three months of the study (Group 1) to the participants that did not gain weight in the first three months (Group 2) (n=12) (Figure 4.3C). We found there was no difference between these two groups. We did a correlation analysis between Δweight gain and Δsoluble CD163 at 6 months and found there was no correlation between these two variables (Figure 4.3D).

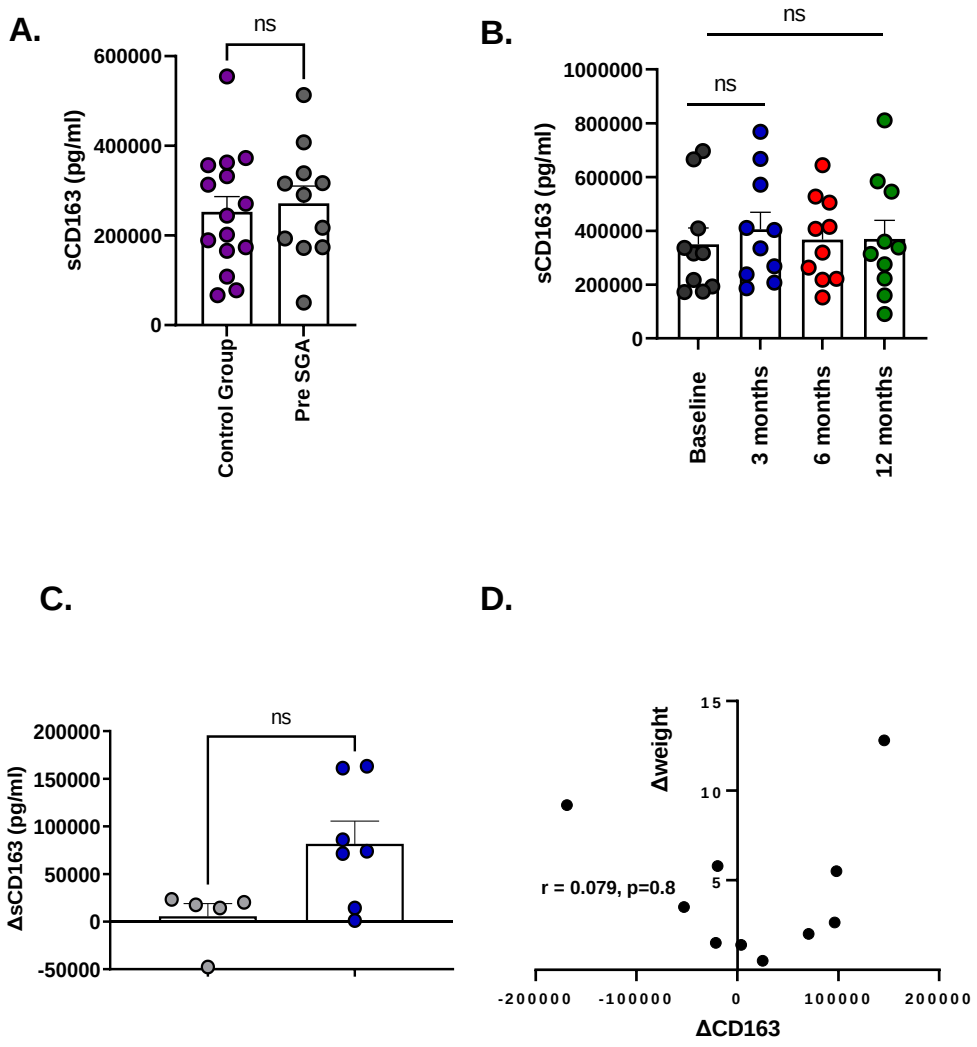


Figure 4.3: Soluble CD163 Levels pre- and post-treatment with SGA medications

Scatter plots showing: **(A)** Levels of soluble CD163 in the serum of healthy controls and the participants in the SGA cohort at baseline prior to commencing treatment. **(B)** Levels of soluble CD163 in the serum of the SGA cohort at baseline, 3, 6, and 12 months after commencing SGA treatment (n=10). **(C)** The change in levels of soluble CD163 in the SGA cohort between baseline and 3 months after commencing SGA treatment. The patients who did not gain weight on SGA treatment (Group 1) are separated from the rest of the cohort who did gain weight (Group 2). **(D)** Correlation graph between the change in weight between baseline and 6 months and the change in sCD163 between baseline and 6 months. Statistical analysis performed using unpaired t-test, Kruskall-Wallis test with multiple comparisons, Spearman correlation coefficient. ns – not significant

4.3.3 Systemic inflammatory cytokine and adipokine profiling: Serum IL-17 Quantification

IL-17 is a pro-inflammatory cytokine that is implicated in insulin resistance. We investigated the effect SGAs had on systemic IL-17 levels and compared the IL-17 at baseline to a healthy control group. Serum IL-17 was measured using high-sensitivity ELISA (*R&D Systems*). There was no difference in IL-17 levels between the healthy control group and the SGA group at baseline (Figure 4.4A). There was no difference in IL-17 in serum in the SGA cohort at baseline, three, six, and twelve months (Figure 4.4B). The data show that SGA treatment did not have a significant effect on IL-17 production as measured in serum.

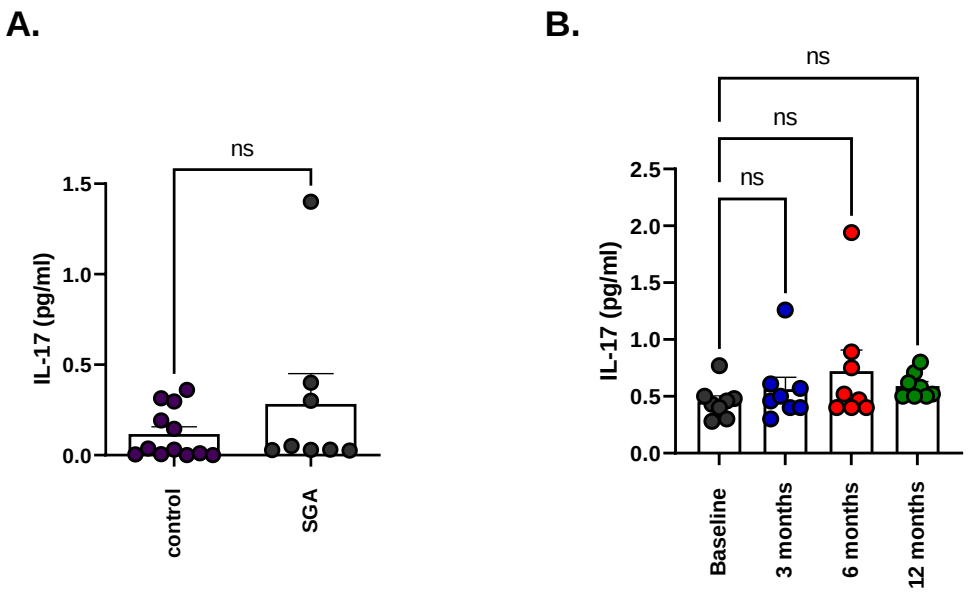


Figure 4.4: IL-17 Levels pre- and post-treatment with SGA medications

Scatter plots showing: **(A)** Levels of IL-17 in the serum of healthy controls and the participants in the SGA cohort at baseline prior to commencing treatment. **(B)** Levels of IL-17 in the serum of the SGA cohort at baseline, 3, 6, and 12 months after commencing SGA treatment (n=8). Statistical analysis performed using Mann Whitney test, Repeated measures ANOVA with multiple comparisons, ns = not significant

4.3.4 Systemic inflammatory cytokine and adipokine profiling: Serum TNF-α Quantification

TNF-α is a pro-inflammatory cytokine that is implicated in insulin resistance. We examined the effect SGAs had on systemic TNF-α levels and compared the TNF-α at baseline to a healthy control group. Serum TNF-α was measured using high-sensitivity ELISA (*R&D Systems*). The SGA cohort had higher baseline levels of serum TNF-α than the healthy control group ($p<0.05$) (Figure 4.5A). There was no difference in TNF-α levels in serum on SGA medication (Figure 4.5B). The data show that SGA treatment did not have a significant effect on TNF-α production as measured in serum.

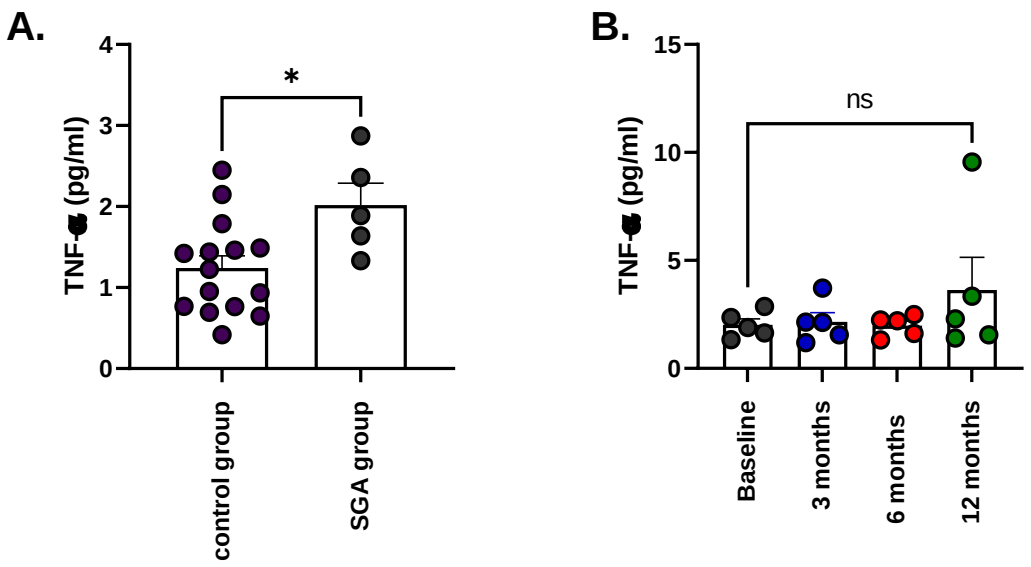


Figure 4.5: TNF-α levels pre- and post-treatment with SGA medications

Scatter plots showing: **(A)** TNF-α levels in the serum of healthy controls and the participants in the SGA cohort at baseline prior to commencing treatment. **(B)** TNF-α levels in the serum of the SGA cohort at baseline, 3, 6, and 12 months after commencing SGA treatment (n =5). Statistical tests performed using unpaired t-test and repeated measures ANOVA, ns= not significant, * = $p<0.05$

4.3.5 Immunophenotyping: T cell identification

Multi-coloured flow cytometry was used to analyse the frequencies of circulating T cells in the SGA cohort. The gating strategy used to identify the T cells is shown in Figure 4.6.

PBMCs isolated from participants were stained using antibodies against surface antigens to appropriate fluorochromes, namely CD3 (VioGreen), CD45RA (PERCP), CD45RO (VioBlue) and CD8 (APCy7) (as per table 2.2). To identify T cells, lymphocytes were selected based on their forward and side scatter and doublets were excluded using FSC-area and FSC-height parameters. T cells were then identified by their positive expression of CD3 (Figure 4.6A).

CD8+ T cells were identified by their positive expression of CD8 and CD4+ T cells were identified by their negative expression of CD8 (Figure 4.6A).

CD45RA+ T cells were identified by their positive expression of CD45RA and CD45RO+ T cells were identified by their positive expression of CD45RO (Figure 4.7A). CD45RA+ T cells are naïve T cells because the CD45RA molecule is present on naïve T cells. CD45RO+ T cells are memory T cells. These cells show stronger helper function for production of antibodies.

4.3.6 T cell profiling before and after treatment with SGA medication

The frequency of circulating lymphocytes and T cells overall did not change from baseline to 12 months after commencing SGA medication. The frequency of circulating CD8 T cells did not change overall from baseline to 12 months after commencing SGA treatment (Figure 4.6C). Similarly, there were no differences between the frequencies of circulating CD4 T cells on SGA treatment (Figure 4.6D). The frequency of CD45RA+ and CD45RO+ T cells were examined and there was no difference in the frequency of these cells on SGA treatment (Figure 4.7C and 4.7D). The data show that SGA treatment did not have a significant effect on T cell frequencies as measured by flow cytometry.

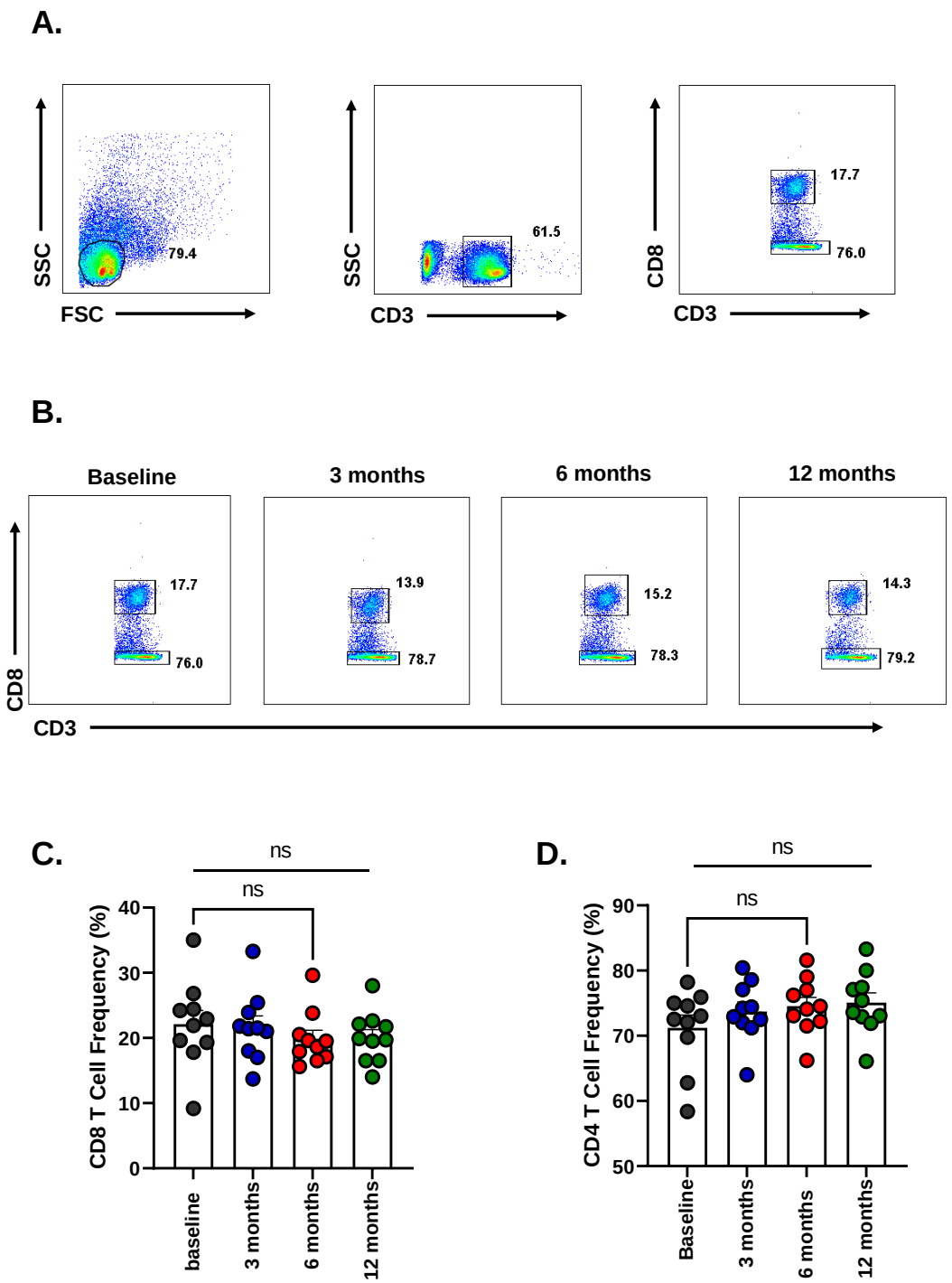


Figure 4.6: CD8 and CD4 T Cell Frequencies pre- and post-treatment with SGA medications

(A) Gating strategy used to identify CD8 T cells and CD4 T cells. **(B)** Representative sample of CD8 and CD4 T cells at all four time points. **(C)** Scatter plot showing CD8 T cell frequency (%). **(D)** Scatter plot showing CD4 T cell frequency (%). Statistical tests performed using repeated measures ANOVA with multiple comparisons for each time point. Ns = not significant

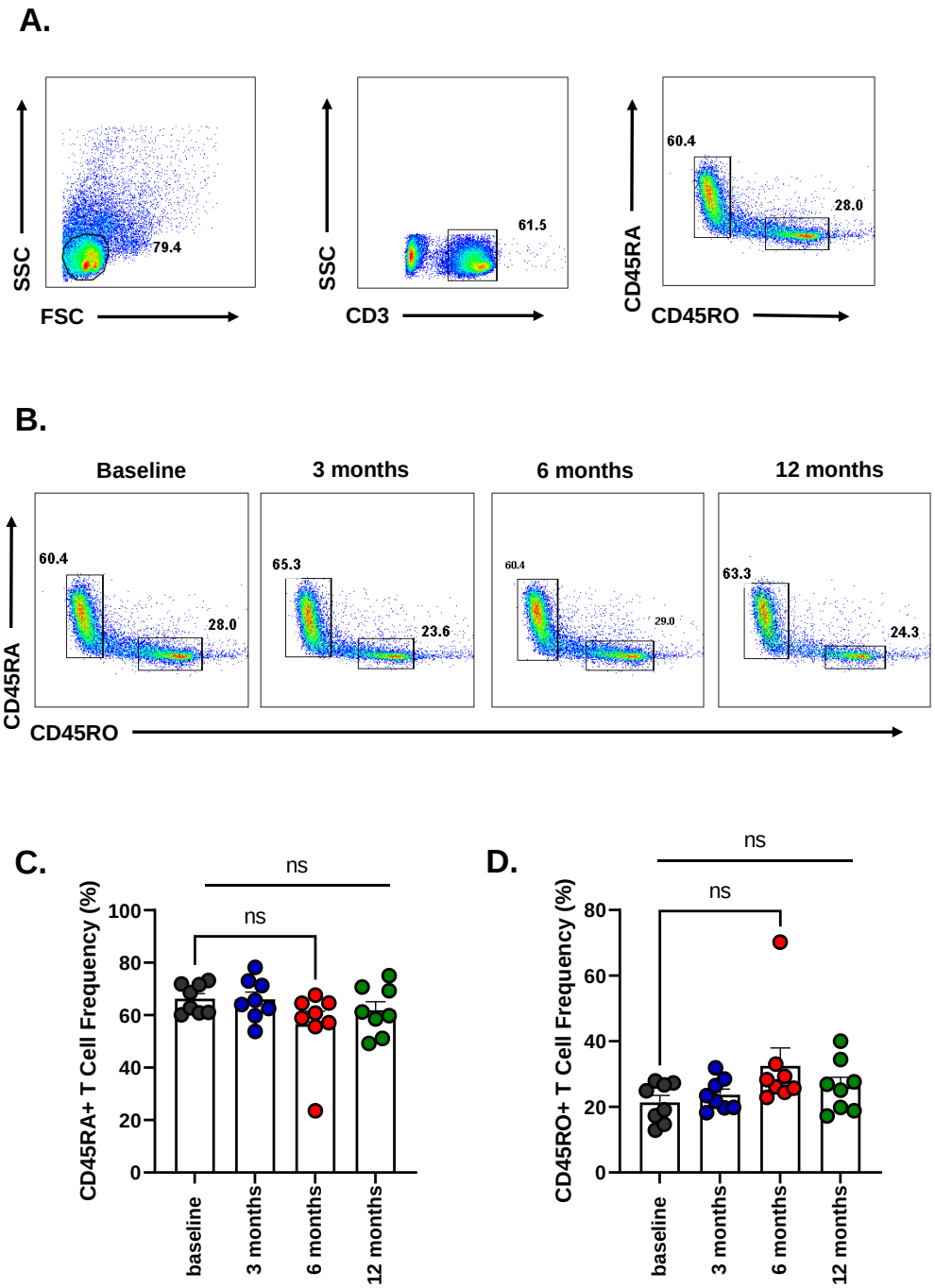


Figure 4.7: CD45RA and CD45RO T Cell Frequencies pre- and post-treatment with SGA medications

(A) Gating strategy used to identify CD45RA T cells and CD45RO T cells. **(B)** Representative sample of CD45RA and CD45RO T cells at all four time points. **(C)** Scatter plot showing CD45RA+ T cell frequency (%). **(D)** Scatter plot showing CD45RO+ T cell frequency (%). Statistical tests performed using repeated measures ANOVA with multiple comparisons for each time point. Ns = not significant

4.3.7 Immunophenotyping: MAIT cell identification

Multi-coloured flow cytometry was used to analyse the frequencies of circulating MAIT cells in the SGA and control cohorts. The gating strategy used to identify the MAIT cells is shown in figure 4.8A.

PBMCs isolated from participants were stained using antibodies against surface antigens to appropriate fluorochromes, namely CD3 (VioGreen), CD161 (APC) and Vα7.2 (PECy7) (as per table 2.2). To identify MAIT cells, lymphocytes were selected based on their forward and side scatter and doublets were excluded using FSC-area and FSC-height parameters. MAIT cells were then identified by their positive expression of CD3, high expression of CD161 and the expression of Vα7.2 TCR on their cell surface (Figure 4.8A).

4.3.8 MAIT Cell profiling before and after treatment with SGA medication

SGA medication resulted in an increase in the frequency of circulating MAIT cells at three and six months (Figure 4.8C & D). The frequency of circulating MAIT cells was reduced at twelve months but remained higher than baseline levels (Figure 4.8C & D). The data show that SGA treatment results in an increase in MAIT cell frequencies as measured by flow cytometry. There was no correlation between the change in MAIT cell frequency from baseline to 6 months and the change in weight (Figure 4.9A), HDL-cholesterol (Figure 4.9B), triglycerides (Figure 4.9C) or soluble CD163 from baseline to 6 months (Figure 4.9D).

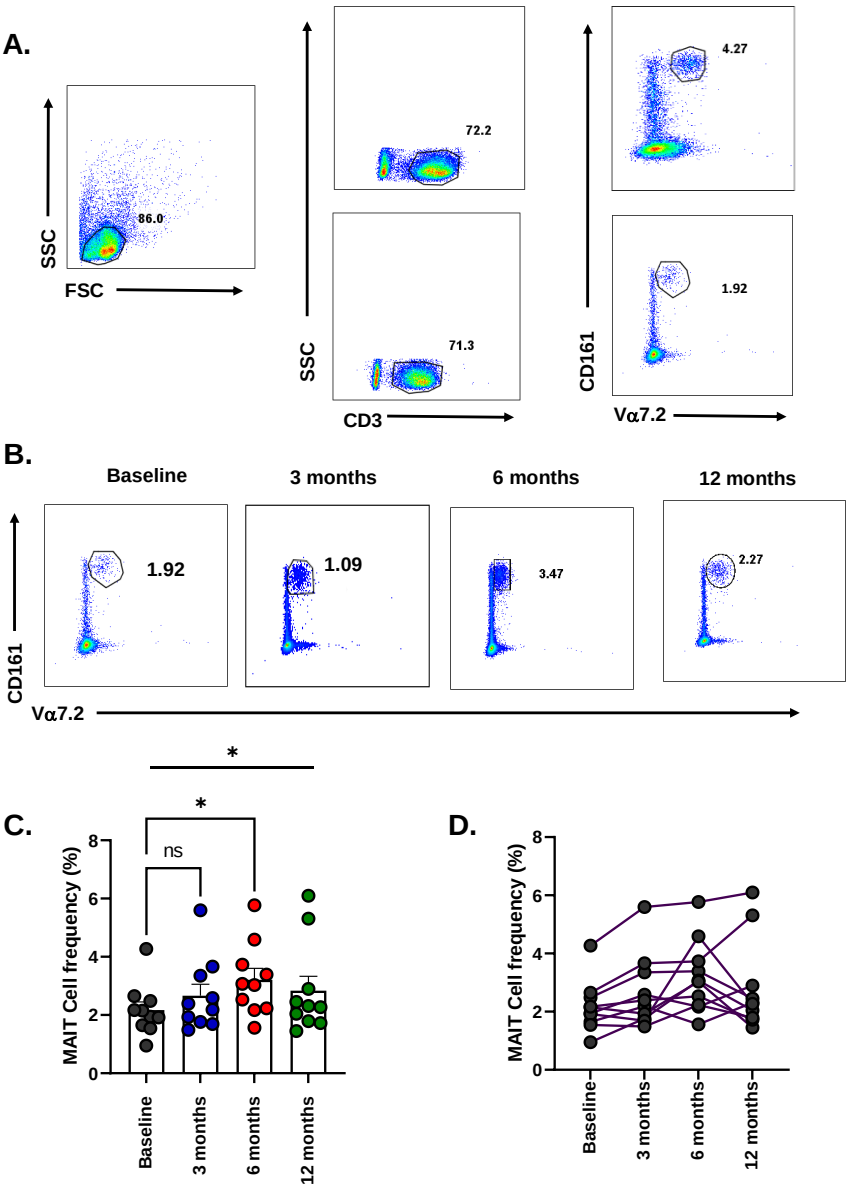


Figure 4.8: MAIT Cell frequencies pre- and post-treatment with SGA medications

(A) Gating strategy for identification of MAIT cells. **(B)** Gating strategies for identification of MAIT cells at all four time points. **(C)** Scatter plot showing MAIT Cell frequencies in the SGA cohort at baseline, 3 months, 6 months, and 12 months. **(D)** Graph from (C) with individual trends shown. Statistical tests performed using Welch's t-test and repeated measures ANOVA with multiple comparisons. Ns = not significant, * = $p<0.05$

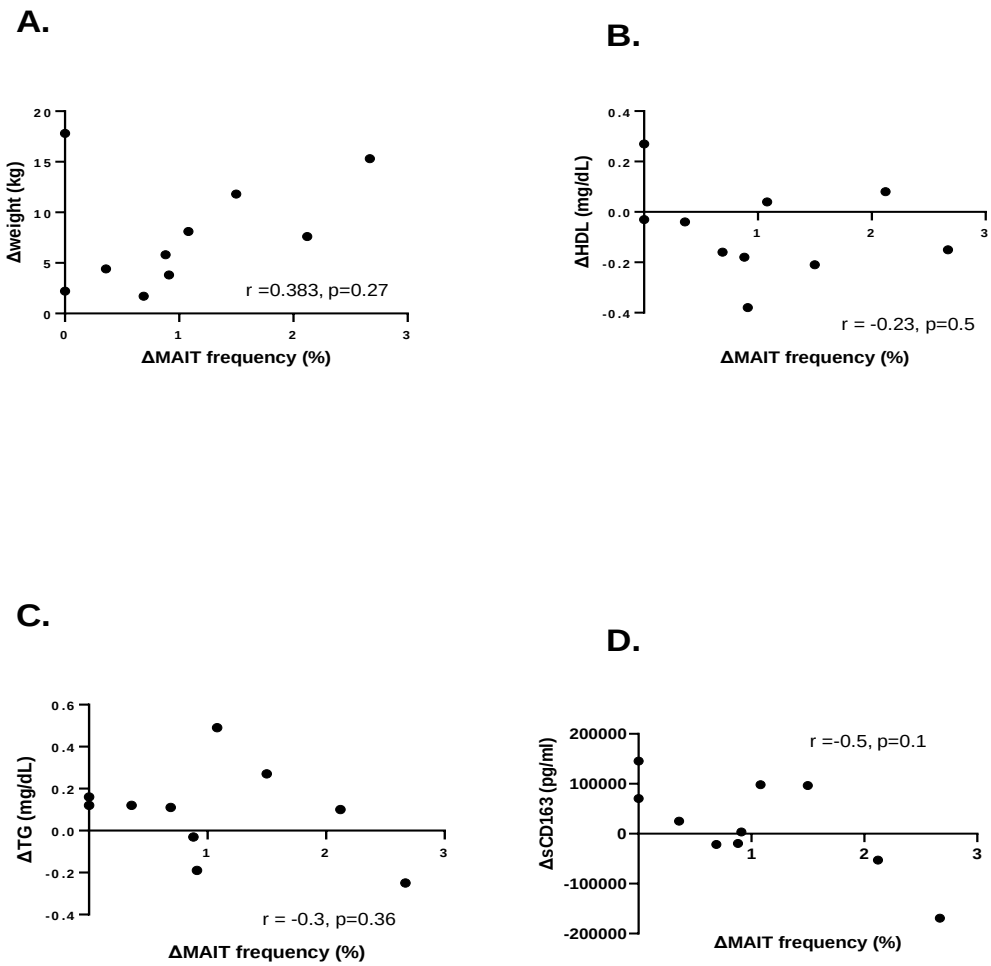


Figure 4.9: ΔMAIT cell frequency and metabolic parameters

Scatter plot of ΔMAIT cell frequency between baseline and 6 months correlation with **(A)** Δ weight, **(B)** Δ HDL-cholesterol, **(C)** Δ triglycerides and **(D)** Δ soluble CD163. Correlation analysis performed using Pearson's correlation coefficient.

4.3.9 Immunophenotyping: Invariant Natural Killer T cell identification

Multi-coloured flow cytometry was used to analyse the frequencies of circulating iNKT cells in the SGA cohort. The gating strategy used to identify the iNKT cells is shown in figure 4.10A.

PBMCs isolated from participants were stained using antibodies against surface antigens to appropriate fluorochromes, namely CD3 (VioGreen) and iNKT Tetramer (PE) (as per table 2.2). A Fluorochrome Minus One control was used by leaving out the iNKT tetramer stain (Figure 4.10A). This made it possible to accurately detect the iNKT cell population.

4.3.10 Invariant Natural Killer T cell profiling before and after treatment with SGA medication

There appeared to be a reducing trend in the frequency of iNKT cells on SGAs, however this trend was not statistically significant (Figure 4.10C). The data show that SGA treatment does not have a significant impact on the frequency of circulating iNKT cells as measured by flow cytometry.

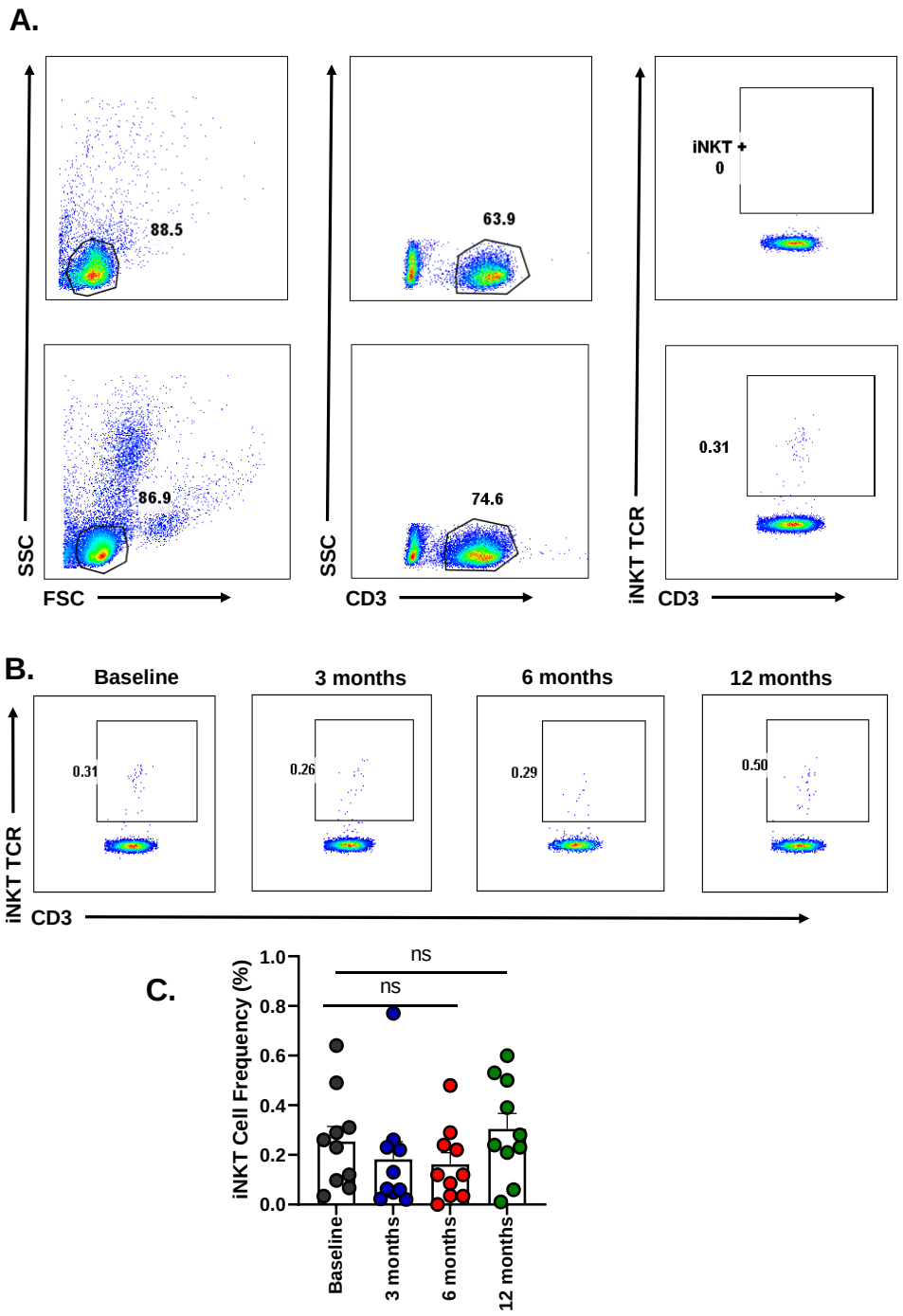


Figure 4.10: Invariant Natural Killer T Cell frequencies pre- and post-treatment with SGA medications

(A) Gating strategy for identification of iNKT cells. **(B)** Gating strategies for identification of iNKT cells at all four time points. **(C)** Scatter plot showing iNKT cell frequencies in the SGA cohort at baseline, 3 months, 6 months, and 12 months. Statistical tests performed using repeated measures ANOVA with multiple comparisons for each time point. Ns = not significant

4.3.11 Immunophenotyping: Natural Killer cell identification

Multi-coloured flow cytometry was used to analyse the frequencies of circulating NK cells in the SGA cohort. The gating strategy used to identify the NK cells is shown in figure 4.11A. PBMCs isolated from participants were stained using antibodies against surface antigens to appropriate fluorochromes namely CD56 (PERCP) (as per table 2.2). To identify NK cells, lymphocytes were selected based on their forward and side scatter and doublets were excluded using FSC-area and FSC-height parameters. NK cells were then identified by their negative expression of CD3, and positive expression of CD56 (Figure 4.11A). NK cells were split into $CD56^{-Bright}$ and $CD56^{-Dim}$ depending on the expression intensity of CD56 (Figure 4.11C).

4.3.12 Natural Killer cell profiling before and after treatment with SGA medication

There was no difference in NK cell frequency on SGA medication (Figure 4.11D).

NK cells were split into $CD56^{-Bright}$ and $CD56^{-Dim}$ depending on the expression intensity of CD56. There was no difference in the frequency of the $CD56^{-Bright}$ population (Figure 4.11E). There was a gradual decreasing trend in the frequency of the $CD56^{-Dim}$ cytotoxic population after commencing SGA treatment, however this trend was not statistically significant (Figure 4.9F). The data show that SGA treatment does not have a significant impact on the frequency of circulating NK cells as measured by flow cytometry. There was no significant correlation between the change in NK cell frequency between baseline and 6 months and the change in weight between baseline and 6 months (Figure 4.12A), the change in HDL-cholesterol between baseline and 6 months (Figure 4.12B) or the change in triglycerides between baseline and 6 months (Figure 4.12C).

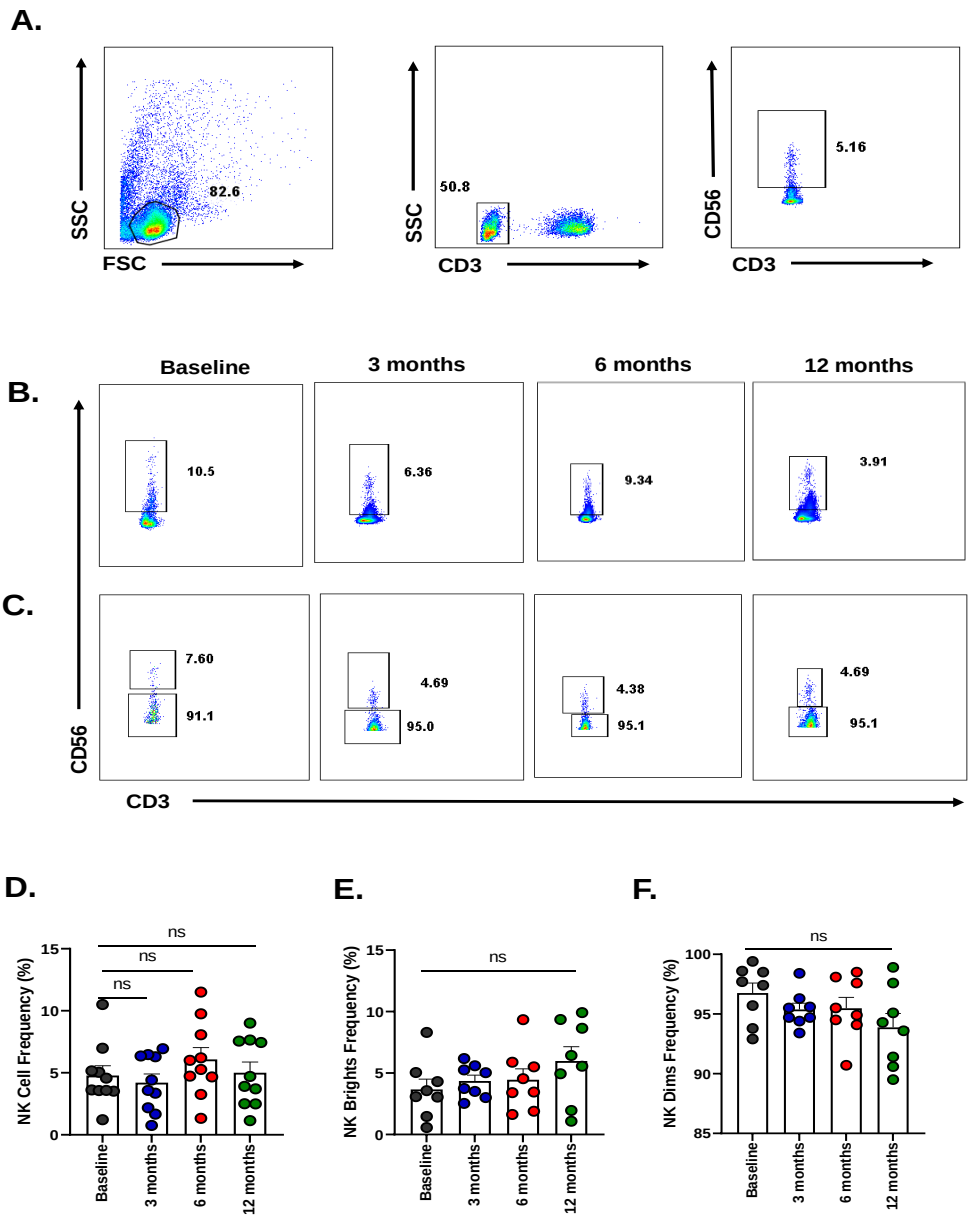
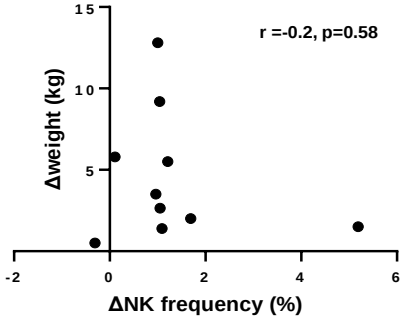


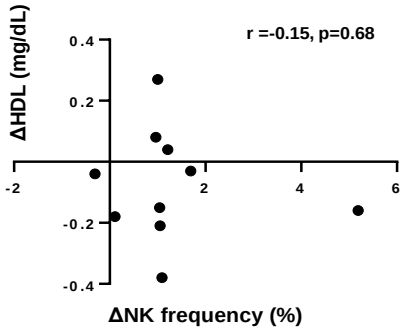
Figure 4.11: Natural Killer Cell frequencies pre- and post-treatment with SGA medications

(A) Gating strategy for identification of NK cells. **(B and C)** Gating strategies for identification of NK cells at all four time points. **(D)** Scatter plot showing NK Cell frequencies in the SGA cohort at baseline, 3 months, 6 months, and 12 months. **(E)** Scatter plot showing NK Brights Cell frequencies in the SGA cohort at baseline, 3 months, 6 months, and 12 months. **(F)** Scatter plot showing NK Dims Cell frequencies in the SGA cohort at baseline, 3 months, 6 months, and 12 months. Statistical tests performed using repeated measures ANOVA with multiple comparisons for each time point. Ns = not significant

A.



B.



C.

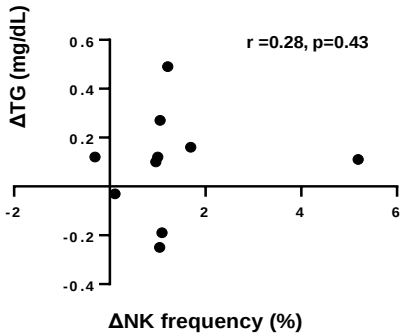


Figure 4.12: ΔNK cell frequency and metabolic parameters

Correlation plot of ΔNK cell frequency between baseline and 6 months correlation with **(A)** Δweight, **(B)** ΔHDL-cholesterol and **(C)** ΔTriglycerides between baseline and 6 months. Correlation analysis performed using Pearson's correlation coefficient.

4.3.13 Immunophenotyping: Monocyte identification

Multi-coloured flow cytometry was used to analyse the frequencies of circulating Monocytes in the SGA cohort. The gating strategy used to identify the Monocytes is shown in figure 4.13A and 4.13B.

PBMCs isolated from participants were stained using antibodies against surface antigens to appropriate fluorochromes namely CD14 (PERCP) (as per table 2.2). To identify monocyte cells, myeloid cells were selected based on their forward and side scatter and doublets were excluded using FSC-area and FSC-height parameters. Monocyte cells were then identified by their positive expression of CD14 (Figure 4.13B). The frequency of CD14+ cells was used to calculate the frequency of circulating monocytes (Figure 4.13C).

4.3.14 Monocyte frequencies before and after treatment with SGA medication

The frequency of circulating monocytes increased within three months of commencing SGA medication (Figure 4.13D). The data show that SGAs initially influenced the frequency of circulating monocytes by causing an initial increase in the first three months which decreased to below baseline levels by six months.

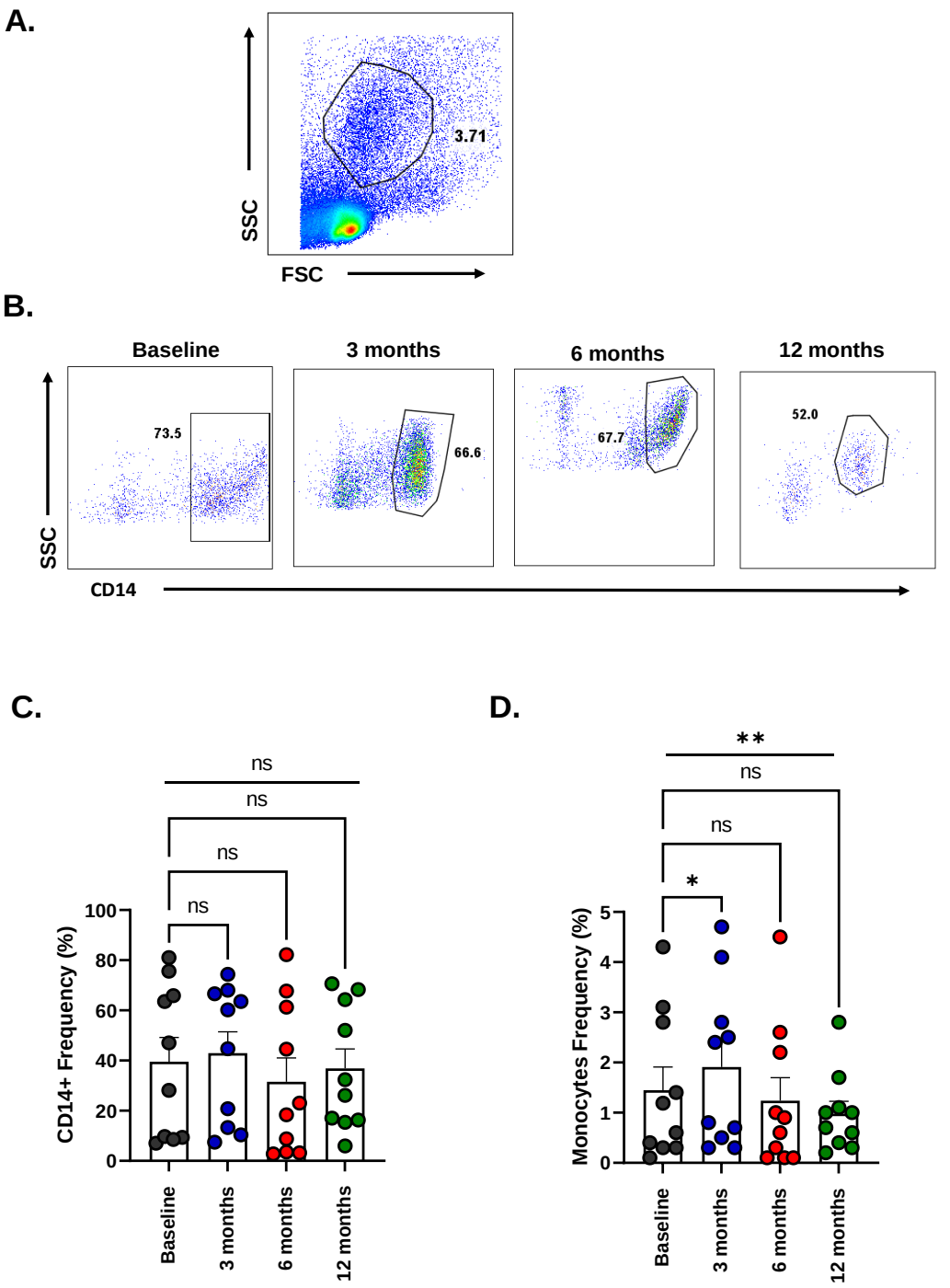


Figure 4.13: Monocyte Cell frequencies pre- and post-treatment with SGA medications

(A) Gating strategy for identification of myeloid cells. **(B)** Gating strategies for identification of monocytes (CD14+ myeloid cells) at all four time points. **(C)** Scatter plot showing CD14+ myeloid cell frequencies at baseline, 3, 6, ad 12 months. **(D)** Scatter plot showing monocyte frequencies at baseline, 3, 6 and 12 months. Statistical analysis performed using Friedman test with multiple comparisons for each time point. Ns = not significant, * = $p<0.05$

4.4 Discussion

SGA medications have the unfortunate side effect of weight gain and metabolic dysregulation. It is well established that obesity is associated with immune system dysregulation and the emergence of a chronic inflammatory state which drives the metabolic complications associated with it. Therefore, in this chapter, our aim was to investigate if SGA medications were associated with changes in either the systemic inflammatory state or immune subset frequencies.

Leptin levels showed an increasing trend on SGA medication that was not statistically significant over the four time points. When we examined the difference between leptin levels at baseline and three months in the larger group of participants (n =12) we found that there was an increase in leptin levels three months after commencing SGAs. There was a correlation between the change in leptin at 6 months and the change in BMI at 6 months. These results indicate that a dysregulation in appetite at the level of the hypothalamus is a key driver of weight gain. It is not surprising that leptin levels increased as the BMI increased because leptin is increased in the expanding adipose tissue associated with obesity (53). The increase in leptin that we found is in line with previous studies which have shown that SGAs are associated with an increase in leptin (119, 123, 124). Higher baseline levels of leptin have been associated with more weight gain on SGAs (88), and we found that the participants with the highest leptin levels at baseline, of approx. 25,000pg/ml and approx. 50,000pg/ml had a high rate of weight gain of 21.6% and 42%. However, the participants in the study who had the highest weight gains of 46.5% and 60% had the lowest and third lowest leptin levels at baseline, at approx. 4,300pg/ml and 3,091pg/ml. We found that leptin levels were higher in the SGA group at baseline compared to the control group. This is likely to be due to the higher BMI percentile in the SGA group at baseline (60.5th percentile) compared to the control group (37th percentile).

There were no significant differences in Soluble CD163 levels on SGA medication. The participants who gained the most weight initially appeared to have higher CD163 levels, but this trend was not statistically significant. Increased soluble CD163 levels result from an increased macrophage activation with polarization towards the M1 pro-inflammatory subtype of

macrophages (54). Moller et al conducted a prospective study of almost 9000 adults and found that increased serum concentrations of soluble CD163 are associated with a greater risk of the development of T2DM (260). They found this risk to be up to five times greater even after adjustment for BMI, lipid profile and physical activity. In our study, there was no correlation between the change in BMI and soluble CD163 from baseline to 6 months.

We found no differences in serum IL-17 and serum TNF-α levels on SGA medication. When we compared baseline TNF-α levels to those in the healthy control group, we found that TNF-α was higher in the SGA group which may indicate a pro-inflammatory state in this group. This is likely to be due to the baseline BMI percentile being higher in the SGA group (60.5^{th} percentile) compared to the control group (37^{th} percentile).

After examining the systemic inflammatory profile of the participants before and after SGA medication, we then examined the frequency of circulating immune cells by flow cytometry. The frequency of CD4 T cells did not change following treatment with SGAs. CD4 T cells are helper cells which help to maintain the balance in the immune system between combating pathogens and self-tolerance (47). There were also no significant differences in the populations of CD45RA and CD45RO T cells. CD45RA is present on naïve T cells and CD45RO is expressed on memory cells following activation (263). This indicates that the activation status of T cells did not change on SGA medication.

The frequency of MAIT cells increased over time on SGAs. MAIT cell frequency has previously been found to be increased in children with obesity (219). Increased MAIT cells are associated with an increase in metabolic disease. A recent study by Toubal et. al showed that an increase in MAIT cells promotes metabolic dysfunction in a mouse model (264). Therefore, increased MAIT cells may be contributing to the future risk of metabolic disease associated with antipsychotic medication use. MAIT cells are few in neonates and absent in germ free mice, which suggests that their development requires the presence of gut microflora (243). SGA treatment has been shown to alter the composition and function of the gut microbiota, which play a role in the regulation of body

weight and inflammatory status (134, 265). It is possible that an altered microbiome in these children, caused by SGA medication, is contributing to the observed increase in MAIT cells. It is possible that an increase in MAIT cell frequency is contributing to the metabolic side effects associated with SGAs.

The frequency of iNKT cells appeared to have a decreasing trend on SGA medication for the first six months of treatment, although this trend was not statistically significant. iNKT cells are a subset of T cells which are thought to be part of both the innate and adaptive immune system and they are also a link between the immune and metabolic systems (249). iNKT cells have been found to be reduced in childhood obesity (54).

There were no differences in the frequencies of NK cells on SGA medication. The frequency of circulating immunoregulatory NK $CD56^{-Bright}$ showed an increasing trend on SGA medication and the frequency of NK $CD56^{-Dim}$ cytotoxic population showed a reducing trend on SGA medication over the 12 months of follow up, that was not statistically significant. Previous studies have shown a reduction in the frequency of $CD56^{-Dim}$ cytotoxic population associated with childhood obesity (221).

Monocytes are an innate immune cell and monocytes in circulation are the precursors for macrophages. There was a small increase in monocyte frequency at 3 months which reduced thereafter.

Overall, our findings from this part of the study were that SGAs do not significantly impact inflammatory markers in the systemic circulation and most circulating immune cells are not impacted by these medications. This indicates that inflammation may not have a causal role to play in weight gain in response to SGAs. We found that MAIT cells increased on SGA medication and increases in MAIT cells are associated with metabolic disease which is a side effect of SGA medication. As SGAs are associated with metabolic side effects and MAIT cells are associated with metabolic disease, it would be useful to examine the effect that SGAs have on cytokine production by MAIT cells and other immune cells. In the next chapter, we investigated the effect SGAs have on immune cell cytokine responses.

CHAPTER 5: Investigating the effects of SGAs on immune cell responses

5.1 Introduction

In chapter 4, we described several alterations in the systemic immunophenotype of children after treatment with SGAs. The systemic immunophenotyping determined the levels of several common inflammatory mediators (e.g., TNF-α and IL-17) and the circulating frequencies of the major peripheral blood immune subsets (e.g., T cells and NK cells). To further investigate these alterations, we examined the impact of SGAs on the immune cell responses. We established an ex-vivo model, where peripheral blood mononuclear cells (PBMC) from cohorts of healthy children, or children before and after SGA treatment were stimulated with either lipopolysaccharide (LPS) (primarily activating monocytes) or microbeads coated with anti-CD3/CD28 antibodies (primarily activating T cells). We assessed both the secreted cytokines using ELISA and the cytokine producing cells using intracellular flow cytometry.

5.1.1 Cytokine producing cells and cytokines investigated in this study

In the following sections the cytokine producing cells and cytokines that were investigated are discussed. These are CD4+ T cells, γδ Tcells, MAIT cells (discussed in section 4.1.5.1), TNF-α, lL-10, IL-17A and IL-17F. As SGAs have an antagonistic effect at dopamine receptors, the effect of dopamine on the immune system was also investigated.

5.1.2 CD4+ T cells

CD4+ T cells mainly serve a helper function in the body and once activated, they produce a range of cytokines that influence a variety of cell types (42, 48, 266). Their main role is in maintaining balance in the immune system between defence against the threat of infections and cancer and maintaining self-tolerance. They play a role in both establishing the immune response and maximising it (42). T helper CD4+ lymphocytes can differentiate into Th1, Th2, Treg or Th17 cells. CD4+ cells themselves do not have a cytotoxic function but they have an important role in directing other cells and in regulating the type of immune response that develops (42, 266). There is a subset of CD4+ T cells that are known as T regulatory cells that limit and suppress the immune response and

play a role in the development of immune tolerance to antigens. Loss of regulatory T cells is associated with autoimmune disease and allergy (42, 266). Circulating regulatory T cells are reduced in obesity (267). Th1 cells are involved in pro-inflammatory responses that lead to secretion of cytokines such as TNF-α and IFN-γ (268). Th2 cells are involved in the secretion of anti-inflammatory cytokines which reduce adipose tissue inflammation and improve insulin resistance (268). Th17 cells produce pro-inflammatory cytokines such as IL-17A and IL-17F. Obesity is associated with an increase in Th17 cells (268).

5.1.3 γδ T cells

γδ T cells are a subset of T lymphocytes that can recognize a broad range of antigens. They act as a bridge between the innate and adaptive immune systems and protect the host from infection, having an important role at the mucosal barrier (269, 270). They induce cytotoxic activity by release of perforins and granzymes and can target cells directly in this way or they can activate other immune cells (271). γδ T cells produce IL-17 and have been implicated in autoimmune disorders (257). People with obesity have a reduced number of γδ T cells which is inversely proportionate to their BMI (269, 272). The remaining γδ T cells are reduced in their ability to secrete cytokines to fight off infections (269, 272).

5.1.4 Cytokines

5.1.4.1 TNF-α

TNF-α is a pro-inflammatory cytokine and is implicated in the development of obesity-related diseases including insulin resistance (273). TNF-α is mainly produced by macrophages and is involved in the regulation of innate immunity, Th1 response, inflammation, cell differentiation and proliferation, energy metabolism and apoptosis (251). In humans, there is a positive correlation between TNF-α expression and body mass index, percentage of body fat, and hyperinsulinemia, whereas weight loss results in a decreased TNF-α level. TNF-α levels were also found to be positively associated with other markers of insulin resistance (53). Numerous studies have demonstrated strong associations

between circulating TNF-α and insulin resistance or other obesity-associated metabolic complications but attempts to block TNF-α function in patients have not yet produced consistent metabolic outcomes (62).

5.1.4.2 IL-10

IL-10 is a cytokine with anti-inflammatory properties that has a role in appropriately limiting the immune response (274, 275). It is produced by dendritic cells, B cells, cytotoxic T cells, γδ T cells, NK cells, mast cells, and neutrophilic and eosinophilic granulocytes (274). It inhibits the release of pro-inflammatory cytokines from monocytes and macrophages, therefore inhibiting secretion of TNF-α and IL-1β (274, 275). People with obesity have been shown to produce more IL-10 than their lean counterparts (276). A potential reason for this is a compensatory mechanism due to the inflammatory environment the cells are in.

5.1.4.3 IL-17A and IL-17F

IL-17A and IL-17F are pro-inflammatory cytokines that play a role in inflammatory disease and autoimmune disease including psoriasis, arthritis, non-alcoholic fatty liver disease and inflammatory bowel disease (48, 257, 277-279). They are members of the IL-17 family of cytokines that regulate both innate and adaptive immunity (279). IL-17A and IL-17F are both produced by several types of immune cells (278). Increases in the production of IL-17 have been reported in children with obesity (219). IL-17 is linked to insulin resistance and metabolic disease (259, 280). Chronic IL-17 activity is implicated in the pathogenesis of autoimmunity and some cancers including cancers involving the colon, skin, pancreas, lung, liver, and myeloma (258).

5.1.5 Dopamine and its effects on inflammation

The mechanism of action of SGAs is antagonistic binding to dopamine receptors. There are five different dopamine receptors, D1-D5. D1 and D5 are called “D1-like” receptors and D2, D3 and D4 are called “D2-like” receptors. D1-like receptors play a role in learning, memory, and regulation of reward systems. D2-like receptors are involved in behavioural and extrapyramidal activity (281). All

SGAs that are currently licensed show antagonistic binding to dopamine D2/D3 receptors to some degree and binding to dopamine D2 receptors at therapeutic doses is critical for their therapeutic action (68). Different SGAs differ in their affinity for the different dopamine receptors (68).

Dopamine appears to play a role in immune system regulation. D2, D3 and D4 dopamine receptors have been identified on human T and B lymphocytes, neutrophils, monocytes, eosinophils, and NK cells (230, 282-284). Studies have shown that dopamine can regulate cytokine secretion during both innate and adaptive immune responses as it suppresses secretion of IFN-γ, TNF-α and IL-1β (230, 231, 285). It has been shown that dopamine suppressed IL-17 production from PBMCs of patients with multiple sclerosis (286, 287).

The levels of dopamine in the body have been shown to determine whether dopamine has a pro- or anti-inflammatory effect. Decreased dopamine levels have been associated with inflammatory processes including neuroinflammation in the brain of Parkinson's disease patients and inflammation in the gut mucosa of patients with inflammatory bowel disease (288). It has been hypothesized that the effects of dopamine on inflammation could be dependent on dopamine levels with low levels being pro-inflammatory and higher levels being anti-inflammatory (287).

5.2 Specific aims for this chapter

The specific aims of this chapter were to narrow our focus on examining the effect of SGAs on the immune system over time by examining the effects of SGAs on immune cell responses and therefore to:

1. Examine the cellular inflammatory profile in the cohort of participants on SGA treatment at each time point, in particular looking at levels of TNF-α, IL-10, IL-17A and IL-17F.
2. Examine the effect of dopamine and risperidone on the production of IL17F and TNF-α by PBMCs.
3. Carry out intracellular immunophenotyping with a focus on examining the frequency of MAIT cells and their production of TNF-α and IL17A over time by intracellular immunophenotyping.

5.3 Results

5.3.1 The pro-inflammatory cytokine TNF-α increases early on following treatment with SGA medication

To measure the levels of production of TNF-α at baseline and over the twelve months of the study period, PBMCs were isolated from participants at each time point. PBMCs were also isolated from a group of healthy controls. The PBMCs were set up on a cell culture plate. PBMCs were either plated alone in CRPMI media or were stimulated with LPS. The PBMCs were at a final concentration of 1 x 10^6 cells per well. The cell supernatants were harvested and TNF-α concentration was examined by ELISA.

Analysis of the supernatants from the basal wells by ELISA showed that levels of TNF- α were higher in the SGA group at baseline compared to the healthy control group (Figure 5.1A). Overall, TNF- α levels as measured in the supernatants of unstimulated PBMCs did not change over the study period (Figure 5.1C and 5.1E).

Analysis of the supernatants from the LPS-stimulated wells by ELISA showed that levels of TNF- α were higher in the SGA group at baseline compared to the healthy control group (Figure 5.1B). When we compared the TNF-α levels at 0 and 3 months in supernatants of LPS-stimulated PBMCs (n=12), we found that TNF-α levels increased significantly at three months (Figure 5.1D). After the three-month time point, TNF-α levels did not increase (Figure 5.1F). The data show that TNF-α increases soon after initiation of SGA medication.

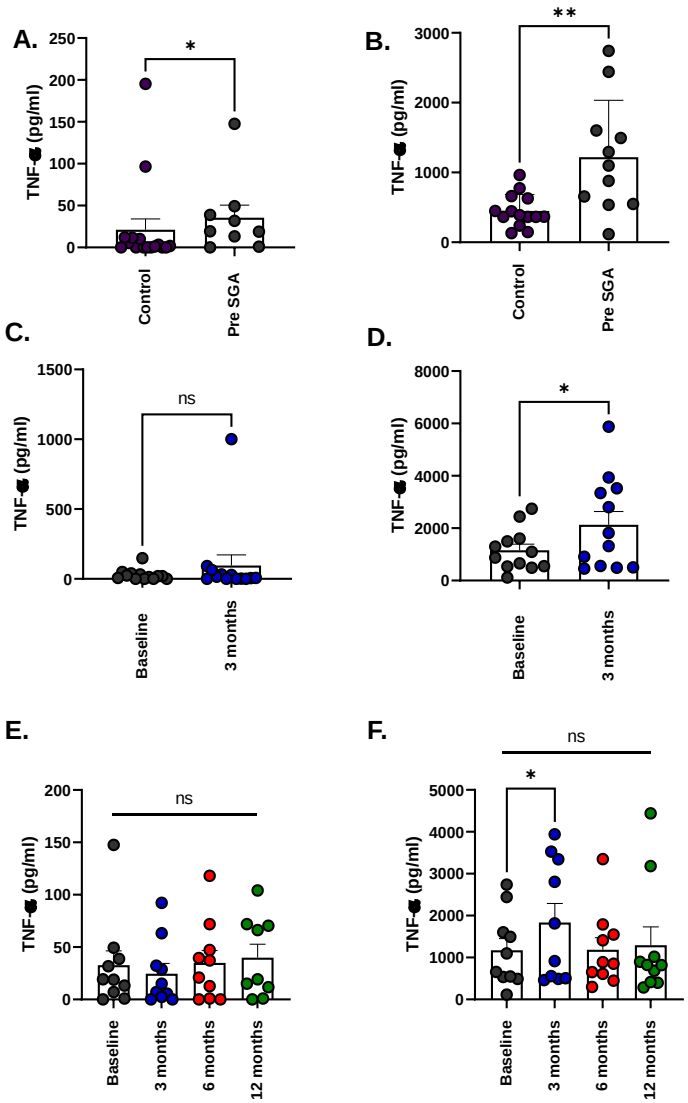


Figure 5.1: TNF-α levels in cell culture supernatants pre- and post-treatment with SGA medications

Scatter plots showing: **(A)** TNF-α levels in the cell culture supernatants of unstimulated PBMCs of healthy controls and the participants in the SGA cohort at baseline prior to commencing treatment. **(B)** TNF-α levels in cell culture supernatants of LPS-stimulated PBMCs of healthy controls and the participants in the SGA cohort at baseline prior to commencing treatment. **(C)** TNF-α levels in cell culture supernatants of unstimulated PBMCs of the SGA group at baseline and 3 months (n=12). **(D)** TNF-α levels in cell culture supernatants of LPS-stimulated PBMCs of the SGA group at baseline and 3 months (n=12). **(E)** TNF-α levels in the SGA cohort at baseline, three months, six months, and twelve months in cell culture supernatants of unstimulated PBMCs (n=10). **(F)** TNF-α levels in the SGA cohort at baseline, three months, six months, and twelve months in cell culture supernatants of LPS- stimulated PBMCs (n=10). Statistical analysis performed using unpaired t- test, paired t-test and repeated measures ANOVA with multiple comparisons, ns – not significant, * = $p<0.05$, ** = $p<0.01$

5.3.2 Dopamine reduces the production of TNF-α by THP-1 cells in vitro

We used a monocyte model to investigate the effect dopamine had on TNF-α production. To do this, we investigated the effect dopamine had on TNF-α production by THP-1 cells. THP-1 cells were plated on a 24 well cell culture plate. To polarize the cells to inflammatory M1 macrophages, phorbol 12-myristate 13-acetate (PMA) was added at a concentration of 100nM and the THP-1 cells were plated at a concentration of 1 x 10^6 cells/ml. Eight wells were set up with cells alone in the first well, cells plus dopamine (100µM concentration) in the second well, cells plus risperidone (SGA) in the third well and cells plus both dopamine and risperidone in the fourth well. The second row of 4 wells were plated as above and after two hours, LPS at a concentration of 100ng/ml was added to these four wells. The supernatants were harvested and TNF-α concentration was examined by ELISA. Dopamine caused a significant reduction in TNF-α production (Figure 5.2A). Risperidone and risperidone and dopamine in combination did not influence TNF-α production (Figure 5.2A). In the LPS stimulated cells, dopamine caused the greatest reduction in the production of TNF-α, although this was not statistically significant (Figure 5.2B).

We also investigated the effect that dopamine and risperidone had on the production of TNF-α by PBMCs in the SGA cohort. As TNF-α production was highest at three months, we used PBMCs collected at this time point for the experiment. PBMCs were thawed after removal from the freezer as described in section 2.10. The PBMCs were plated at a concentration of 500,000 cells per 0.5ml. In order to stimulate cytokine production from T cells, we stimulated the PBMCs with TCR beads. The first well had CRPMI and TCR beads at a concentration of 25ng/ml. The second well had CRPMI, TCR beads (25ng/ml) plus dopamine at a concentration of 100µM. The third well had CRPMI, TCR beads (25ng/ml) plus risperidone (1µg/ml). The supernatants were harvested and TNF-α concentration was examined by ELISA. Dopamine caused a significant reduction in TNF-α production (Figure 5.2C). Risperidone caused a significant reduction in TNF-α production (Figure 5.2C).

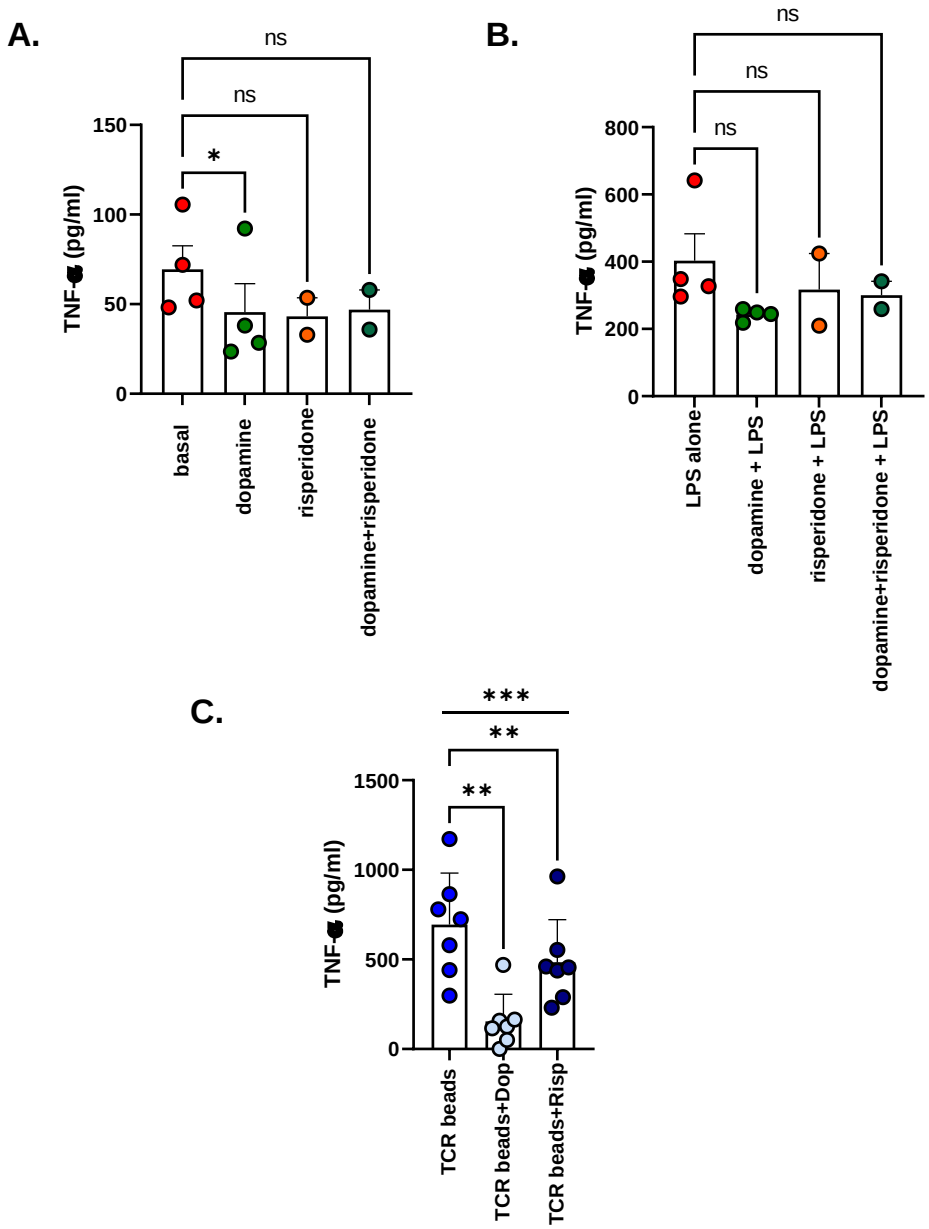


Figure 5.2: TNF-α levels in cell culture supernatants of THP-1 cells

Scatter plots showing: **(A)** TNF-α levels in cell culture supernatants of THP-1 cells, THP-1 cells treated with dopamine, THP-1 cells treated with risperidone and THP-1 cells treated with dopamine and risperidone. **(B)** TNF-α levels in cell culture supernatants of THP-1 cells, THP-1 cells treated with dopamine, THP-1 cells treated with risperidone and THP-1 cells treated with dopamine and risperidone, and LPS added to all wells after two hours. **(C)** TNF-α levels in cell culture supernatants of PBMCs stimulated with TCR beads alone, TCR beads + dopamine and TCR beads + risperidone. Statistical analysis performed using repeated measures ANOVA with multiple comparisons. ns – not significant, * = $p<0.05$, ** = $p<0.01$, *** = $p<0.001$

5.3.3 IL-10 increases on SGA treatment

IL-10 is a cytokine that is considered to be anti-inflammatory (51, 274, 275). However, it has been shown to increase in people with obesity (276). Production of IL-10 from PBMCs was measured by ELISA. Levels of IL-10 were measured in the supernatants of PBMCs that did not have any stimulant added and in the supernatants of PBMCs that were stimulated with LPS. The unstimulated PBMCs showed increased IL-10 production at 3 months (n=12) (Figure 5.3A & Figure 5.3B). IL-10 levels increased at each time point in unstimulated PBMCs (Figure 5.3C). There were no differences in IL-10 levels in the supernatants of PBMCs that were stimulated with LPS (Figure 5.3D and Figure 5.3E). These data show that IL-10 levels increase on SGA medication, as measured in the supernatants of unstimulated PBMCs.

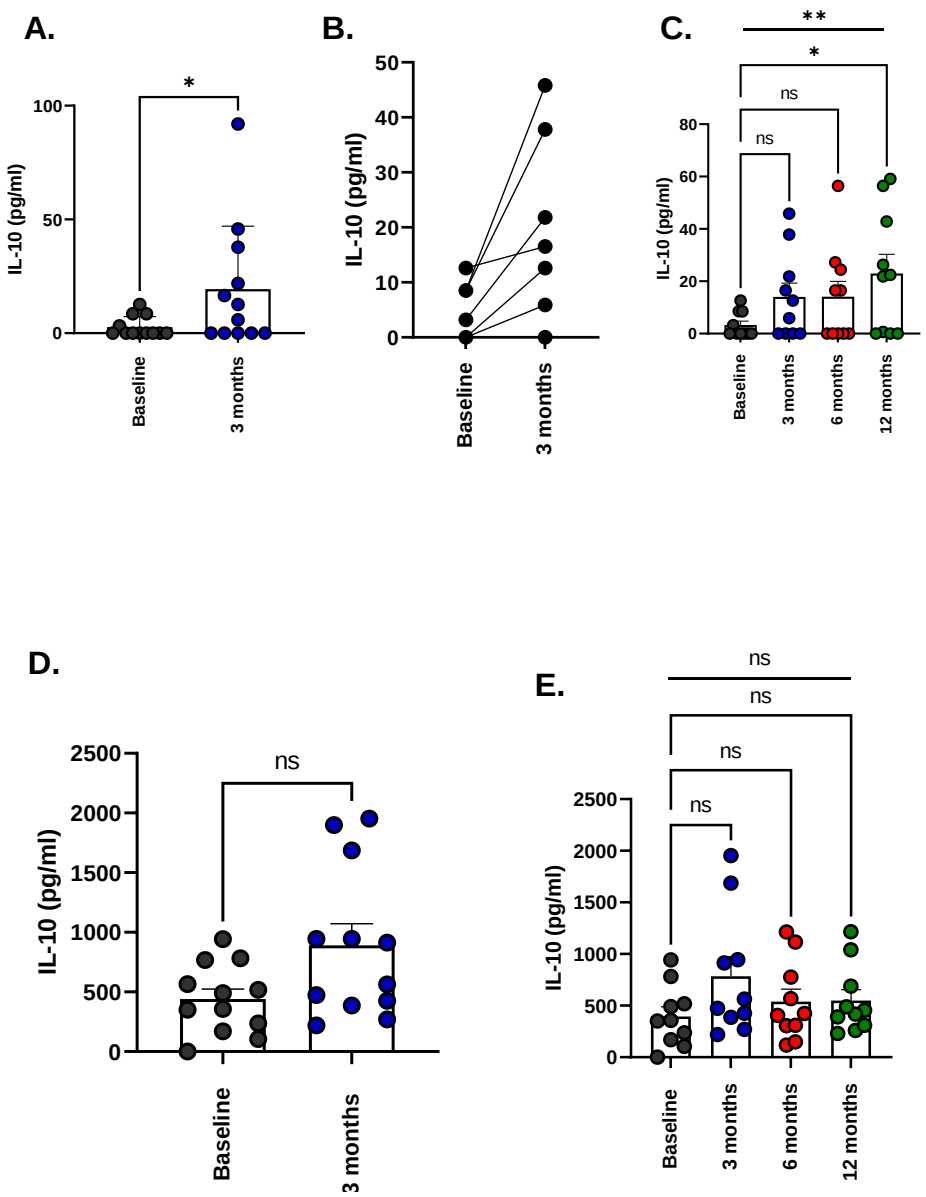


Figure 5.3: IL-10 levels in cell culture supernatants pre- and post-treatment with SGA medications

Scatter plots showing: **(A)** IL-10 levels in the cell culture supernatants of unstimulated PBMCs of the SGA group at baseline and 3 months (n=12). **(B)** IL-10 levels in the cell culture supernatants of unstimulated PBMCs of the SGA group at baseline and 3 months (n=12), this graph shows that the general trend of IL-10 was upwards for each participant. **(C)** IL-10 levels in the SGA cohort at baseline, three months, six months, and twelve months in cell culture supernatants of unstimulated PBMCs (n=10). **(D)** IL-10 levels in cell culture supernatants of LPS-stimulated PBMCs of the SGA group at baseline and 3 months (n=12). **(E)** IL-10 levels in the SGA cohort at baseline, three months, six months, and twelve months in cell culture supernatants of LPS- stimulated PBMCs (n=10). Statistical analysis performed using Wilcoxon test and Friedman test with multiple comparisons, ns – not significant, * = $p<0.05$, ** = $p<0.01$

5.3.4 The production of IL-17A by PBMCs shows an increasing trend on SGA medication

IL-17A is a pro-inflammatory cytokine that is associated with insulin resistance (259, 280). To measure the production of IL-17A at baseline and over the twelve months of the study period, PBMCs were isolated from participants at each time point. PBMCs were also isolated from a group of healthy controls. The PBMCs were set up on a cell culture plate and stimulated with TCR beads. The PBMCs were at a final concentration of 1×10^6 cells per well. The cell supernatants were harvested and IL-17A concentration was examined by ELISA.

The healthy control groups had higher levels of IL17A as measured in cell culture supernatants of PBMCs than the SGA group (Figure 5.4A).

In the SGA group (n =12), IL-17A production increased at three months (Figure 5.4B). There were no differences in IL-17A production in the SGA cohort that completed the twelve months of follow up (n=10) (Figure 5.4C). We separated the participants (n=12) into those that gained weight between baseline and three months and the participants that did not gain weight and we found there were no differences in IL-17A between the two groups (Figure 5.4D). The data show that IL-17A increases early on with SGA treatment.

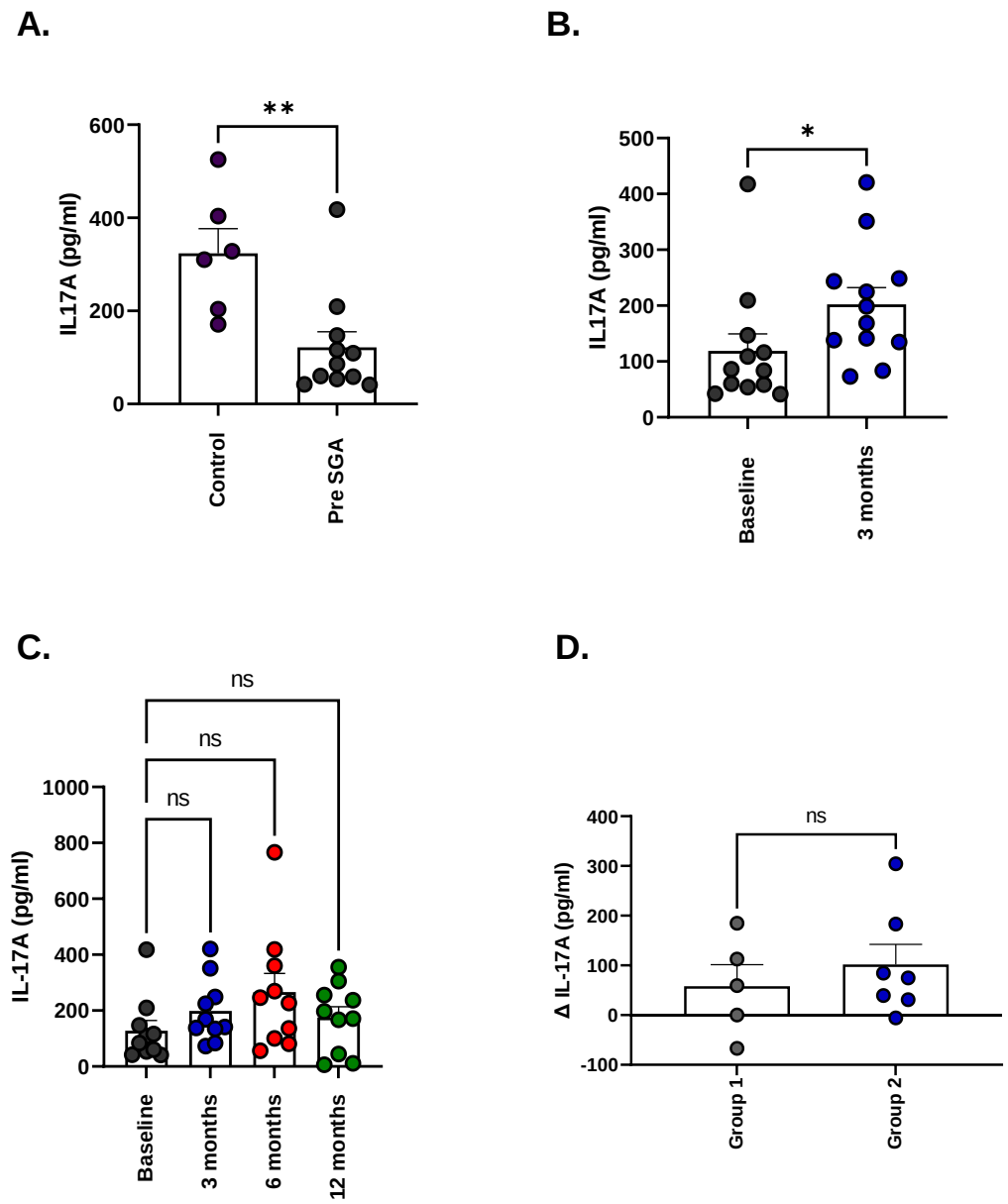


Figure 5.4: IL-17A levels in cell culture supernatants pre- and post-treatment with SGA medications

Scatter plots showing: **(A)** IL-17A levels in the cell culture supernatants of TCR-stimulated PBMCs of the SGA group at baseline compared to a healthy control group. **(B)** IL-17A levels in the cell culture supernatants of TCR-stimulated PBMCs of the SGA group at baseline and 3 months (n=12). **(C)** IL-17A levels in the SGA cohort at baseline, three months, six months and twelve months in cell culture supernatants of TCR-stimulated PBMCs (n=10). **(D)** IL-17A levels in the SGA cohort at baseline and at 3 months after commencing SGA treatment. The patients who did not gain weight (Group 1) on SGA treatment are separated from the rest of the cohort who did gain weight (Group 2). Statistical analysis performed using Mann Whitney test, Wilcoxon Test and repeated measures ANOVA with multiple comparisons, ns – not significant, * = $p<0.05$, ** = $p<0.01$

5.3.5 The production of IL-17F by PBMCs shows an increasing trend on SGA medication

IL-17F is a pro-inflammatory cytokine that is associated with insulin resistance (259, 280). To measure the levels of production of IL-17F at baseline and over the twelve months of the study period, PBMCs were isolated from participants at each time point. PBMCs were also isolated from a group of healthy controls. The PBMCs were set up in a cell culture plate and stimulated with TCR beads. The PBMCs were at a final concentration of 1×10^6 cells per well. The cell supernatants were harvested and IL-17F concentration was examined by ELISA.

There were no differences in levels of IL17F as measured in cell culture supernatants of PBMCs between the SGA group at baseline and the healthy control group (Figure 5.5A).

In the group of participants that completed 3 months of follow up (n=12), there was no difference in IL-17F levels at 3 months compared to baseline (Figure 5.5B).

In the SGA group (n =10), IL-17F production increased at three months and six months, this was not statistically significant. IL-17F production reduced at twelve months but remained higher than baseline levels (Figure 5.5C).

We separated the participants (n=12) into those that gained weight between baseline and three months and the participants that did not gain weight and we found there were no differences in IL-17F between the two groups (Figure 5.5D).

We investigated the effect that dopamine and risperidone had on the production of IL-17F by PBMCs in the SGA cohort. As IL-17F production was highest at six months, we used PBMCs collected at this time point for the experiment. PBMCs were thawed after removal from the freezer as described in section 2.10. The PBMCs were plated at a concentration of 500,000 cells per 0.5ml. The first well had CRPMI and TCR beads. The second well had CRPMI, TCR beads plus dopamine at a concentration of 100µM. The third well had CRPMI, TCR beads plus risperidone at a concentration of 1µg/ml. The supernatants were harvested and IL-17F concentration was examined by ELISA. Dopamine caused a reduction

in IL-17F production, that was not statistically significant, and Risperidone had less of an influence on IL-17F production (Figure 5.5E).

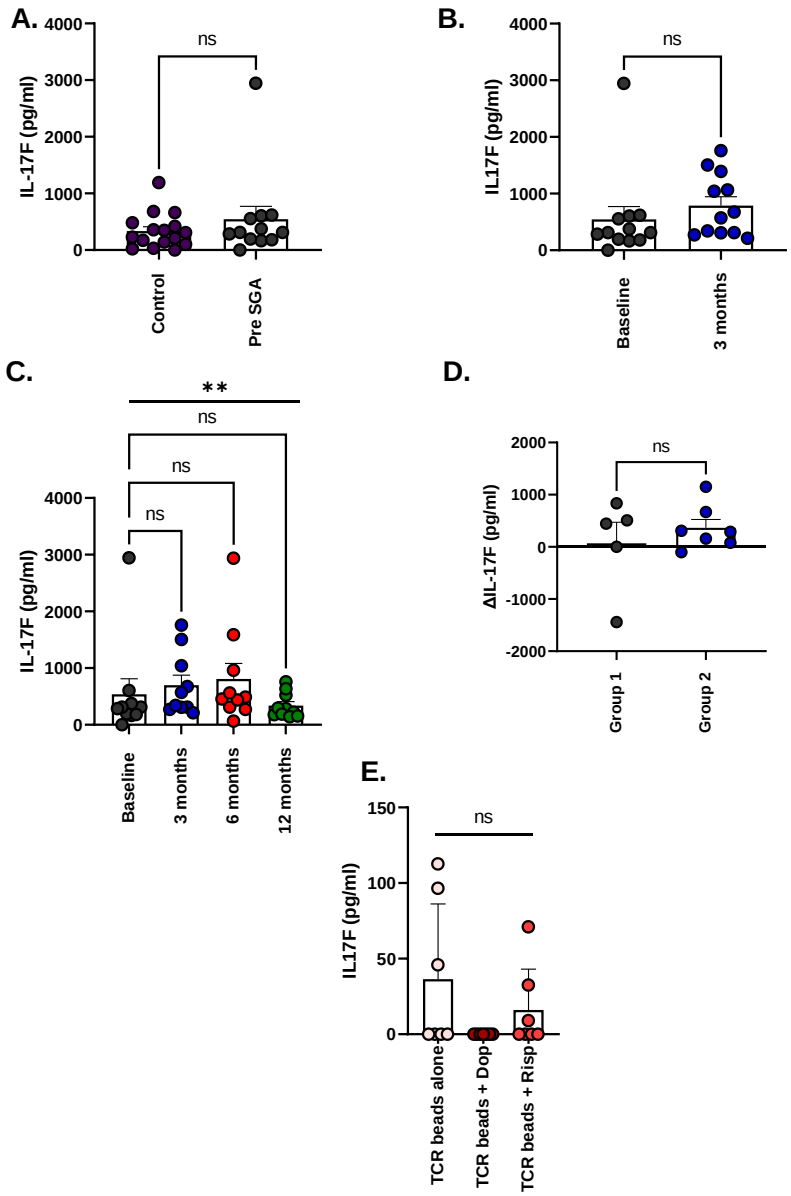


Figure 5.5: IL-17F levels in cell culture supernatants pre- and post-treatment with SGA medications

Scatter plots showing: **(A)** IL-17F levels in the cell culture supernatants of TCR-stimulated PBMCs of the SGA group at baseline compared to a healthy control group. **(B)** IL-17F levels in the cell culture supernatants of TCR-stimulated PBMCs of the SGA group at baseline and 3 months (n=12). **(C)** IL-17F levels in the SGA cohort at baseline, three months, six months and twelve months in cell culture supernatants of TCR-stimulated PBMCs (n=10). **(D)** IL-17F levels in the SGA cohort at baseline and at 3 months after commencing SGA treatment. The patients who did not gain weight on SGA treatment are separated from the rest of the cohort who did gain weight. **(E)** IL-17F levels in the cell culture supernatants of PBMCs treated with TCR beads alone, TCR beads + dopamine and TCR beads + risperidone. Statistical analysis performed using Mann Whitney test, Wilcoxon Test and Friedman test with multiple comparisons, ns – not significant, * = $p<0.05$, ** = $p<0.01$

5.3.6 Intracellular immunophenotyping at baseline, 3 months and 6 months in the SGA cohort

PBMCs were removed from the -80°C freezer and thawed as described in Section 2.10. The PBMCs were plated on a 96 well cell culture plate. We investigated IL-17 and TNF-α production from MAIT cells, CD4 T cells and γδ cells. We examined the effects that dopamine hydrochloride and Cell Stimulation Cocktail (phorbol 12-myristate 13-acetate (PMA) and ionomycin) had on the production of these cytokines.

PBMCs were plated at a concentration of 500,000 PBMCs per 0.5ml. The first well contained PBMCs and CRPMI media. Cell Stimulation Cocktail was added to the second well (stock at 500X, used at 1X). Dopamine hydrochloride was added to the third well at a concentration of 100µM. Dopamine hydrochloride and Cell Stimulation Cocktail were added to the fourth well. All wells also had Cell Inhibitor cocktail (*Invitrogen eBioscience*), protein transport inhibitor, added to keep the cytokines inside the cell (stock at 500X, used at 1X). The PBMCs were left in an incubator overnight.

5.3.7 MAIT cell identification

In section 4.3.8, we showed that MAIT cell frequency increased on SGA medication. We also showed that the cytokines, TNF-αand IL-17A increased on SGA medication. To determine the cellular source of these cytokines, we carried out intracellular flow cytometry to investigate if MAIT cells were contributing to the elevated levels of TNF-α and IL-17 in children treated with SGAs. The gating strategy used to identify the MAIT cells is shown in figure 5.6.

PBMCs isolated from participants were stained using antibodies against surface antigens to appropriate fluorochromes, namely CD3 (VioGreen), CD161 (FITC), Vα7.2 (PECy7), IL-17 (PE) and TNF (APC) (as per table 2.3). To identify MAIT cells, lymphocytes were selected based on their forward and side scatter and doublets were excluded using FSC-area and FSC-height parameters. MAIT cells were then identified by their positive expression of CD3, high expression of CD161 and the expression of Vα7.2 TCR on their cell surface (Figure 5.6A). IL-17 and TNF-α producing MAIT cells were also identified (Figure 5.6 B and Figure 5.6C).

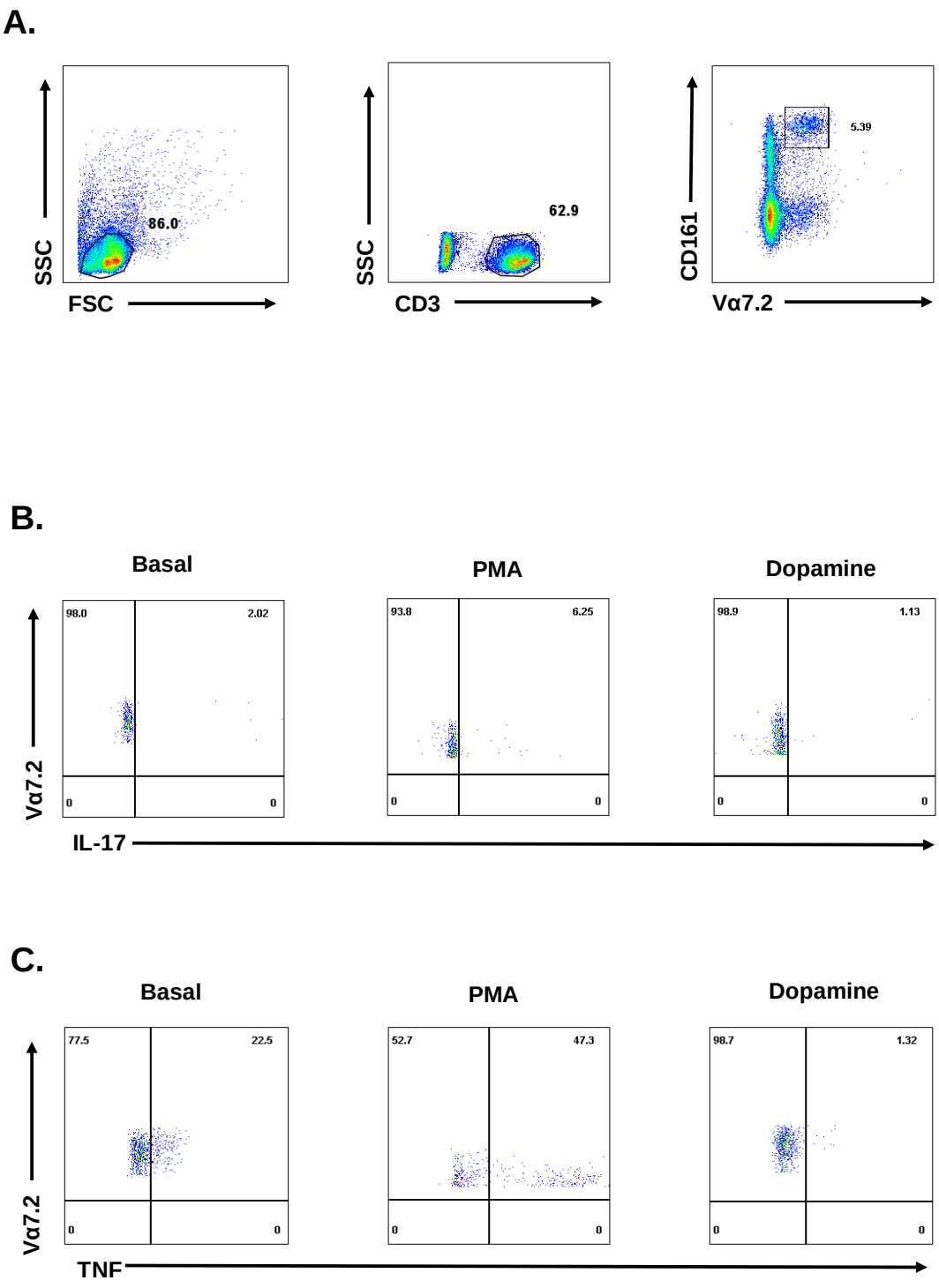


Figure 5.6: MAIT cell identification

(A) Representative sample showing gating strategy for identification of MAIT cells. **(B)** Representative sample showing gating strategy for identification of IL-17 producing MAIT cells in unstimulated cells, cells stimulated with PMA and cells cultured in dopamine hydrochloride. **(C)** Representative sample showing gating strategy for identification of TNF-α producing MAIT cells in unstimulated cells, cells stimulated with PMA and cells cultured in dopamine hydrochloride

5.3.8 MAIT cell frequencies are increased with SGA treatment and are a source of IL-17 and TNF-α

MAIT cell frequencies increased on SGA medications (Figure 5.7A).

The frequency of IL-17 producing MAIT cells showed an increasing trend at three months on SGA medication that was not statistically significant (Figure 5.7B).

The frequency of TNF-α producing MAIT cells showed no difference on SGA medication (Figure 5.7C).

When stimulated with PMA, both IL-17 (Figure 5.7D) and TNF-α production increased significantly (Figure 5.7E).

5.3.9 Dopamine causes a reduction in TNF-α production from MAIT cells but does not affect IL-17 production

Dopamine caused a significant reduction in TNF-α production from MAIT cells on SGAs at the three-month time point (Figure 5.8A).

The combination of dopamine and PMA together did not have a significant impact on TNF-α production from MAIT cells, therefore dopamine did not exert a dopamine anti-inflammatory effect in the presence of a PMA stimulant (Figure 5.8B).

Dopamine did not influence IL-17 production (Figure 5.8C). Dopamine and PMA in combination caused a reduction in IL-17 producing MAIT cells at the three-month time-point when compared to PBMCs stimulated with PMA alone (Figure 5.8D).

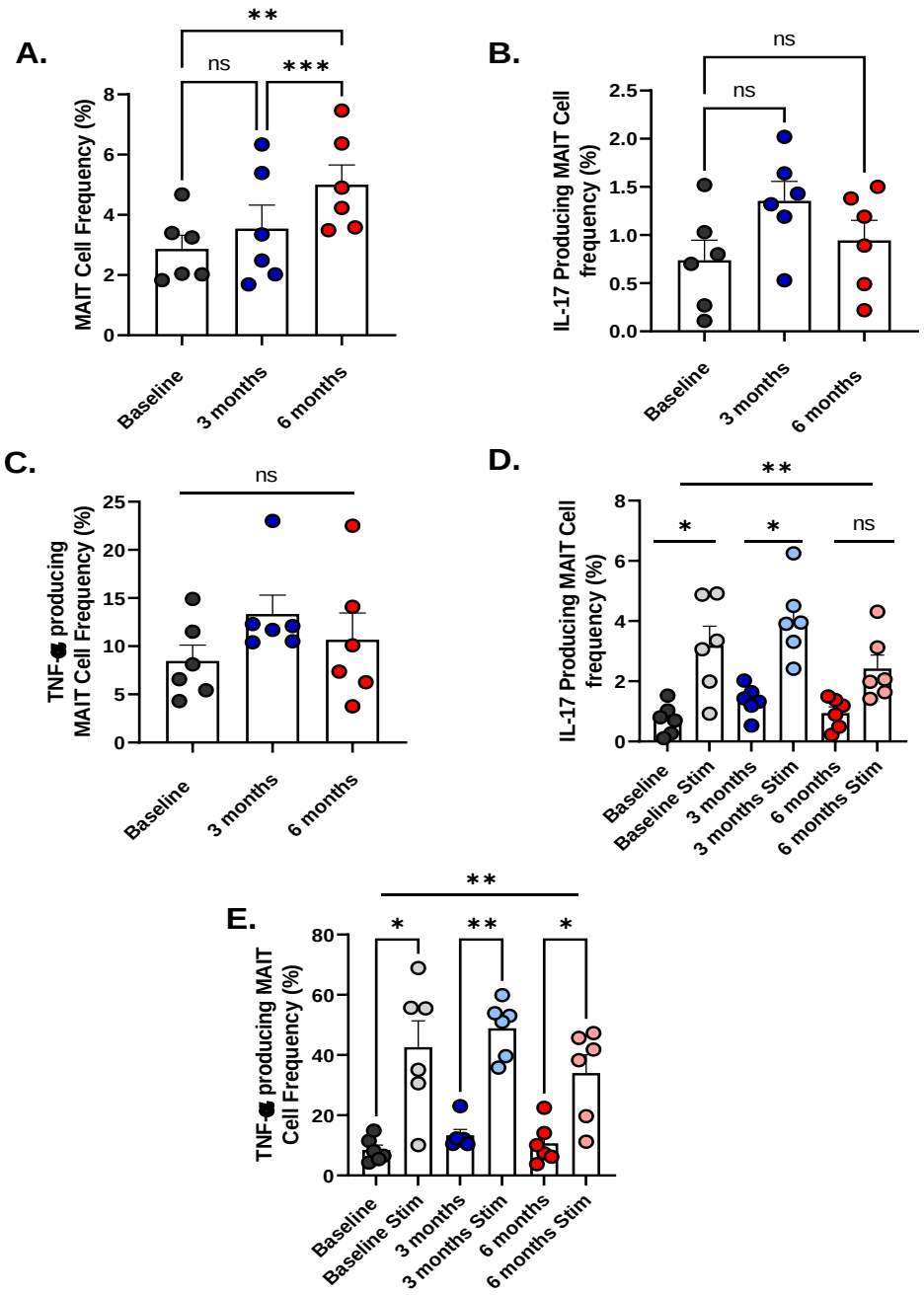


Figure 5.7: Cytokine production by MAIT Cells

Scatter plots showing: **(A)** MAIT Cell frequencies in the SGA cohort at baseline, 3 months, and 6 months. **(B)** IL-17 producing MAIT Cell frequencies in the SGA cohort at baseline, 3 months, and 6 months. **(C)** TNF-α producing MAIT Cell frequencies in the SGA cohort at baseline, 3 months, and 6 months. **(D)** IL-17 producing MAIT Cell frequencies in the SGA cohort at baseline, 3 months, and 6 months in unstimulated PBMCs and PBMCs stimulated with PMA. **(E)** TNF-α producing MAIT Cell frequencies in the SGA cohort at baseline, 3 months, and 6 months in unstimulated PBMCs and PBMCs stimulated with PMA. Statistical analysis performed using repeated measures ANOVA with multiple comparisons, ns – not significant, * = $p<0.05$, ** = $p<0.01$, *** = $p<0.001$

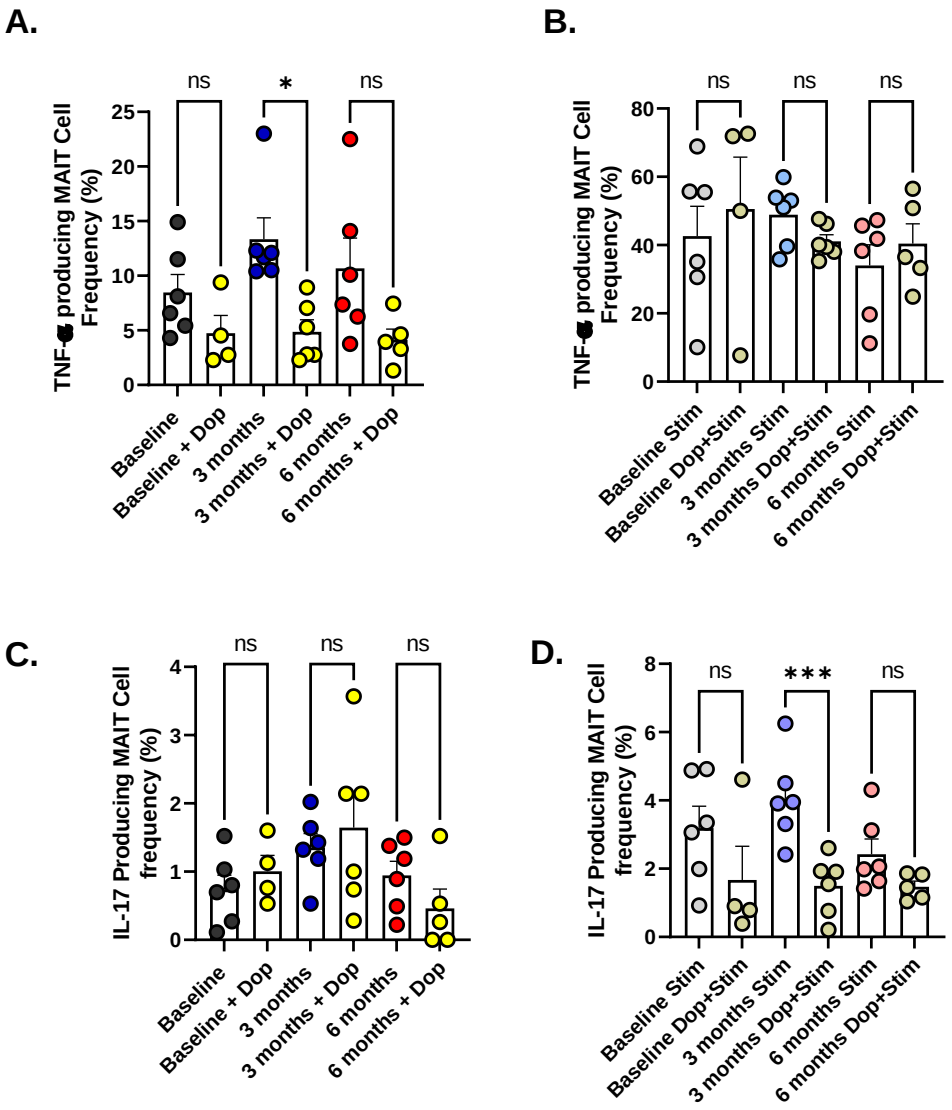


Figure 5.8: Cytokine production by MAIT cells stimulated with dopamine

Scatter plots showing: **(A)** TNF- α producing MAIT Cell frequencies in the SGA cohort at baseline, 3 months, and 6 months in unstimulated PBMCs and PBMCs stimulated with dopamine hydrochloride. **(B)** TNF-α producing MAIT Cell frequencies in the SGA cohort at baseline, 3 months, and 6 months in PMA-stimulated PBMCs and PBMCs stimulated with dopamine hydrochloride + PMA. **(C)** IL-17 producing MAIT Cell frequencies in the SGA cohort at baseline, 3 months, and 6 months in unstimulated PBMCs and PBMCs stimulated with dopamine hydrochloride. **(D)** IL-17 producing MAIT Cell frequencies in the SGA cohort at baseline, 3 months, and 6 months in PMA-stimulated PBMCs and PBMCs stimulated with dopamine hydrochloride + PMA. Statistical analysis performed using repeated measures ANOVA with multiple comparisons, ns – not significant, * = $p<0.05$, ** = $p<0.01$

5.3.10 CD4 T Cell identification

Multi-coloured flow cytometry was used to analyse the frequencies of circulating CD4 T cells and their production of IL-17 and TNF-α. The gating strategy used to identify the CD4 T cells is shown in figure 5.9.

PBMCs isolated from participants were stained using antibodies against surface antigens to appropriate fluorochromes, namely CD3 (VioGreen), CD4 (PEVio615), IL-17 (PE) and TNF (APC) (as per table 2.3). To identify CD4 cells, lymphocytes were selected based on their forward and side scatter and doublets were excluded using FSC-area and FSC-height parameters. CD4 cells were then identified by their positive expression of CD3 and CD4 on their cell surface (Figure 5.9A). IL-17 and TNF producing CD4 T cells were also identified (Figure 5.9B and Figure 5.9C).

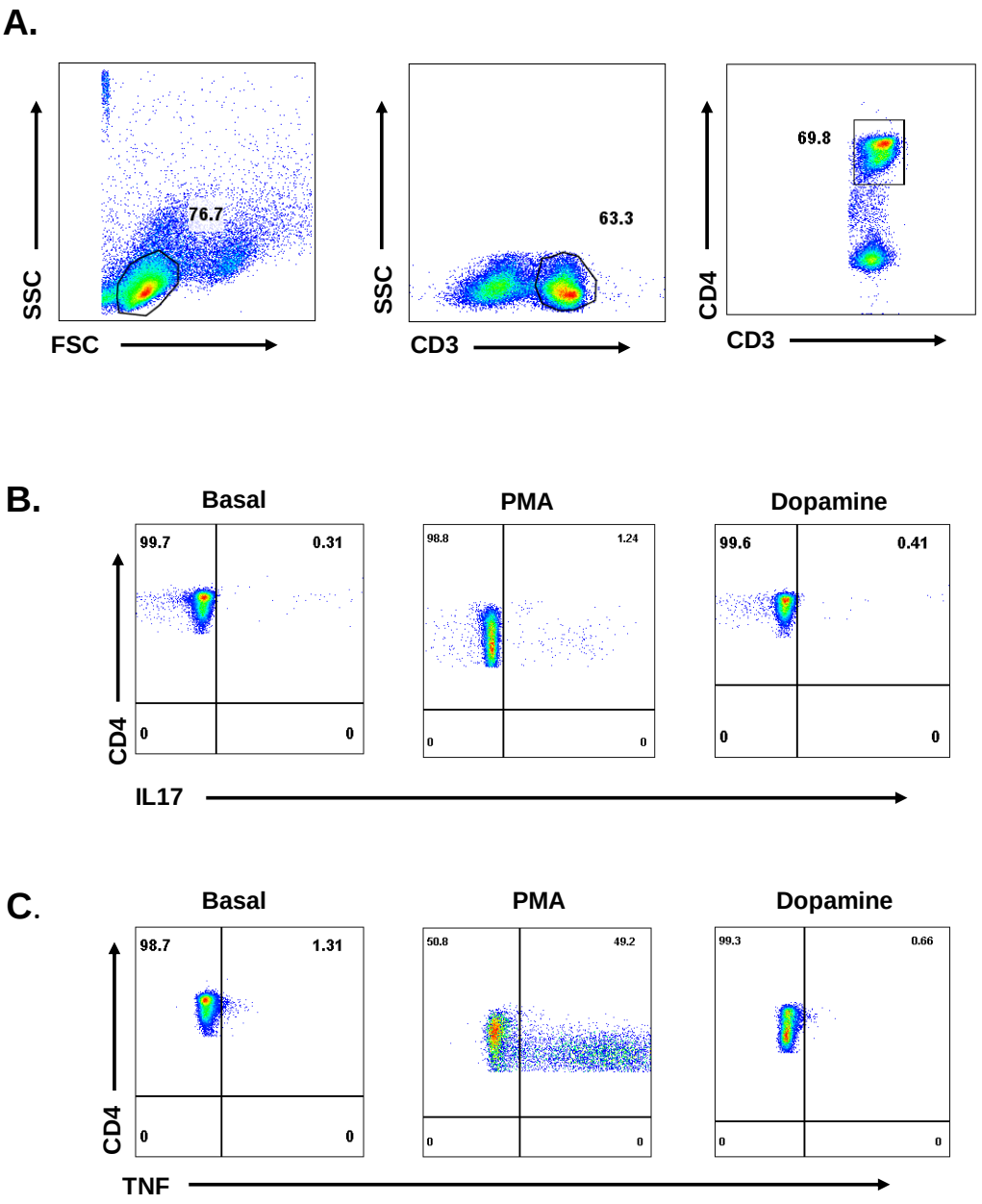


Figure 5.9: CD4 T cell identification

(A) Representative sample showing gating strategy for identification of CD4 T cells. **(B)** Representative sample showing gating strategy for identification of IL-17 producing CD4 T cells. **(C)** Representative sample showing gating strategy for identification of TNF-α producing CD4 T cells in unstimulated cells, cells stimulated with PMA and cells cultured in dopamine hydrochloride

5.3.11 CD4 T cell frequencies and their production of IL-17 and TNF-α do not change with SGA medication

CD4 T cell frequencies did not change on SGA medications (Figure 5.10A). The frequency of IL-17 producing CD4 T cells also showed no differences on SGA medication (Figure 5.10B).

The frequency of TNF-α producing CD4 T cells also showed no differences on SGA medication (Figure 5.10AC).

When stimulated with PMA, both IL-17 (Figure 5.10D) and TNF-α production increased (Figure 5.10E).

5.3.12 Dopamine does not affect IL-17 or TNF-α production from CD4 T cells

Dopamine did not cause a reduction in TNF-α production from CD4 T cells (Figure 5.11A). Dopamine did not cause a reduction in IL-17 production at any time point (Figure 5.11B). Dopamine and PMA in combination also had no effect on IL-17 (Figure 5.11C) and TNF-α production (Figure 5.11D) by CD4 T cells.

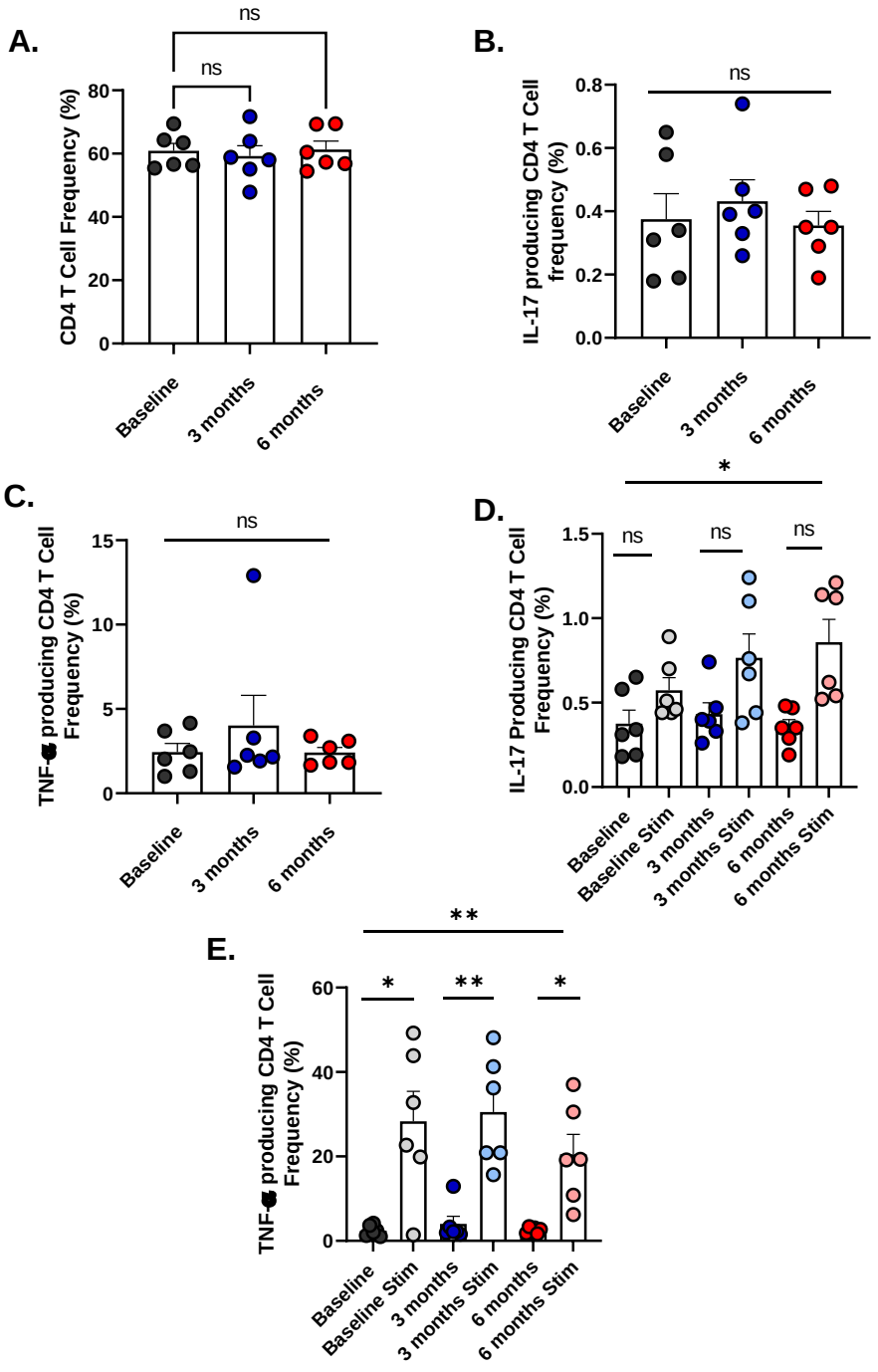


Figure 5.10: Cytokine production by CD4 T Cells

Scatter plots showing: **(A)** CD4 T Cell frequencies in the SGA cohort at baseline, 3 months, and 6 months. **(B)** IL-17 producing CD4 T Cell frequencies in the SGA cohort at baseline, 3 months, and 6 months. **(C)** TNF-α producing CD4 T Cell frequencies in the SGA cohort at baseline, 3 months, and 6 months. **(D)** IL-17 producing CD4 T Cell frequencies in the SGA cohort at baseline, 3 months, and 6 months in unstimulated PBMCs and PBMCs stimulated with PMA. **(E)** TNF-α producing CD4 T Cell frequencies in the SGA cohort at baseline, 3 months, and 6 months in unstimulated PBMCs and PBMCs stimulated with PMA. Statistical analysis performed using repeated measures ANOVA with multiple comparisons, ns – not significant, * = $p<0.05$

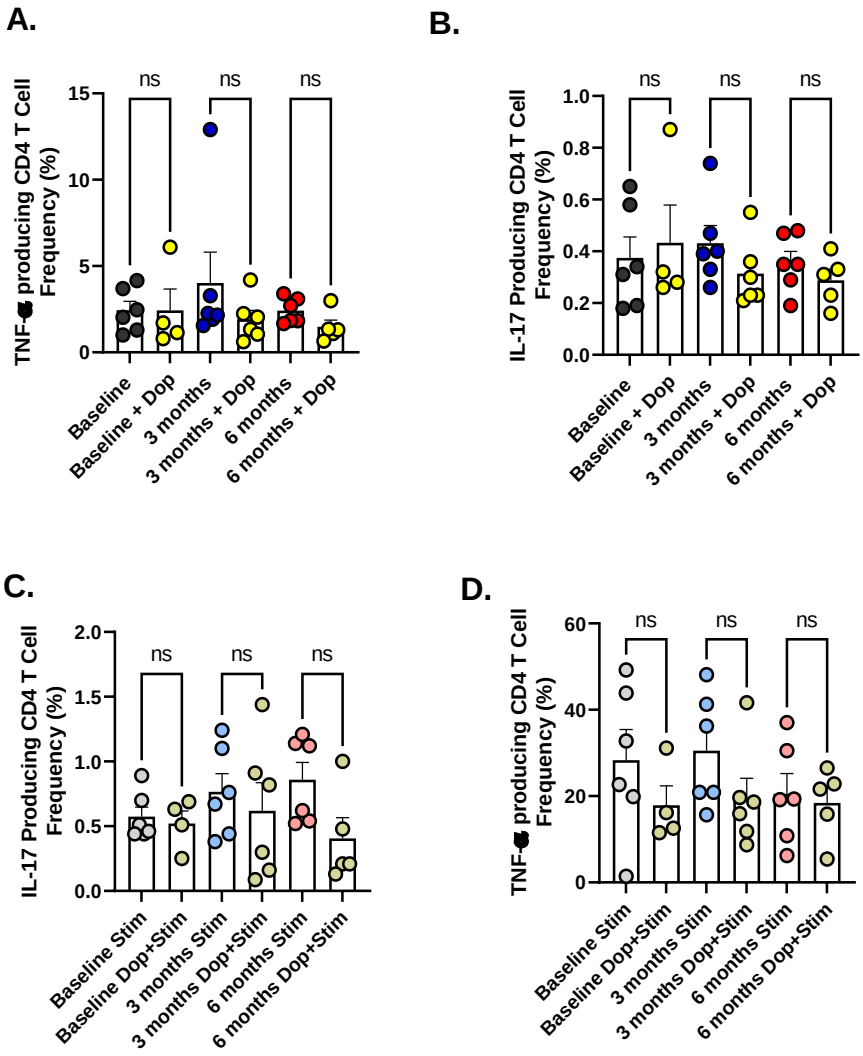


Figure 5.11: Cytokine production by CD4 T Cells stimulated with dopamine

Scatter plots showing: **(A)** TNF- α producing CD4 T Cell frequencies in the SGA cohort at baseline, 3 months, and 6 months in unstimulated PBMCs and PBMCs stimulated with dopamine hydrochloride. **(B)** IL-17 producing CD4 T Cell frequencies in the SGA cohort at baseline, 3 months, and 6 months in unstimulated PBMCs and PBMCs stimulated with dopamine hydrochloride. **(C)** IL-17 producing CD4 T Cell frequencies in the SGA cohort at baseline, 3 months, and 6 months in PMA-stimulated PBMCs and PBMCs stimulated with dopamine hydrochloride + PMA. **(D)** TNF-α producing CD4 T Cell frequencies in the SGA cohort at baseline, 3 months, and 6 months in PMA-stimulated PBMCs and PBMCs stimulated with dopamine hydrochloride + PMA. Statistical analysis performed using repeated measures ANOVA with multiple comparisons, ns – not significant, * = $p<0.05$, ** = $p<0.01$

5.3.13 Gamma Delta (γδ) cell identification

Multi-coloured flow cytometry was used to analyse the frequencies of circulating γδ cells and their production of IL-17 and TNF-α. The gating strategy used to identify the γδ cells is shown in figure 5.12.

PBMCs isolated from participants were stained using antibodies against surface antigens to appropriate fluorochromes, namely CD3 (VioGreen), γδ (APCy7), IL-17 (PE) and TNF (APC) (as per table 2.3). To identify γδ cells, lymphocytes were selected based on their forward and side scatter and doublets were excluded using FSC-area and FSC-height parameters. γδ cells were then identified by their positive expression of CD3 and γδ on their cell surface (Figure 5.12A). IL-17 and TNF producing γδ cells were also identified (Figure 5.12B and Figure 5.12C).

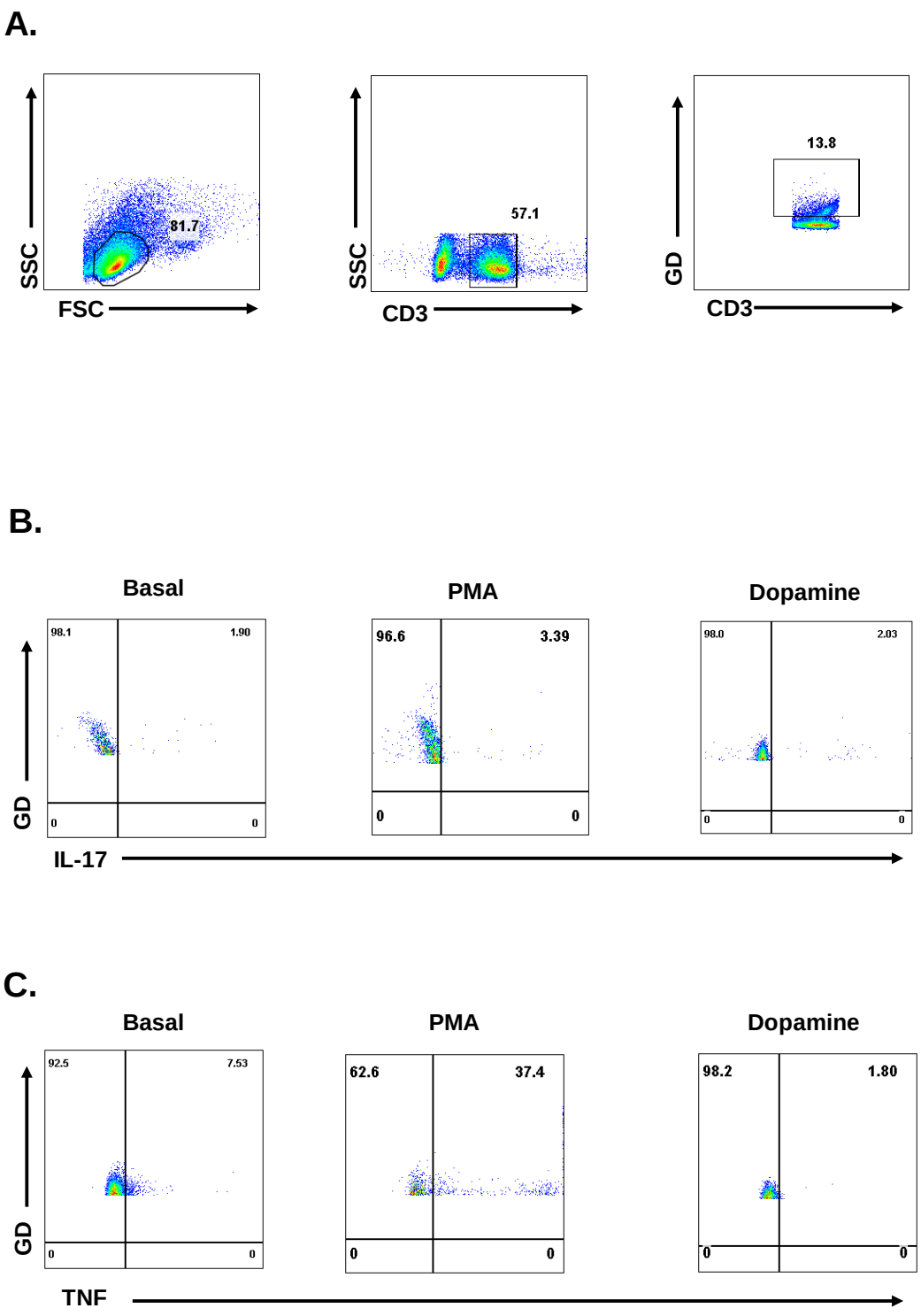


Figure 5.12: γδ T cell identification

(A) Representative sample showing gating strategy for identification of γδ T cells. **(B)** Representative sample showing gating strategy for identification of IL-17 producing γδ T cells. **(C)** Representative sample showing gating strategy for identification of TNF-α producing γδ T cells in unstimulated cells, cells stimulated with PMA and cells cultured in dopamine hydrochloride

5.3.14 γδ cell frequencies are unchanged with SGA treatment and SGA treatment does not affect IL-17 and TNF-α production by γδ cells

γδ cell frequencies did not change significantly on SGA treatment (Figure 5.13A).

There were no differences in the frequency of IL-17 producing γδ cells on SGA medication (Figure 5.13B).

There were no differences in the frequency of TNF-α producing γδ cells on SGA medication (Figure 5.13C).

When stimulated with PMA, TNF-α production increased (Figure 5.13D) but IL-17 production did not (Figure 5.13E).

5.3.15 Dopamine does not affect IL-17 or TNF production from γδ cells

Dopamine did not affect IL-17 (Figure 5.14A) or TNF-α (Figure 5.14B) production from γδ cells on SGAs. Dopamine and PMA in combination also did not affect IL-17 (Figure 5.14C) or TNF-α production (Figure 5.14D) from γδ cells.

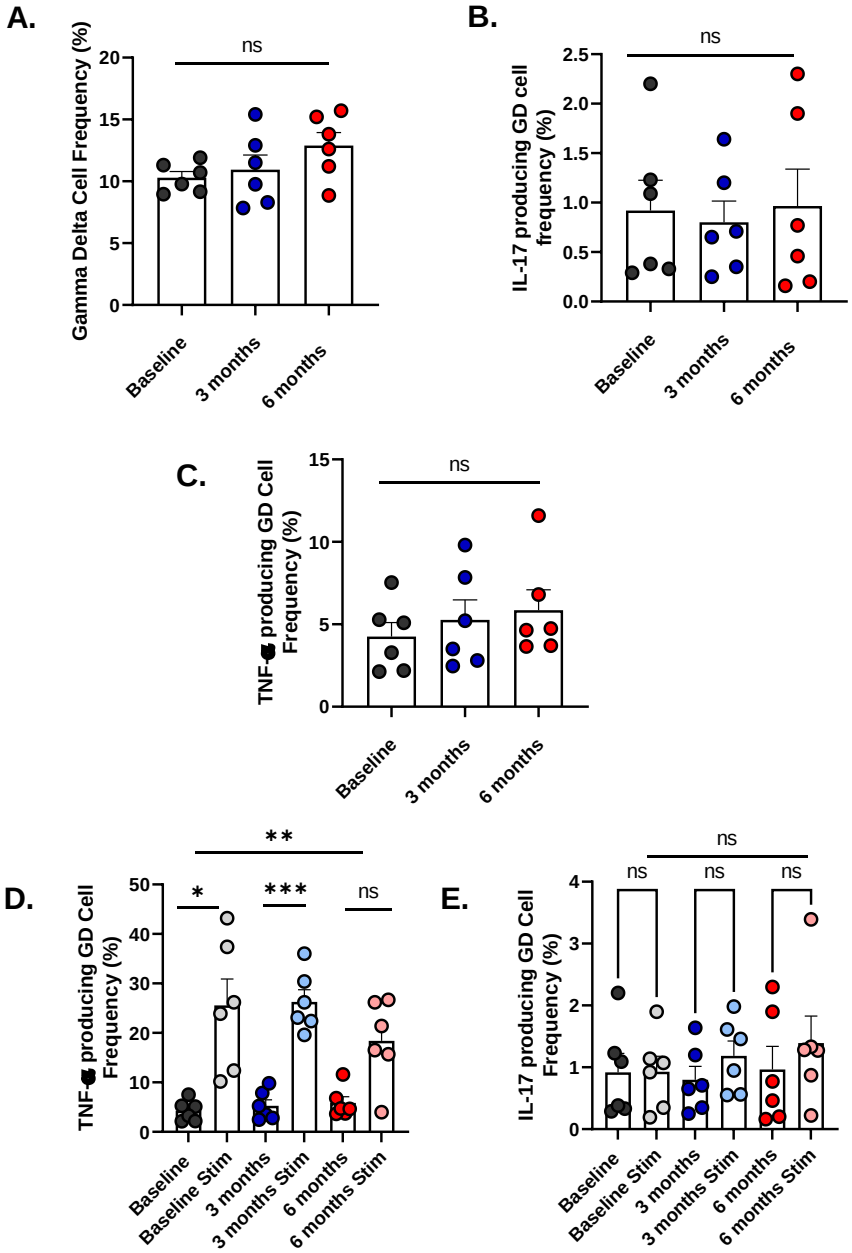


Figure 5.13: Cytokine production by γδ T Cells

Scatter plots showing: **(A)** γδ T Cell frequencies in the SGA cohort at baseline, 3 months, and 6 months. **(B)** IL-17 producing γδ T Cell frequencies in the SGA cohort at baseline, 3 months, and 6 months. **(C)** TNF-α producing γδ T Cell frequencies in the SGA cohort at baseline, 3 months, and 6 months. **(D)** TNF-α producing γδ T Cell frequencies in the SGA cohort at baseline, 3 months, and 6 months in unstimulated PBMCs and PBMCs stimulated with PMA. **(E)** IL-17 producing γδ T Cell frequencies in the SGA cohort at baseline, 3 months, and 6 months in PMA-stimulated PBMCs and PBMCs with PMA. Statistical analysis performed using repeated measures ANOVA with multiple comparisons, ns – not significant

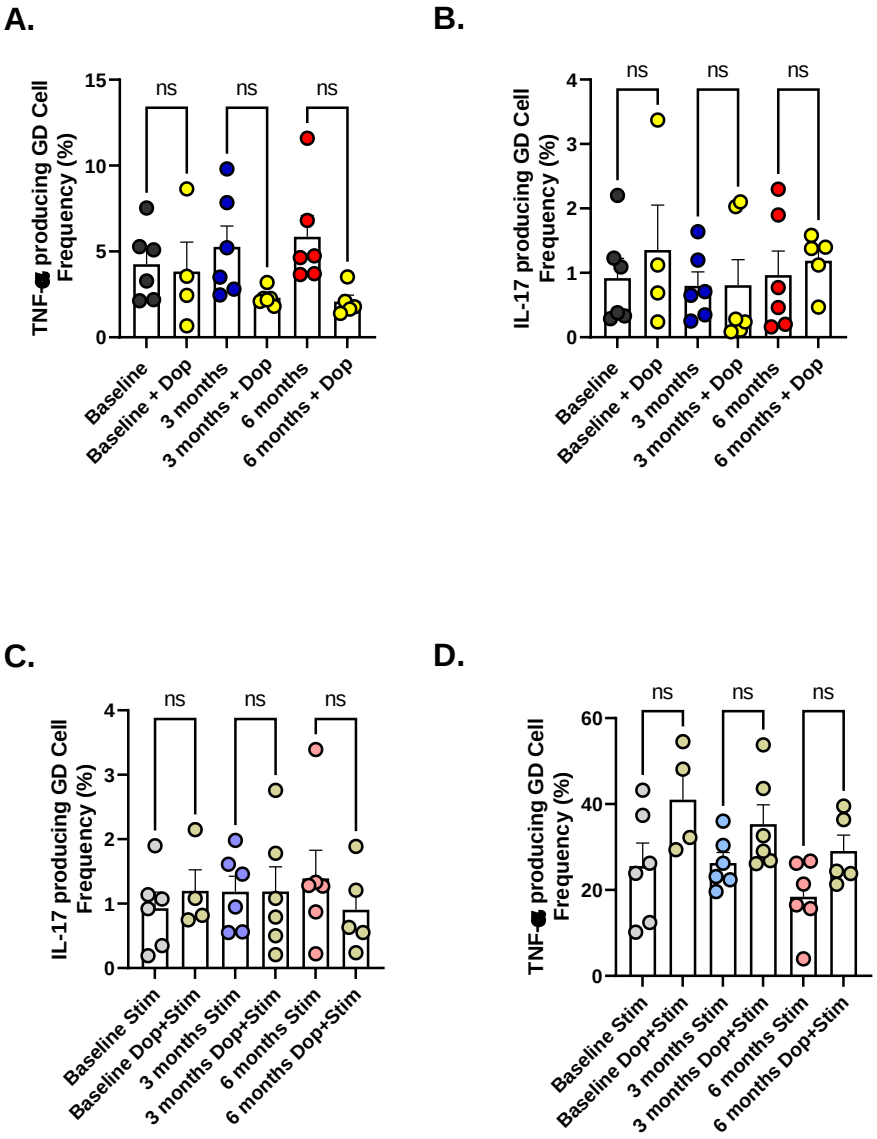


Figure 5.14: Cytokine production by γδ T Cells stimulated with dopamine

Scatter plots showing: **(A)** TNF- α producing γδ T Cell frequencies in the SGA cohort at baseline, 3 months, and 6 months in unstimulated PBMCs and PBMCs stimulated with dopamine hydrochloride. **(B)** IL-17 producing γδ T Cell frequencies in the SGA cohort at baseline, 3 months, and 6 months in unstimulated PBMCs and PBMCs stimulated with dopamine hydrochloride. **(C)** IL-17 producing γδ T Cell frequencies in the SGA cohort at baseline, 3 months, and 6 months in PMA-stimulated PBMCs and PBMCs stimulated with dopamine hydrochloride + PMA. **(D)** TNF-α producing γδ T Cell frequencies in the SGA cohort at baseline, 3 months, and 6 months in PMA-stimulated PBMCs and PBMCs stimulated with dopamine hydrochloride + PMA. Statistical analysis performed using repeated measures ANOVA with multiple comparisons, ns – not significant, * = $p<0.05$, ** = $p<0.01$, *** = $p<0.001$

5.4 Discussion

We determined in Chapter 3 that SGA medications caused significant weight gain from as early as three months after commencement, which is well established in the literature (76, 80, 81). In chapter 4, we found that the frequency of MAIT cells increased on SGA treatment. MAIT cell frequency is increased in children with obesity (219), and IL-17 is also increased in obesity and is associated with metabolic disease (259, 280).

In this chapter we investigated the effect of SGAs on immune cell responses. To do this, we established the cellular inflammatory profile of the participants at baseline and at the three follow up time points on SGA medication to further investigate if SGAs cause the development of an inflammatory phenotype.

We found that the pro-inflammatory cytokine TNF-α is increased early on, three months after commencing SGAs. In the supernatants of the PBMCs that were stimulated with LPS, TNF-α levels increased at three months indicating increasing inflammation. TNF-α levels reduced at six months and were below baseline levels by the end of the study which indicates that the inflammatory process plateaued at six months and reduced further at twelve months. Weight gain associated with SGAs tends to plateau from six months, although it remains elevated relative to same-age peers (75, 76). We also found that TNF-α concentration in PBMC supernatants was higher in the SGA group compared to a healthy control group at baseline. This was possibly due to the SGA group having a higher BMI percentile than the control group. This could also indicate a possible pro-inflammatory state in the SGA group which would warrant further investigation in future studies. We found that TNF-α concentration in PBMC supernatants that were stimulated with LPS had the same pattern as the finding of weight gain in chapter 3 (3.3.4). TNF-α concentration significantly increased at three months and then plateaued, indicating a link between increased TNF- α and weight gain.

We found that in-vitro dopamine treatment reduces the production of TNF- α, both from THP-1 cells and from PBMCs cultured from the participants in this study. These results are in line with previous studies investigating the effect of dopamine on inflammation (230, 231, 285). Risperidone also caused a reduction in TNF- α

compared to basal PBMCs and also caused a reduction in TNF- α production by THP-1 cells which also shows that SGAs may have an anti-inflammatory effect. This is in line with studies which found that SGAs may have anti-inflammatory effects on THP-1 cells and PBMCs, findings that are conflicting with the results of other studies (162, 164). The differences in the Sarsenbayeva et al study may be because of a different approach; they investigated the effect of SGAs on genes associated with cytokine production rather than measuring actual levels of cytokine production. There are studies demonstrating that SGAs may have pro-inflammatory effects and cause an increase in TNF-α production (148, 165).

We found that the anti-inflammatory cytokine, IL-10 increased on SGA treatment. Although IL-10 is thought to be anti-inflammatory it has been shown to be increased in people with obesity (276). Our results are consistent with those of two previous studies that have reported an increase in IL-10 associated with SGAs (125, 138, 289). In one study IL-10 was measured in unstimulated and LPS-stimulated rat primary glial cell cultures. IL-10 was markedly elevated in the unstimulated cultures which is similar to the finding in this study of IL-10 increasing in unstimulated PBMCs (289). The other study found that IL-10 production from PBMCs increased on risperidone treated participants (138). Again however, there is no clear pattern of results; a number of other studies have found SGAs cause a reduction in IL-10 (150, 158, 162, 166). Borovcanin et al measured cytokine levels in serum and not from cells. Duarte et al examined the effect of ziprasidone which is a different SGA to those prescribed in our study. They also used PBMCs from healthy volunteers that they cultured in ziprasidone rather than using PBMCs isolated from patients on SGAs.

The pro-inflammatory cytokines, IL-17A and IL-17F showed an increasing trend on SGAs up to six months. Levels reduced at twelve months which is when weight gain and other inflammatory markers such as TNF-α were plateauing and reducing. IL-17 is increased in both adults and children with obesity and is associated with the development of insulin resistance (219, 259, 280). Although we found that dopamine inhibits the production of TNF-α in vitro, we also found it did not significantly affect the production of IL17F. Risperidone appeared to cause a non-significant reduction in IL17F which is in line with other studies showing that risperidone may have anti-inflammatory effects (144, 162). Subanna et al

conducted a three month follow up study of patients with schizophrenia that commenced SGAs and found that IL-17 reduced on SGA treatment (290).

As two pro-inflammatory cytokines TNF-α and IL-17 were increased on SGA treatment, we further investigated a cellular source for these cytokines. We found that MAIT cell frequencies were increased on SGA treatment and were a source of TNF-α and IL-17. Increased MAIT cells are associated with an increase in metabolic disease and MAIT cell frequency and cytokine production are altered in people with obesity (219, 264). It is possible that an increase in MAIT cell frequency is contributing to the metabolic side effects associated with SGAs, and as discussed in chapter 4, it is possible that an altered microbiome in these children, caused by SGA medication, is contributing to the observed increase in MAIT cells (134, 265).

The frequency of CD4 T cells and γδ T cells did not change on SGA medication, and SGAs did not affect the production of IL-17 or TNF-α from CD4 T or γδ T cells. Th17 cells produce pro-inflammatory cytokines such as IL-17A and IL-17F. Obesity is associated with an increase in Th17 cells (268). Obesity is also associated with a reduced number of γδ T cells which is inversely proportionate to their BMI in studies on an adult population (269, 272). However, one year of SGA treatment did not have a similar effect on CD4 T cells or γδ T cells in this study population of children and adolescents.

We found that dopamine caused a reduction in TNF-α production by MAIT cells, but it did not cause a reduction in the production of IL-17 from these cells. Previous research has reported similar results regarding TNF-α, where dopamine has been shown to regulate cytokine secretion as it suppresses secretion of IFN-γ, TNF-α and IL-1β (230, 231, 285). Melnicov et. al found that dopamine suppressed IL-17 production from PBMCs of patients with multiple sclerosis, however we did not find that dopamine influenced IL-17 production in this study population (286). However, dopamine did have an anti-inflammatory effect on PMA stimulated cells, causing a decrease in IL-17. We found that dopamine did not affect IL-17 or TNF-α production by CD4 T cells and γδ cells.

These results show that PBMCs of participants on SGA treatment are primed to show hyperresponsiveness to stimulants akin to obesity. The results also showed

anti-inflammatory effects of SGAs on PBMCs as we also showed an increase in IL-10.

This study has found that within three months of starting an antipsychotic these children and adolescents developed an inflammatory phenotype. This is like the inflammation observed in children with obesity that occurs before the metabolic dysfunction emerges. The same driver of metabolic disease in the form of an inflammatory phenotype happens with SGA medications. This indicates that early inflammation could be one possible mechanism for the weight gain and metabolic dysfunction that occurs in patients who are on SGA medications. By understanding more about the immunological underpinnings of the metabolic complications associated with SGAs, future research could possibly identify novel targets to combat this inflammation and may lead to a reduction in metabolic side effects.

However, these findings need to be taken in the context that this is a very small part of a complex area. SGA related weight gain occurs due to central and peripheral factors, in the context of mental illness and also in the context of co-occurring medical conditions. Therefore, further research is needed in order to understand more about the causes of SGA related weight gain and the resulting complications.

CHAPTER 6: Discussion

6.1 General Discussion

Obesity is a complex disease that is currently a global health concern termed “globesity”. It is a major challenge for health services as it is associated with a raft of medical disorders including hypertension, dyslipidaemia, cardiovascular disease, insulin resistance, Type 2 Diabetes Mellitus (T2DM), autoimmune disease and an increased risk of developing malignancies (2, 25). Obesity is associated with the development of a chronic inflammatory state that drives the metabolic complications associated with it (54, 55). Medications associated with weight gain and obesity are modifiable risk factors that contribute to obesity in a small but significant proportion of children.

Second Generation Antipsychotics (SGAs) are associated with rapid weight gain in the short-term and significant cardiometabolic complications in the longer term. Despite these complications, SGA prescribing rates have increased in both child and adult populations in many countries over the past two decades. Children are a particularly vulnerable group; firstly, they are at higher risk of SGA-associated weight gain than adults (85, 86), and secondly children that are overweight or obese are likely to be overweight or obese as adults (5, 19, 291).

In this research, the aim was to prospectively investigate the effect of SGAs on weight gain and the immune system over time in youth. Children and adolescents were followed up at three time points over a twelve-month period to investigate the impact of SGAs on cardiometabolic health, inflammatory markers, immunophenotyping, and immune cell responses. This is important to investigate because weight gain is associated with a chronic inflammatory state, and rapid weight gain is associated with a worse pro-inflammatory profile. Up to this point, there is a striking lack of studies investigating the effects of SGAs on inflammatory markers associated with weight gain and metabolic dysregulation.

In the work presented in this thesis, I presented the results of three main studies, first a clinical investigation of weight and cardiometabolic risk factors in relation to SGA medication, second a study of the effect of SGAs on the immunophenotype in children and adolescents and third an investigation of SGAs on immune cell responses. We started from a clinical perspective and examined

weight gain and cardiometabolic risk factors at baseline and on SGAs. Next, we gained a broad overview of the immune system at baseline and on SGAs. We did this by measuring adipokines and cytokines in serum and analysing different populations of immune cells that are important in obesity. We then narrowed the focus and examined the impact of SGAs on immune cell responses. This involved stimulating PBMCs at each time point and measuring the secreted cytokines by ELISA and investigating the cytokine producing cells by flow cytometry.

6.1.1 Main results and significance of Study 1

From a clinical perspective, we looked at the cardiometabolic risk factors in the cohort at baseline and then at each time point while on SGA medication (see chapter 3). We found that at baseline, 30% of the participants recruited had a BMI percentile that was greater than or equal to the 80th percentile. There were also abnormalities in lipid profile detected at baseline, with 40% having a high total cholesterol, 30% having a raised LDL-cholesterol, 10% having raised triglycerides and 40% having low HDL-cholesterol. These findings are concerning as they show that participants in this cohort were already in a metabolically vulnerable position and at risk of complications before they commenced SGA medication. This profile worsened on SGAs and by the end of the study, 70% of the participants had a BMI percentile that was greater than or equal to the 80th percentile. This represented significant weight gain in this cohort on SGAs and most of the weight gain occurred within the first three months of treatment. This is typical of SGA-related weight gain as it usually occurs within the first few months of treatment (75, 76). The rapid acceleration in weight did plateau and reduce slightly over time in the study cohort, but overall, the BMI percentile remained higher than baseline levels. This is also typical of SGA-related weight gain as it tends to plateau over time, but it remains elevated compared to same age peers (75, 76). In relation to lipid profile, there were concerning results in that at the end of the study 60% of participants had raised total cholesterol levels, and 80% of participants had low HDL-levels. As low HDL is a risk factor for the metabolic syndrome, this highlights that SGA treatment further pushed this already metabolically vulnerable cohort towards developing T2DM.

We also found concerning results in relation to glucose regulation. By 12 months, 20% of study participants had raised fasting glucose levels. At baseline, 20% of the participants in our study had raised fasting insulin levels, which is an early marker for cardiovascular and metabolic disease risk. This highlights that these children were at risk of metabolic disease before they commenced SGA medication. Children with mental illness and neurodevelopmental disorders such as ASD are also at increased risk for obesity, metabolic syndrome, T2DM, cardiovascular disease (CVD), coronary heart disease, and cerebrovascular disease in comparison with the general population and independent of SGA prescribing (92, 98). On SGA medication there was a 50% increase in elevated fasting insulin levels by the end of the study. This shows that SGAs further increased the already elevated risk of metabolic disease in this cohort.

In this study, we found that having a lower BMI percentile at baseline was a risk factor for increased weight gain on SGAs. This is in keeping with two previous studies (94, 100). Conversely, two other studies have reported that a *higher* BMI at baseline is a risk factor for greater weight gain on SGAs (93, 97). Although there is not yet consistency in the literature about whether low baseline BMI percentile is a risk factor for greater SGA-associated weight gain, education for clinicians is urgently needed because at present clinicians are not aware of this potential risk factor, and often may not be as concerned about weight gain when commencing SGAs in patients with low baseline BMI.

Medication type, weight gain and BMI percentile at baseline did not influence lipid profile or glucose regulation. Due to the small sample size, it is likely that this study was underpowered to detect differences in lipid profile or glucose regulation between medications.

There was a very significant increase in prolactin levels on SGAs, in particular risperidone. This is concerning due to the negative impact of raised prolactin on bone health. Although most clinicians are aware of the high risk of elevated prolactin with risperidone, few seem to be aware of the negative impact of hyperprolactinaemia on bone health. It is important that levels of prolactin are closely monitored, and that hyperprolactinaemia is appropriately managed, either by reducing medication, switching medication or addition of low-dose

aripiprazole, a partial dopamine agonist that causes prolactin levels to decrease (209, 210).

There has been a striking lack of longitudinal studies investigating the effect of SGAs on weight gain and metabolic health in children and adolescents. As this study is a twelve month longitudinal study investigating this, it provides novel insights into the effect of SGAs on youth from a clinical perspective over time.

6.1.2 Main results and significance of Study 2

We also investigated the immunological effect of SGAs (see chapter 4). Firstly, we explored the systemic effects of SGAs through measurement of serum cytokines. Secondly, we investigated the effect of SGAs on immunophenotype, looking at SGA-associated changes in immune cell frequencies.

Leptin increased significantly in serum samples obtained from the cohort between baseline and three months, coinciding with rapid weight gain. Notably, leptin was higher in the SGA group at baseline compared to the healthy control group which reflects the fact that some of the participants in the SGA group were overweight at baseline. There were not significant differences in leptin between baseline and 12 months. These findings are in keeping with the literature because leptin has been found to increase in people with obesity (292), and the most significant increase in leptin was at three months when the rapid weight gain occurred. This finding is important because elevated leptin levels cause leptin resistance by desensitizing hypothalamic neurons in the hunger centre which leads to more weight gain (98, 110, 120). The effect of SGAs on leptin have not been extensively investigated longitudinally. Leptin has also been shown to increase the production of the pro-inflammatory cytokine TNF-α contributing to the inflammatory state in obesity (121). Therefore, the increased leptin found at three months on SGAs could potentially have long term implications in relation to weight gain and metabolic health due to a contribution to inflammation and further weight gain.

We did not find significant differences in soluble CD163, IL-17 or TNF-α over 12 months. Regarding IL-17 and TNF- α, it is likely because the levels of these cytokines in serum are very small.

Next, we investigated the immunophenotype of PBMCs, which are the major cells in human immunity. We found that SGAs caused an increase in the frequency of MAIT cells and monocytes. It is well established that MAIT cell frequencies are altered in people with obesity, in that they are increased in children with obesity and reduced in adults with obesity, which is hypothesized to be due to exhaustion in these cells causing cell death (219, 241). Childhood obesity is associated with increased frequencies of MAIT cells, which were also observed in association with SGA use in the study cohort. This shows that study participants developed an immunophenotype like that seen in childhood obesity within three months of commencing SGAs.

SGA treatment did not cause a significant change to the other immune cell populations we examined: T cells, NK cellsiNKT or monocytes cells. There were trends that appeared to be like what is seen in childhood obesity. There was a non-significant increase in NK and iNKT cells and a non-significant decrease in NK dim cells.

Statistically significant changes in these cells have been observed in childhood obesity. The changes observed here were non-significant, likely due to limited power to detect differences in the frequencies of these cell populations.

6.1.3 Main results and significance of Study 3

In study 3, we further investigated the effects of SGAs on immune cell responses (see chapter 5). The main finding was that pro-inflammatory cytokines TNF-α, IL-17F and IL-10 increased on antipsychotic treatment.

TNF-α and IL-17F are cytokines that are associated with the development of insulin resistance (259, 273, 280). The increase in these pro-inflammatory

cytokines could potentially be contributing to the metabolic dysregulation that is associated with SGA medications, which leads to insulin resistance and T2DM. TNF-α levels reduced at six months which indicates that the inflammatory process plateaued at six months. This is possibly because the rapid weight gain occurred in the first three months and another possibility is adaptation of the body to the inflammation. Other cytokines examined in this study such as serum IL-17, IL-17A and IL-17F in cell culture supernatants also started reducing at six months. IL-17F levels increased at three and six months and were reduced at the twelve month time-point. This plateau in inflammatory cytokines is possibly due to adaptation of the body over time.

IL-10 also increased on SGA treatment. While IL-10 is considered to be an anti-inflammatory cytokine (274), it has been shown to increase in people with obesity (276). The mechanism for this increase is unknown but it is potentially a compensatory mechanism to try to dampen the inflammatory environment. Overall, the increase in the cytokines IL-17F, IL-10 and TNF-α indicate that SGAs cause the development of an inflammatory phenotype like that which is seen in people with obesity.

We were interested to explore the potential cell types that were releasing the IL-17 and TNF-α. We carried out intracellular immunophenotyping to investigate the immune cells that were releasing these cytokines, and examined three specific cell types, MAIT cells, CD4 T cells and γδ T cells. We looked at these cells because they are known to produce TNF-α and IL-17. The increase in TNF-α and IL-17 appeared to be coming from the MAIT cells that were increased in this population on SGAs. The other cells studied, CD4 T cells and γδ T cells, did not show an increase in release of these cytokines, leading to our theory that it is MAIT cell frequency and cytokine production that is one of the first abnormalities occurring on SGA medications.

MAIT cell frequencies are increased in children with obesity (219) and they have a role in the development of metabolic disease due to their production of pro-inflammatory cytokines which lead to insulin resistance. It has been shown that MAIT cell development requires the presence of gut microflora (243). SGA treatment has been shown to alter the composition and function of the gut

microflora (134, 265). There is a hypothesis suggesting that the antagonistic effects of SGAs on receptors such as serotonin 5HT2C, Histamine H1, and muscarinic α1 adrenergic receptors alter the composition of the gut microbiota which leads to weight gain (265). There is also a study suggesting that changes in gut bacteria can cause a deterioration of host metabolic health (293). It is possible that an altered microbiome in the participants of this study, caused by SGA medication, is contributing to the observed increase in MAIT cells, which is having a negative impact on metabolic health.

We know from other studies that the early immune changes found in this study predict development of serious metabolic disease; for example, increased MAIT cells have been shown to cause metabolic dysfunction in a mouse model (264). MAIT cells have been implicated in the development of several metabolic diseases including obesity, NAFLD, and T1DM and the altered frequency of MAIT cells found in children and adults with obesity is associated with insulin resistance and T2DM (241, 243).

In the final analysis of this study, we investigated the effect of dopamine on immune cells. We were particularly interested in this as the main mode of action of SGAs is antagonism at dopamine receptors. We had hypothesised that dopamine would cause a reduction in TNF-α production.

We conducted an in vitro study using a monocyte model in a leukaemia cell line (THP-1 cells) and found that dopamine reduced the production of TNF-α by THP-1 cells in vitro. We then investigated this in our study sample showing that TNF-α production reduced in PBMCs. To investigate the specific immune cells affected by dopamine, we added dopamine to the intracellular flow cytometry experiment, and found that the addition of dopamine caused a reduction in TNF-α production from MAIT cells. Previous research has reported similar results regarding TNF-α, where dopamine has been shown to reduce secretion of TNF-α by neutrophils and monocytes (230, 231, 285). The effect of dopamine on TNF-α production by MAIT cells has not been investigated previously.

We found no effect of dopamine on IL-17 production by MAIT cells, nor did it influence IL-17 or TNF-α production by CD4 T cells or γδ T cells. Contrary to our results, Melnicov et. al found that dopamine suppressed IL-17 production from

PBMCs of patients with multiple sclerosis (286). It is interesting that in the study presented in this thesis, the main immune cells affected by dopamine were MAIT cells. In this study we also found that MAIT cell frequencies increased on SGAs, and they were a source of TNF-α and IL-17. In parallel, MAIT cells are also increased in children with obesity. We found that the increase in TNF-α was not just due to weight gain but also possibly a direct effect of dopamine antagonism. The dopamine blocking action of SGAs could be one of the potential mechanisms driving the increase in pro-inflammatory cytokines found in this study.

This research has led to an increased understanding of the biological mechanisms underpinning cardio-metabolic dysfunction associated with SGA medication in children and adolescents. The experiments carried out in this study demonstrate possible immune mechanistic factors as to how SGA medications cause adverse metabolic side effects. The novel findings from this research have the capacity to pave the way for the development of interventions targeting specific aspects of the inflammatory response that would reduce metabolic dysregulation. There is currently great optimism about the use of interventions such as GLP-1 analogues. GLP-1 analogues are a good example of the types of interventions that could result from an increased understanding of the pathophysiology of immune dysregulation associated with obesity and SGAs. It also provides new knowledge that could be useful in identifying and preventing proinflammatory states early in treatment or downstream to inform personalised approaches to care.

6.2 Strengths of this research work

To date, most research investigating the relationship between paediatric obesity, inflammation and alterations in immune cells has been conducted using cross-sectional studies. This study has two key strengths; firstly, it has a prospective longitudinal design, and secondly it involves a study cohort who were not obese to begin with but were exposed to a risk factor for weight gain. This gives us unique insights into the effects of weight gain on the immune system over a one-year period and allows us to observe the evolution of the immune system following treatment with SGAs.

Similarly, most research investigating SGAs and possible causes for the metabolic side effects are either cross-sectional studies, or longitudinal studies with a small follow up window of less than four months. As this study took place over twelve months, we were able to investigate the timeframe when most weight gain and metabolic side effects occur, and we were also able to investigate when the changes in inflammation and immune cells occurred.

To our knowledge, this is the first study that looked at longitudinal changes in IL-17F induced by SGAs. Our finding that this pro-inflammatory cytokine increased on SGA treatment is important. We know from the literature that IL-17 increases in obesity and drives metabolic dysregulation through interruption of insulin signalling and development of insulin resistance. This study has shown that SGA treatment induces a similar inflammatory phenotype in a short time-frame.

6.3 Study Limitations

This research study does have limitations that are worthy of discussion. As there is limited literature published on this area to date, the research design was largely exploratory. Ideally, sample sizes should have been calculated to detect minimum significant differences in each parameter investigated.

The main limitation of this study is the small sample size, and because of this the study may be underpowered to detect differences in immune parameters. There were a few challenges in recruitment of children starting SGA medication. Firstly, despite the global increase in prescription of SGA medication, there were very low rates of SGA prescribing in the Irish clinical services collaborating in this study. This may reflect the growing understanding about the serious cardiometabolic side effects of SGAs. Secondly, the study included stringent inclusion/exclusion criteria and many potential participants, in particular those from Intellectual Disability services, were not eligible due to the presence of genetic / medical conditions. Thirdly, the requirement for a baseline blood sample caused difficulty as several children were started on SGA medication before the clinician alerted the researcher to a potential participant. Finally, this study was mainly carried out by a single researcher who travelled to get the samples at the clinical sites and transported the sample to the lab to be processed. This had to

be done within a few hours and it meant participants were not recruited during periods of leave or if transport of the sample was not going to be possible within the required timeframe.

It is also important to note that these children were on different SGAs and had different diagnoses. Therefore, we could not examine the effect that the different SGAs had on the immune system, nor could we investigate the influence of diagnosis.

Another limitation is that the experiments were done on different days which could have influenced results.

Finally, this research study only used blood samples to examine the effects of SGAs on the immune system. This does not give us a complete understanding of how SGAs influence the immune system and investigation of other tissues such as adipose tissue and lymph nodes would give a more complete picture of how SGAs affected the immune system in these participants. However, collecting these tissue samples would not have been feasible in this patient cohort.

6.4 The impact of the COVID-19 pandemic on the study

The COVID-19 pandemic occurred in the final year of this study. At the beginning of the pandemic there was no access to the lab for a period of four months. After this period there was limited access to the lab and due to social distancing, there was very little lab support and lab supervision for the rest of the project. Overall, however it was possible to complete all data analysis as originally planned.

6.5 The impact of the study

The findings of this research study have implications for clinical practice. There are two major impacts. Firstly, there is an impact for clinical practice in that we have shown early immune dysregulation paralleling the widely recognised weight gain that has not previously been reported in a prospective longitudinal SGA-naïve cohort. This is important because it should result in a change in clinical practice. There should be less SGAs prescribed and better monitoring of patients

that are on SGAs. Currently, many children in Ireland are being prescribed SGAs. Recent changes in service provision for children and adolescents with neurodevelopmental disorders including autism spectrum disorder and intellectual disability have resulted in a reduction of availability of non-pharmacological interventions for behaviours that challenge. As a result, clinicians are coming under increased pressure to prescribe medication to try to reduce the severity of these behaviours (294). Often in these difficult cases no treatment may result in exclusion from school or residential care placement breaking down. This lack of access to appropriate disability services is driving increasing rates of prescribing of SGAs in a vulnerable patient cohort. It is essential that monitoring guidelines are put in place specific to children and adolescents to reduce cardiometabolic side effects.

The second impact is that this study has contributed significantly to understanding the pathophysiology of immune dysregulation associated with SGAs and obesity. This is important because it is essential to fully understand the pathophysiology to develop rational targeted treatments and interventions.

6.6 Future Directions

This study has reported several novel findings in relation to SGA-associated weight gain and metabolic dysfunction, however much is still unknown. To build on our research, future studies could investigate the mechanisms underpinning immune dysregulation associated with SGAs. Future work could also focus on the use of adjunct pharmacological treatments such as GLP-1 in the prevention of SGA-related weight gain. From a clinical perspective, there is an urgent need for formal guidelines for SGA prescribing and monitoring in youth. These future directions are discussed in more detail below.

This study has found that MAIT cells increase on SGA medication, a finding which has also been reported in children with obesity (219). Future studies are needed to investigate the mechanism causing the increase in frequency of MAIT cells and their increased production of TNF-α and IL-17F, which are both pro-inflammatory cytokines implicated in insulin resistance. If potential mechanisms

are discovered, it could result in novel targets to prevent MAIT cells from increasing in frequency and producing pro-inflammatory cytokines.

We found that the pro-inflammatory cytokine TNF-α was increased in supernatants of PBMCs in the SGA group at baseline compared to a healthy control group. This was in both PBMCs cultured alone without any stimulants added to the cell culture media and in PBMCs stimulated with LPS. This could indicate a pro-inflammatory state in the SGA group which would warrant further investigation in future studies. ASD and schizophrenia have both been found to be associated with dysregulation of inflammatory markers (138, 139). However, the participants in this study group had other diagnoses such as Emotionally Unstable Personality Disorder as well as ASD and EOP. Therefore, it was not possible to accurately investigate the presence of a pro-inflammatory state in children with ASD or EOP.

As an alternative to starting SGA medication may not be an option in many of these cases, adjunct pharmacological treatments could be considered, and the results of this study support the use of GLP-1 analogues. Further research into the use of GLP-1 analogues, possibly as adjunctive treatments with SGAs is required. To date, GLP-1 analogues have been found to reduce weight gain and are anti-inflammatory, reducing the activation of inflammatory macrophages (36, 39) and levels of IL-17 (295). GLP-1 analogues have now been approved in adolescents in oral form. They are taken once weekly and have been shown to cause weight loss in adolescents with obesity (296). A recent randomized controlled trial in adults with obesity found that semaglutide (a GLP-1 analogue) caused a 14.9% reduction in body weight in the treatment group compared to a 2.4% reduction in body weight in the placebo group from baseline to the end of the study (40). These results provide hope for prescribing clinicians; it is often impossible to find an alternative treatment for severe behaviours that challenge, and the immediate risks associated with these behaviours are often judged to outweigh the longer-term cardio-metabolic risks. An effective treatment for these cardio-metabolic risks would be extremely valuable. It will be important for future studies to investigate the effect of using GLP-1 as an adjunct treatment with SGAs to determine whether the severity of SGA associated weight gain and metabolic complications can be reduced.

Another pharmacological approach that has been trialled to manage SGA related weight gain is metformin. There has been a recent clinical trial comparing metformin treatment, switching antipsychotics to aripiprazole and continuing antipsychotics with lifestyle interventions for youth treated with SGAs (109). This study found that adding metformin or switching SGA medication to aripiprazole resulted in a significantly decreased BMI z-score compared to continuing SGA treatment with lifestyle interventions. However, there were no significant differences between metformin and the switching to aripiprazole in terms of reduction in BMI. A downside of metformin use was that it was associated with significantly more adverse effects than switching to aripiprazole. A clinical trial comparing metformin to placebo in participants with ASD on SGAs found that in the first 16 weeks of the study the participants on metformin experienced more weight loss compared to the participants on placebo (177). However, in the second 16 weeks of the study, the participants in the placebo arm experienced weight loss and there was no further weight loss in the participants on metformin (176). The weight loss in the placebo group was comparable to that achieved previously in the metformin group. This indicates that adjunct treatment with metformin may not be effective in the long term for patients on SGAs. Another downside of adjunct treatment with metformin are the side effects such as adverse gastrointestinal effects including diarrhoea and also irritability and agitation which can make it difficult to tolerate, particularly for children with ASD. It takes up to 12 weeks to achieve adequate dose titration with metformin in children meaning there is a delay before there are any changes in body weight. Taking the above into consideration, it is likely that adjunct treatment with GLP-1 could be a more effective approach to managing SGA-related weight gain. Overall, it is more beneficial to limit prescribing of SGAs and adding in additional medications to treat side-effects is not always ideal or desirable, but it is sometimes necessary.

Increased prescribing of SGAs is often a consequence of poor MDT service provision particularly in relation to ASD. A future direction in this regard would be to investigate the prescribing rates of SGAs in those receiving MDT interventions and those who are not.

Despite the growing evidence showing that children and adolescents are more at risk of SGA-related complications than adults, there are still no formal guidelines for monitoring and management of SGA-related complications in youth, and these are urgently needed. In terms of future directions of this work, there are a few steps that should be taken to ensure guideline development is prioritised in Ireland. Work at many levels is required for successful development and implementation of guidelines relating to SGA prescribing. In the first instance, formal Irish guidelines need to be developed. This would require the input of psychiatrists in the area with an interest in physical health and a knowledge of SGA monitoring. There is a special interest group on physical health within the College of Psychiatrists of Ireland. A systematic review of current recommendations and guidelines for SGA monitoring and management is required; if such a task was undertaken by the members of this special interest group, a position paper on guidelines could subsequently be developed, and brought to the attention of the Department of Health and HSE policy makers. Collaborative work between members of the College of Psychiatrists of Ireland and members of other colleges such as the Royal College of Physicians of Ireland, Faculty of Paediatrics would also be advantageous in development and implementation of guidelines. Adequate clinician training would form an essential part of successful guideline implementation. The British Association of Psychopharmacology has published guidelines on the management of weight gain, metabolic disturbance, and cardiovascular risk in adult patients on SGAs (167). Similar guidelines are urgently required for children and adolescents on SGAs.

6.7 Conclusion

Overall, this study highlights that SGA-related weight gain occurs early on within three months of commencement of the medication. This is concerning given the current “globesity” epidemic and the high rates of prescription of SGA medications as well as the greater risks of overweight and obesity in children and adolescents with mental health disorders. This study has provided insights into the mechanisms of SGA-associated immune dysregulation over time. It also gives us novel insights into the pathophysiology of obesity related immune

dysregulation given that the study participants gained a significant amount of weight in a short period of time and were then followed up longitudinally. This study also provides scope for the development of future treatments to help to prevent weight gain and metabolic dysregulation. This would ultimately lead to improved quality of life for this group of vulnerable young people.

CHAPTER 7: Bibliography

1. Organization WH. Consideration of the evidence on childhood obesity for the Commission on Ending Childhood Obesity: report of the ad hoc working group on science and evidence for ending childhood obesity, Geneva, Switzerland. 2016.
2. Juonala M, Magnussen CG, Berenson GS, Venn A, Burns TL, Sabin MA, et al. Childhood adiposity, adult adiposity, and cardiovascular risk factors. N Engl J Med. 2011;365:1876-85.
3. Sabin M, Shield J. Childhood obesity. Obesity and metabolism. 36: Karger Publishers; 2008. p. 85-96.
4. Sabin MA, Ford AL, Holly JM, Hunt LP, Crowne EC, Shield JP. Characterisation of morbidity in a UK, hospital based, obesity clinic. Archives of disease in childhood. 2006;91(2):126-30.
5. Tirosh A, Shai I, Afek A, Dubnov-Raz G, Ayalon N, Gordon B, et al. Adolescent BMI trajectory and risk of diabetes versus coronary disease. New England Journal of Medicine. 2011;364(14):1315-25.
6. Kearns K, Dee A, Fitzgerald AP, Doherty E, Perry IJ. Chronic disease burden associated with overweight and obesity in Ireland: the effects of a small BMI reduction at population level. BMC Public Health. 2014;14(1):143.
7. Doherty E, Dee A, O'Neill C. Estimating the amount of overweight and obesity related health-care use in the Republic of Ireland using SLAN data. The Economic and Social Review. 2012;43(2, Summer):227-50.
8. Trandafir L, Temneanu O. Pre and post-natal risk and determination of factors for child obesity. Journal of medicine and life. 2016;9(4):386.
9. Rogero MM, Calder PC. Obesity, inflammation, toll-like receptor 4 and fatty acids. Nutrients. 2018;10(4):432.
10. Cole TJ, Lobstein T. Extended international (IOTF) body mass index cut-offs for thinness, overweight and obesity. Pediatric obesity. 2012;7(4):284-94.
11. Wang Y, Wang J. A comparison of international references for the assessment of child and adolescent overweight and obesity in different populations. European journal of clinical nutrition. 2002;56(10):973-82.
12. Hajian-Tilaki K, Heidari B. A comparison between International Obesity Task Force and Center for Disease Control references in assessment of overweight and obesity among adolescents in Babol, northern Iran. International journal of preventive medicine. 2013;4(2):226.

13. Keaver L, Webber L, Dee A, Shiely F, Marsh T, Balanda K, et al. Application of the UK foresight obesity model in Ireland: the health and economic consequences of projected obesity trends in Ireland. PloS one. 2013;8(11):e79827.
14. Olshansky SJ, Passaro DJ, Hershow RC, Layden J, Carnes BA, Brody J, et al. A potential decline in life expectancy in the United States in the 21st century. New England Journal of Medicine. 2005;352(11):1138-45.
15. Layte R, McCrory C. Growing Up in Ireland: National longitudinal study of children: overweight and obesity among 9-year-olds: Government Publications; 2011.
16. Heinen M. The Childhood Obesity Surveillance Initiative (COSI) in the Republic of Ireland: Findings from 2008, 2010 and 2012. Health. 2014;1:03.
17. Keane E, Kearney PM, Perry IJ, Kelleher CC, Harrington JM. Trends and prevalence of overweight and obesity in primary school aged children in the Republic of Ireland from 2002-2012: a systematic review. BMC public health. 2014;14(1):974.
18. Health Do, Children. Obesity-the Policy Challenges: The Report of the National Taskforce on Obesity 2005: Department of Health and Children; 2005.
19. Skinner AC, Steiner MJ, Henderson FW, Perrin EM. Multiple markers of inflammation and weight status: cross-sectional analyses throughout childhood. Pediatrics. 2010;125(4):e801-e9.
20. Daousi C, Casson I, Gill G, MacFarlane I, Wilding J, Pinkney J. Prevalence of obesity in type 2 diabetes in secondary care: association with cardiovascular risk factors. Postgraduate medical journal. 2006;82(966):280-4.
21. Anderson EL, Howe LD, Jones HE, Higgins JP, Lawlor DA, Fraser A. The prevalence of non-alcoholic fatty liver disease in children and adolescents: a systematic review and meta-analysis. PloS one. 2015;10(10):e0140908.
22. Verhulst SL, Schrauwen N, Haentjens D, Suys B, Rooman RP, Van Gaal L, et al. Sleep-disordered breathing in overweight and obese children and adolescents: prevalence, characteristics and the role of fat distribution. Archives of disease in childhood. 2007;92(3):205-8.
23. Crowson CS, Matteson EL, Davis III JM, Gabriel SE. Contribution of obesity to the rise in incidence of rheumatoid arthritis. Arthritis care & research. 2013;65(1):71-7.

24. Willis SA, Bawden SJ, Malaikah S, Sargeant JA, Stensel DJ, Aithal GP, et al. The role of hepatic lipid composition in obesity-related metabolic disease. Liver International. 2021;41(12):2819-35.
25. Renehan AG, Tyson M, Egger M, Heller RF, Zwahlen M. Body-mass index and incidence of cancer: a systematic review and meta-analysis of prospective observational studies. The lancet. 2008;371(9612):569-78.
26. Avgerinos KI, Spyrou N, Mantzoros CS, Dalamaga M. Obesity and cancer risk: Emerging biological mechanisms and perspectives. Metabolism. 2019;92:121-35.
27. Fuentes E, Fuentes F, Vilahur G, Badimon L, Palomo I, #xe1. Mechanisms of Chronic State of Inflammation as Mediators That Link Obese Adipose Tissue and Metabolic Syndrome. Mediators of Inflammation. 2013;2013:11.
28. Sjo CP, Stenstrom AD, Bojesen AB, Frolich JS, Bilenberg N. Development of Metabolic Syndrome in Drug-Naive Adolescents After 12 Months of Second-Generation Antipsychotic Treatment. J Child Adolesc Psychopharmacol. 2017;27(10):884-91.
29. Cooke AA, Connaughton RM, Lyons CL, McMorrow AM, Roche HM. Fatty acids and chronic low grade inflammation associated with obesity and the metabolic syndrome. European journal of pharmacology. 2016;785:207-14.
30. Russell-Mayhew S, McVey G, Bardick A, Ireland A. Mental health, wellness, and childhood overweight/obesity. Journal of obesity. 2012;2012.
31. Eisenberg ME, Neumark-Sztainer D, Story M. Associations of weight-based teasing and emotional well-being among adolescents. Archives of pediatrics & adolescent medicine. 2003;157(8):733-8.
32. Goodman E, Whitaker RC. A prospective study of the role of depression in the development and persistence of adolescent obesity. Pediatrics. 2002;110(3):497-504.
33. Bray GA, Frühbeck G, Ryan DH, Wilding JP. Management of obesity. The Lancet. 2016;387(10031):1947-56.
34. Daniels SR, Arnett DK, Eckel RH, Gidding SS, Hayman LL, Kumanyika S, et al. Overweight in children and adolescents: pathophysiology, consequences, prevention, and treatment. Circulation. 2005;111(15):1999-2012.

35. Kendall D, Vail A, Amin R, Barrett T, Dimitri P, Ivison F, et al. Metformin in obese children and adolescents: the MOCA trial. The Journal of Clinical Endocrinology & Metabolism. 2013;98(1):322-9.
36. Hogan A, Tobin A, Ahern T, Corrigan M, Gaoatswe G, Jackson R, et al. Glucagon-like peptide-1 (GLP-1) and the regulation of human invariant natural killer T cells: lessons from obesity, diabetes and psoriasis. Diabetologia. 2011;54(11):2745.
37. Chadda KR, Cheng TS, Ong KK. GLP-1 agonists for obesity and type 2 diabetes in children: Systematic review and meta-analysis. Obesity Reviews. 2020:1-10. https://doi.org/.1111/obr.13177.
38. Bacha F. FDA approval of GLP-1 receptor agonist (liraglutide) for use in children. The Lancet Child & Adolescent Health. 2019;3(9):595-7.
39. Hogan AE, Gaoatswe G, Lynch L, Corrigan MA, Woods C, O'Connell J, et al. Glucagon-like peptide 1 analogue therapy directly modulates innate immune-mediated inflammation in individuals with type 2 diabetes mellitus. Diabetologia. 2014;57(4):781-4.
40. Wilding JP, Batterham RL, Calanna S, Davies M, Van Gaal LF, Lingvay I, et al. Once-Weekly Semaglutide in Adults with Overweight or Obesity. New England Journal of Medicine. 2021.
41. Warrington R, Watson W, Kim HL, Antonetti FR. An introduction to immunology and immunopathology. Allergy, Asthma & Clinical Immunology. 2011;7(S1):S1.
42. Marshall JS, Warrington R, Watson W, Kim HL. An introduction to immunology and immunopathology. Allergy, Asthma & Clinical Immunology. 2018;14(2):1-10.
43. Netea MG, Joosten LA, Latz E, Mills KH, Natoli G, Stunnenberg HG, et al. Trained immunity: a program of innate immune memory in health and disease. Science. 2016;352(6284).
44. O'Sullivan TE, Sun JC, Lanier LL. Natural killer cell memory. Immunity. 2015;43(4):634-45.
45. Turvey SE, Broide DH. Innate immunity. Journal of Allergy and Clinical Immunology. 2010;125(2):S24-S32.

46. Napier RJ, Adams EJ, Gold MC, Lewinsohn DM. The Role of Mucosal Associated Invariant T Cells in Antimicrobial Immunity. Frontiers in Immunology. 2015;6(344).
47. Chaplin DD. Overview of the immune response. Journal of Allergy and Clinical Immunology. 2010;125(2):S3-S23.
48. Bonilla FA, Oettgen HC. Adaptive immunity. Journal of Allergy and Clinical Immunology. 2010;125(2):S33-S40.
49. Lanier LL. Shades of grey—the blurring view of innate and adaptive immunity. Nature Reviews Immunology. 2013;13(2):73.
50. Godfrey DI, MacDonald HR, Kronenberg M, Smyth MJ, Van Kaer L. NKT cells: what's in a name? Nature Reviews Immunology. 2004;4(3):231-7.
51. Kourtzelis I, Hajishengallis G, Chavakis T. Phagocytosis of apoptotic cells in resolution of inflammation. Frontiers in immunology. 2020;11.
52. Lumeng CN, Saltiel AR. Inflammatory links between obesity and metabolic disease. The Journal of clinical investigation. 2011;121(6):2111-7.
53. Bai Y, Sun Q. Macrophage recruitment in obese adipose tissue. obesity reviews. 2015;16(2):127-36.
54. Carolan E, Hogan AE, Corrigan M, Gaotswe G, O'connell J, Foley N, et al. The impact of childhood obesity on inflammation, innate immune cell frequency, and metabolic microRNA expression. The Journal of Clinical Endocrinology & Metabolism. 2014;99(3):E474-E8.
55. Mraz M, Haluzik M. The role of adipose tissue immune cells in obesity and low-grade inflammation. Journal of Endocrinology. 2014;222(3):R113-R27.
56. Shi H, Kokoeva MV, Inouye K, Tzameli I, Yin H, Flier JS. TLR4 links innate immunity and fatty acid–induced insulin resistance. The Journal of clinical investigation. 2006;116(11):3015-25.
57. Chang E-J, Lee SK, Song YS, Jang YJ, Park HS, Hong JP, et al. IL-34 is associated with obesity, chronic inflammation, and insulin resistance. The Journal of Clinical Endocrinology & Metabolism. 2014;99(7):E1263-E71.
58. Hotamisligil GS. Inflammation and metabolic disorders. Nature. 2006;444(7121):860-7.
59. Cinkajzlová A, Mráz M, Haluzík M. Lymphocytes and macrophages in adipose tissue in obesity: markers or makers of subclinical inflammation? Protoplasma. 2017;254(3):1219-32.

60. Ghazarian M, Luck H, Revelo XS, Winer S, Winer DA. Immunopathology of Adipose Tissue during Metabolic Syndrome. Turkish Journal of Pathology. 2015;31.
61. McLaughlin T, Ackerman SE, Shen L, Engleman E. Role of innate and adaptive immunity in obesity-associated metabolic disease. The Journal of clinical investigation. 2017;127(1):5-13.
62. Cao H. Adipocytokines in obesity and metabolic disease. Journal of endocrinology. 2014;220(2):T47-T59.
63. McGillicuddy FC, Harford KA, Reynolds CM, Oliver E, Claessens M, Mills KH, et al. Lack of interleukin-1 receptor I (IL-1RI) protects mice from high-fat diet–induced adipose tissue inflammation coincident with improved glucose homeostasis. Diabetes. 2011;60(6):1688-98.
64. Vandanmagsar B, Youm Y-H, Ravussin A, Galgani JE, Stadler K, Mynatt RL, et al. The NLRP3 inflammasome instigates obesity-induced inflammation and insulin resistance. Nature medicine. 2011;17(2):179-88.
65. Esser N, Legrand-Poels S, Piette J, Scheen AJ, Paquot N. Inflammation as a link between obesity, metabolic syndrome and type 2 diabetes. Diabetes research and clinical practice. 2014;105(2):141-50.
66. Nakamura K, Fuster JJ, Walsh K. Adipokines: a link between obesity and cardiovascular disease. Journal of cardiology. 2014;63(4):250-9.
67. Fawcett KA, Barroso I. The genetics of obesity: FTO leads the way. Trends in genetics. 2010;26(6):266-74.
68. Kaar SJ, Natesan S, McCutcheon R, Howes OD. Antipsychotics: mechanisms underlying clinical response and side-effects and novel treatment approaches based on pathophysiology. Neuropharmacology. 2020;172:107704.
69. Christensen DL, Braun KVN, Baio J, Bilder D, Charles J, Constantino JN, et al. Prevalence and characteristics of autism spectrum disorder among children aged 8 years—autism and developmental disabilities monitoring network, 11 sites, United States, 2012. MMWR Surveillance Summaries. 2018;65(13):1.
70. Daly P. An Evaluation of Education Provision for Students with Autism Spectrum Disorder in Ireland National Council for Special

Education. NCSE, Editor National Council for Special Education: Dublin. 2016

71. Amminger GP, Henry LP, Harrigan SM, Harris MG, Alvarez-Jimenez M, Herrman H, et al. Outcome in early-onset schizophrenia revisited: findings from the Early Psychosis Prevention and Intervention Centre long-term follow-up study. Schizophrenia research. 2011;131(1-3):112-9.
72. Schimmelmann BG, Conus P, Cotton S, McGorry PD, Lambert M. Pre-treatment, baseline, and outcome differences between early-onset and adult-onset psychosis in an epidemiological cohort of 636 first-episode patients. Schizophrenia research. 2007;95(1-3):1-8.
73. Olfson M, Blanco C, Liu S-M, Wang S, Correll CU. National Trends in the Office-Based Treatment of Children, Adolescents, and Adults With AntipsychoticsNational Trends in Treatment With Antipsychotics. JAMA Psychiatry. 2012;69(12):1247-56.
74. Authority HPR. [Online] Available at: https://wwwhpraie/. 2020.
75. Calarge CA, Xie D, Fiedorowicz JG, Burns TL, Haynes WG. Rate of weight gain and cardiometabolic abnormalities in children and adolescents. J Pediatr. 2012;161(6):1010-5.
76. Correll CU, Carlson HE. Endocrine and metabolic adverse effects of psychotropic medications in children and adolescents. J Am Acad Child Adolesc Psychiatry. 2006;45(7):771-91.
77. Panagiotopoulos C, Ronsley R, Kuzeljevic B, Davidson J. Waist circumference is a sensitive screening tool for assessment of metabolic syndrome risk in children treated with second-generation antipsychotics. The Canadian Journal of Psychiatry. 2012;57(1):34-44.
78. Ronsley R, Scott D, Warburton WP, Hamdi RD, Louie DC, Davidson J, et al. A Population-Based Study of Antipsychotic Prescription Trends in Children and Adolescents in British Columbia, from 1996 to 2011. The Canadian Journal of Psychiatry. 2013;58(6):361-9.
79. Maayan L, Correll CU. Weight gain and metabolic risks associated with antipsychotic medications in children and adolescents. J Child Adolesc Psychopharmacol. 2011;21(6):517-35.
80. Krill RA, Kumra S. Metabolic consequences of second-generation antipsychotics in youth: appropriate monitoring and clinical management. Adolesc Health Med Ther. 2014;5:171-82.

81. Ronsley R, Nguyen D, Davidson J, Panagiotopoulos C. Increased risk of obesity and metabolic dysregulation following 12 months of second-generation antipsychotic treatment in children: a prospective cohort study. The Canadian Journal of Psychiatry. 2015;60(10):441-50.
82. Correll CU, Manu P, Olshanskiy V, Napolitano B, Kane JM, Malhotra AK. Cardiometabolic risk of second-generation antipsychotic medications during first-time use in children and adolescents. JAMA. 2009;302(16):1765-73.
83. O'Donoghue B, Schafer MR, Becker J, Papageorgiou K, Amminger GP. Metabolic changes in first-episode early-onset schizophrenia with second-generation antipsychotics. Early Interv Psychiatry. 2014;8(3):276-80.
84. Patel NC, Hariparsad M, Matias-Akthar M, Sorter MT, Barzman DH, Morrison JA, et al. Body mass indexes and lipid profiles in hospitalized children and adolescents exposed to atypical antipsychotics. J Child Adolesc Psychopharmacol. 2007;17(3):303-11.
85. Caneja D, Pina Camacho L, Fraguas D, Saiz Martínez PA. Are children treated with second-generation antipsychotics at higher risk of weight gain than adults? European Neuropsychopharmacology, 29. 2016.
86. Nielsen RE, Laursen MF, Lammers Vernal D, Bisgaard C, Jakobsen H, Steinhausen H-C, et al. Risk of Diabetes in Children and Adolescents Exposed to Antipsychotics: A Nationwide 12-Year Case-Control Study. Journal of the American Academy of Child & Adolescent Psychiatry. 2014;53(9):971-9.e6.
87. Kryzhanovskaya LA, Xu W, Millen BA, Acharya N, Jen KY, Osuntokun O. Comparison of long-term (at least 24 weeks) weight gain and metabolic changes between adolescents and adults treated with olanzapine. Journal of child and adolescent psychopharmacology. 2012;22(2):157-65.
88. Baeza I, Vigo L, de la Serna E, Calvo-Escalona R, Merchan-Naranjo J, Rodriguez-Latorre P, et al. The effects of antipsychotics on weight gain, weight-related hormones and homocysteine in children and adolescents: a 1-year follow-up study. Eur Child Adolesc Psychiatry. 2017;26(1):35-46.
89. Martinez-Ortega JM, Funes-Godoy S, Diaz-Atienza F, Gutierrez-Rojas L, Perez-Costillas L, Gurpegui M. Weight gain and increase of body mass index among children and adolescents treated with antipsychotics: a critical review. Eur Child Adolesc Psychiatry. 2013;22(8):457-79.

90. Bobo WV, Cooper WO, Stein CM, Olfson M, Graham D, Daugherty J, et al. Antipsychotics and the risk of type 2 diabetes mellitus in children and youth. JAMA psychiatry. 2013;70(10):1067-75.
91. Pramyothin P, Khaodhiar L. Type 2 diabetes in children and adolescents on atypical antipsychotics. Curr Diab Rep. 2015;15(8):53.
92. De Hert M, Dobbelaere M, Sheridan EM, Cohen D, Correll CU. Metabolic and endocrine adverse effects of second-generation antipsychotics in children and adolescents: A systematic review of randomized, placebo controlled trials and guidelines for clinical practice. Eur Psychiatry. 2011;26(3):144-58.
93. Gebhardt S, Haberhausen M, Heinzel-Gutenbrunner M, Gebhardt N, Remschmidt H, Krieg JC, et al. Antipsychotic-induced body weight gain: predictors and a systematic categorization of the long-term weight course. J Psychiatr Res. 2009;43(6):620-6.
94. Ratzoni G, Gothelf D, Brand-Gothelf A, Reidman J, Kikinzon L, Gal G, et al. Weight gain associated with olanzapine and risperidone in adolescent patients: a comparative prospective study. Journal of the American Academy of Child & Adolescent Psychiatry. 2002;41(3):337-43.
95. de Hoogd S, Overbeek WA, Heerdink ER, Correll CU, de Graeff ER, Staal WG. Differences in body mass index z-scores and weight status in a Dutch pediatric psychiatric population with and without use of second-generation antipsychotics. J Child Adolesc Psychopharmacol. 2012;22(2):166-73.
96. Reeves GM, Keeton C, Correll CU, Johnson JL, Hamer RM, Sikich L, et al. Improving metabolic parameters of antipsychotic child treatment (IMPACT) study: rationale, design, and methods. Child and Adolescent Psychiatry and Mental Health. 2013;7(1):31.
97. van der Esch CCL, Kloosterboer SM, van der Ende J, Reichart CG, Kouijzer MEJ, de Kroon MMJ, et al. Risk factors and pattern of weight gain in youths using antipsychotic drugs. Eur Child Adolesc Psychiatry. 2020.
98. Lopuszanska UJ, Skorzynska-Dziduszko K, Lupa-Zatwarnicka K, Makara-Studzinska M. Mental illness and metabolic syndrome--a literature review. Ann Agric Environ Med. 2014;21(4):815-21.
99. van der Esch CC, Kloosterboer SM, van der Ende J, Reichart CG, Kouijzer ME, de Kroon MM, et al. Risk factors and pattern of weight gain in youths using antipsychotic drugs. European Child & Adolescent Psychiatry. 2020:1-9.

100. Saddichha S, Ameen S, Akhtar S. Predictors of antipsychotic-induced weight gain in first-episode psychosis: conclusions from a randomized, double-blind, controlled prospective study of olanzapine, risperidone, and haloperidol. J Clin Psychopharmacol. 2008;28(1):27-31.
101. Vandenberghe F, Najar-Giroud A, Holzer L, Conus P, Eap CB, Ambresin AE. Second-Generation Antipsychotics in Adolescent Psychiatric Patients: Metabolic Effects and Impact of an Early Weight Change to Predict Longer Term Weight Gain. J Child Adolesc Psychopharmacol. 2018;28(4):258-65.
102. McIntyre RS, Jerrell JM. Metabolic and cardiovascular adverse events associated with antipsychotic treatment in children and adolescents. Archives of pediatrics & adolescent medicine. 2008;162(10):929-35.
103. Jarskog LF, Hamer RM, Catellier DJ, Stewart DD, Lavange L, Ray N, et al. Metformin for weight loss and metabolic control in overweight outpatients with schizophrenia and schizoaffective disorder. Am J Psychiatry. 2013;170(9):1032-40.
104. Hirsch L, Yang J, Bresee L, Jette N, Patten S, Pringsheim T. Second-generation antipsychotics and metabolic side effects: a systematic review of population-based studies. Drug safety. 2017;40(9):771-81.
105. Hirsch L, Patten SB, Bresee L, Jette N, Pringsheim T. Second-generation antipsychotics and metabolic side-effects: Canadian population-based study. BJPsych open. 2018;4(4):256-61.
106. Jerrell JM, McIntyre RS, Tripathi A. Childhood treatment with psychotropic medication and development of comorbid medical conditions in adolescent-onset bipolar disorder. Hum Psychopharmacol. 2011;26(7):451-9.
107. Shedlock K, Susi A, Gorman GH, Hisle-Gorman E, Erdie-Lalena CR, Nylund CM. Autism Spectrum Disorders and Metabolic Complications of Obesity. J Pediatr. 2016;178:183-7.e1.
108. Whicher CA, Price HC, Holt RIG. Mechanisms in endocrinology: Antipsychotic medication and type 2 diabetes and impaired glucose regulation. Eur J Endocrinol. 2018;178(6):R245-R58.
109. Correll CU, Sikich L, Reeves G, Johnson J, Keeton C, Spanos M, et al. Metformin add-on vs. antipsychotic switch vs. continued antipsychotic treatment plus healthy lifestyle education in overweight or obese youth with severe mental illness: results from the IMPACT trial. World Psychiatry. 2020;19(1):69-80.

110. Baptista T, Zarate J, Joober R, Colasante C, Beaulieu S, Paez X, et al. Drug induced weight gain, an impediment to successful pharmacotherapy: focus on antipsychotics. Current Drug Targets. 2004;5(3):279-99.
111. Cuerda C, Merchan-Naranjo J, Velasco C, Gutierrez A, Leiva M, de Castro MJ, et al. Influence of resting energy expenditure on weight gain in adolescents taking second-generation antipsychotics. Clin Nutr. 2011;30(5):616-23.
112. Fonseka TM, Müller DJ, Kennedy SH. Inflammatory cytokines and antipsychotic-induced weight gain: review and clinical implications. Molecular neuropsychiatry. 2016;2(1):1-14.
113. Fountaine RJ, Taylor AE, Mancuso JP, Greenway FL, Byerley LO, Smith SR, et al. Increased food intake and energy expenditure following administration of olanzapine to healthy men. Obesity (Silver Spring). 2010;18(8):1646-51.
114. Teff KL, Rickels MR, Grudziak J, Fuller C, Nguyen H-L, Rickels K. Antipsychotic-Induced Insulin Resistance and Postprandial Hormonal Dysregulation Independent of Weight Gain or Psychiatric Disease. Diabetes. 2013;62(9):3232.
115. Britvic D, Maric NP, Doknic M, Pekic S, Andric S, Jasovic-Gasic M, et al. Metabolic issues in psychotic disorders with the focus on first-episode patients: a review. Psychiatria Danubina. 2013;25(4):410-5.
116. Ardizzone TD, Bradley RJ, Freeman Iii AM, Dwyer DS. Inhibition of glucose transport in PC12 cells by the atypical antipsychotic drugs risperidone and clozapine, and structural analogs of clozapine. Brain research. 2001;923(1-2):82-90.
117. Vestri HS, Maianu L, Moellering DR, Garvey WT. Atypical antipsychotic drugs directly impair insulin action in adipocytes: effects on glucose transport, lipogenesis, and antilipolysis. Neuropsychopharmacology. 2007;32(4):765-72.
118. Wang G-J, Volkow ND, Logan J, Pappas NR, Wong CT, Zhu W, et al. Brain dopamine and obesity. The Lancet. 2001;357(9253):354-7.
119. Baptista T, Beaulieu S. Are Leptin and Cytokines Involved in Body Weight Gain during Treatment with Antipsychotic Drugs? The Canadian Journal of Psychiatry. 2002;47(8):742-9.
120. Reynolds GP, Kirk SL. Metabolic side effects of antipsychotic drug treatment–pharmacological mechanisms. Pharmacology & therapeutics. 2010;125(1):169-79.

121. Leonard BE, Schwarz M, Myint AM. The metabolic syndrome in schizophrenia: is inflammation a contributing cause? Journal of psychopharmacology. 2012;26(5_suppl):33-41.
122. Sapra M, Lawson D, Iranmanesh A, Varma A. Adiposity-independent hypoadiponectinemia as a potential marker of insulin resistance and inflammation in schizophrenia patients treated with second generation antipsychotics. Schizophrenia research. 2016;174(1-3):132-6.
123. Bartoli F, Crocamo C, Clerici M, Carra G. Second-generation antipsychotics and adiponectin levels in schizophrenia: A comparative meta-analysis. Eur Neuropsychopharmacol. 2015;25(10):1767-74.
124. Drzyzga Ł, Obuchowicz E, Marcinowska A, Herman ZS. Cytokines in schizophrenia and the effects of antipsychotic drugs. Brain, behavior, and immunity. 2006;20(6):532-45.
125. Singh R, Bansal Y, Medhi B, Kuhad A. Antipsychotics-induced metabolic alterations: recounting the mechanistic insights, therapeutic targets and pharmacological alternatives. European journal of pharmacology. 2019;844:231-40.
126. Kroeze WK, Hufeisen SJ, Popadak BA, Renock SM, Steinberg S, Ernsberger P, et al. Reply: H1-histamine Receptor Affinity Predicts Short-term Weight Gain for Typical and Atypical Antipsychotic Drugs. Neuropsychopharmacology. 2003;28(12):2210-1.
127. Gohlke J, Dhurandhar E, Correll C, Morrato E, Newcomer J, Remington G, et al. Recent advances in understanding and mitigating adipogenic and metabolic effects of antipsychotic drugs. Frontiers in Psychiatry. 2012;3(62).
128. Davoodi N, Kalinichev M, Clifton PG. Comparative effects of olanzapine and ziprasidone on hypophagia induced by enhanced histamine neurotransmission in the rat. Behavioural pharmacology. 2008;19(2):121-8.
129. Hahn M, Chintoh A, Giacca A, Xu L, Lam L, Mann S, et al. Atypical antipsychotics and effects of muscarinic, serotonergic, dopaminergic and histaminergic receptor binding on insulin secretion in vivo: an animal model. Schizophrenia research. 2011;131(1-3):90-5.
130. Kirk SL, Glazebrook J, Grayson B, Neill JC, Reynolds GP. Olanzapine-induced weight gain in the rat: role of 5-HT2C and histamine H1 receptors. Psychopharmacology. 2009;207(1):119.

131. Davey KJ, Cotter PD, O'Sullivan O, Crispie F, Dinan TG, Cryan JF, et al. Antipsychotics and the gut microbiome: olanzapine-induced metabolic dysfunction is attenuated by antibiotic administration in the rat. Translational Psychiatry. 2013;3:e309.
132. Bahr SM, Tyler BC, Wooldridge N, Butcher BD, Burns TL, Teesch LM, et al. Use of the second-generation antipsychotic, risperidone, and secondary weight gain are associated with an altered gut microbiota in children. Transl Psychiatry. 2015;5(10):e652.
133. Bahr SM, Weidemann BJ, Castro AN, Walsh JW, Burnett CM, Pearson NA, et al. Risperidone-induced weight gain is mediated through shifts in the gut microbiome and suppression of energy expenditure. EBioMedicine. 2015;2(11):1725-34.
134. Flowers SA, Evans SJ, Ward KM, McInnis MG, Ellingrod VL. Interaction Between Atypical Antipsychotics and the Gut Microbiome in a Bipolar Disease Cohort. Pharmacotherapy. 2017;37(3):261-7.
135. Yuan X, Zhang P, Wang Y, Liu Y, Li X, Kumar BU, et al. Changes in metabolism and microbiota after 24-week risperidone treatment in drug naïve, normal weight patients with first episode schizophrenia. Schizophr Res. 2018;201:299-306.
136. Skonieczna-Żydecka K, Łoniewski I, Misera A, Stachowska E, Maciejewska D, Marlicz W, et al. Second-generation antipsychotics and metabolism alterations: a systematic review of the role of the gut microbiome. Psychopharmacology. 2019;236(5):1491-512.
137. Calarge CA, Ivins SD, Motyl KJ, Shibli-Rahhal AA, Bliziotes MM, Schlechte JA. Possible mechanisms for the skeletal effects of antipsychotics in children and adolescents. Therapeutic advances in psychopharmacology. 2013;3(5):278-93.
138. Cazzullo CL, Sacchetti E, Galluzzo A, Panariello A, Adorni A, Pegoraro M, et al. Cytokine profiles in schizophrenic patients treated with risperidone: A 3-month follow-up study. Progress in Neuro-Psychopharmacology and Biological Psychiatry. 2002;26(1):33-9.
139. Momtazmanesh S, Zare-Shahabadi A, Rezaei N. Cytokine alterations in schizophrenia: an updated review. Frontiers in Psychiatry. 2019;10.
140. Ashwood P, Krakowiak P, Hertz-Picciotto I, Hansen R, Pessah I, Van de Water J. Elevated plasma cytokines in autism spectrum disorders provide

evidence of immune dysfunction and are associated with impaired behavioral outcome. Brain, behavior, and immunity. 2011;25(1):40-5.

141. Vargas DL, Nascimbene C, Krishnan C, Zimmerman AW, Pardo CA. Neuroglial activation and neuroinflammation in the brain of patients with autism. Annals of Neurology: Official Journal of the American Neurological Association and the Child Neurology Society. 2005;57(1):67-81.

142. Haring L, Koido K, Vasar V, Leping V, Zilmer K, Zilmer M, et al. Antipsychotic treatment reduces psychotic symptoms and markers of low-grade inflammation in first episode psychosis patients, but increases their body mass index. Schizophrenia Research. 2015;169(1-3):22-9.

143. Upthegrove R, Manzanares-Teson N, Barnes NM. Cytokine function in medication-naive first episode psychosis: a systematic review and meta-analysis. Schizophrenia research. 2014;155(1-3):101-8.

144. Noto C, Ota VK, Gouvea ES, Rizzo LB, Spindola LM, Honda PH, et al. Effects of risperidone on cytokine profile in drug-naive first-episode psychosis. The international journal of neuropsychopharmacology. 2014;18(4).

145. Pillinger T, Osimo EF, Brugger S, Mondelli V, McCutcheon RA, Howes OD. A meta-analysis of immune parameters, variability, and assessment of modal distribution in psychosis and test of the immune subgroup hypothesis. Schizophrenia bulletin. 2019;45(5):1120-33.

146. Beumer W, Drexhage RC, De Wit H, Versnel MA, Drexhage HA, Cohen D. Increased level of serum cytokines, chemokines and adipokines in patients with schizophrenia is associated with disease and metabolic syndrome. Psychoneuroendocrinology. 2012;37(12):1901-11.

147. Di Nicola M, Cattaneo A, Hepgul N, Di Forti M, Aitchison KJ, Janiri L, et al. Serum and gene expression profile of cytokines in first-episode psychosis. Brain, behavior, and immunity. 2013;31:90-5.

148. Song X, Fan X, Li X, Zhang W, Gao J, Zhao J, et al. Changes in pro-inflammatory cytokines and body weight during 6-month risperidone treatment in drug naive, first-episode schizophrenia. Psychopharmacology. 2014;231(2):319-25.

149. Miller BJ, Buckley P, Seabolt W, Mellor A, Kirkpatrick B. Meta-analysis of cytokine alterations in schizophrenia: clinical status and antipsychotic effects. Biological psychiatry. 2011;70(7):663-71.

150. Kao Y-C, Ko C-Y, Wang S-C, Liu Y-P. Protective effects of quetiapine on metabolic and inflammatory abnormalities in schizophrenic patients during exacerbated stage. Chin J Physiol. 2016;59(2):69-77.
151. Kelsven S, de la Fuente-Sandoval C, Achim CL, Reyes-Madrigal F, Mirzakhanian H, Domingues I, et al. Immuno-inflammatory changes across phases of early psychosis: The impact of antipsychotic medication and stage of illness. Schizophrenia research. 2020;226:13-23.
152. Capuzzi E, Bartoli F, Crocamo C, Clerici M, Carra G. Acute variations of cytokine levels after antipsychotic treatment in drug-naive subjects with a first-episode psychosis: a meta-analysis. Neuroscience & Biobehavioral Reviews. 2017;77:122-8.
153. Varun CN, Venkataswamy MM, Ravikumar R, Nagaraju R, Debnath M, Varambally S, et al. Th17 and MAIT cell mediated inflammation in antipsychotic free schizophrenia patients. Schizophrenia research. 2019;212:47-53.
154. Fernandes BS, Steiner J, Bernstein HG, Dodd S, Pasco JA, Dean OM, et al. C-reactive protein is increased in schizophrenia but is not altered by antipsychotics: meta-analysis and implications. Mol Psychiatry. 2016;21(4):554-64.
155. Russell A, Ciufolini S, Gardner-Sood P, Bonaccorso S, Gaughran F, Dazzan P, et al. Inflammation and metabolic changes in first episode psychosis: preliminary results from a longitudinal study. Brain, behavior, and immunity. 2015;49:25-9.
156. Miller BJ, Mellor A, Buckley P. Total and differential white blood cell counts, high-sensitivity C-reactive protein, and the metabolic syndrome in non-affective psychoses. Brain, behavior, and immunity. 2013;31:82-9.
157. Schwarz MJ. Cytokines, neurophysiology, neuropsychology, and psychiatric symptoms. Dialogues in clinical neuroscience. 2003;5(2):139.
158. Borovcanin M, Jovanovic I, Radosavljevic G, Dejanovic SD, Stefanovic V, Arsenijevic N, et al. Antipsychotics can modulate the cytokine profile in schizophrenia: attenuation of the type-2 inflammatory response. Schizophrenia research. 2013;147(1):103-9.
159. Romeo B, Brunet-Lecomte M, Martelli C, Benyamina A. Kinetics of cytokine levels during antipsychotic treatment in schizophrenia: a meta-analysis. International Journal of Neuropsychopharmacology. 2018;21(9):828-36.

160. O'Connell KE, Thakore J, Dev KK. Pro-inflammatory cytokine levels are raised in female schizophrenia patients treated with clozapine. Schizophrenia research. 2014;156(1):1-8.
161. Himmerich H, Schönherr J, Fulda S, Sheldrick AJ, Bauer K, Sack U. Impact of antipsychotics on cytokine production in-vitro. Journal of Psychiatric Research. 2011;45(10):1358-65.
162. Stapel B, Sieve I, Falk CS, Bleich S, Hilfiker-Kleiner D, Kahl KG. Second generation atypical antipsychotics olanzapine and aripiprazole reduce expression and secretion of inflammatory cytokines in human immune cells. Journal of psychiatric research. 2018;105:95-102.
163. Stefanović V, Mihajlović G, Nenadović M, Đukić-Dejanović S, Borovčanin M, Trajković G. The effect of antipsychotic drugs on nonspecific inflammation markers in the first episode of schizophrenia. Vojnosanitetski pregled. 2015;72(12):1085-92.
164. Sarsenbayeva A, Marques-Santos CM, Thombare K, Di Nunzio G, Almby KE, Lundqvist M, et al. Effects of second-generation antipsychotics on human subcutaneous adipose tissue metabolism. Psychoneuroendocrinology. 2019;110:104445.
165. Sárvári AK, Veréb Z, Uray IP, Fésüs L, Balajthy Z. Atypical antipsychotics induce both proinflammatory and adipogenic gene expression in human adipocytes in vitro. Biochemical and biophysical research communications. 2014;450(4):1383-9.
166. Duarte T, Barbisan F, do Prado-Lima PAS, Azzolin VF, da Cruz Jung IE, Duarte MMMF, et al. Ziprasidone, a second-generation antipsychotic drug, triggers a macrophage inflammatory response in vitro. Cytokine. 2018;106:101-7.
167. Cooper SJ, Reynolds GP, Barnes T, England E, Haddad PM, Heald A, et al. BAP guidelines on the management of weight gain, metabolic disturbances and cardiovascular risk associated with psychosis and antipsychotic drug treatment. J Psychopharmacol. 2016;30(8):717-48.
168. Barnes TR, Drake R, Paton C, Cooper SJ, Deakin B, Ferrier IN, et al. Evidence-based guidelines for the pharmacological treatment of schizophrenia: Updated recommendations from the British Association for Psychopharmacology. Journal of Psychopharmacology. 2020;34(1):3-78.

169. Findling R, Drury S, Jensen P, Rapoport J, Bukstein O, Walter H, et al. Practice parameter for the use of atypical antipsychotic medications in children and adolescents. J Am Acad Child Adolesc Psychiatry. 2011.
170. Pringsheim T, Panagiotopoulos C, Davidson J, Ho J. Evidence-based recommendations for monitoring safety of second generation antipsychotics in children and youth. J Can Acad Child Adolesc Psychiatry. 2011;20(3):218-33.
171. Excellence NIfC. Psychosis and schizophrenia in children and young people: Recognition and management (NICE Clinical Guideline CG115). London, United Kingdom: NICE. 2016.
172. Correll CU. Monitoring and management of antipsychotic-related metabolic and endocrine adverse events in pediatric patients. Int Rev Psychiatry. 2008;20(2):195-201.
173. Alvarez-Jiménez M, González-Blanch C, Vázquez-Barquero JL, Pérez-Iglesias R, Martínez-García O, Pérez-Pardal T, et al. Attenuation of antipsychotic-induced weight gain with early behavioral intervention in drug-naive first-episode psychosis patients: A randomized controlled trial. J Clin Psychiatry. 2006;67(8):1253-60.
174. Dayabandara M, Hanwella R, Ratnatunga S, Seneviratne S, Suraweera C, de Silva VA. Antipsychotic-associated weight gain: management strategies and impact on treatment adherence. Neuropsychiatr Dis Treat. 2017;13:2231-41.
175. Green CA, Yarborough BJH, Leo MC, Yarborough MT, Stumbo SP, Janoff SL, et al. The STRIDE weight loss and lifestyle intervention for individuals taking antipsychotic medications: a randomized trial. American Journal of Psychiatry. 2015;172(1):71-81.
176. Handen BL, Anagnostou E, Aman MG, Sanders KB, Chan J, Hollway JA, et al. A Randomized, Placebo-Controlled Trial of Metformin for the Treatment of Overweight Induced by Antipsychotic Medication in Young People With Autism Spectrum Disorder: Open-Label Extension. J Am Acad Child Adolesc Psychiatry. 2017;56(10):849-56 e6.
177. Anagnostou E, Aman MG, Handen BL, Sanders KB, Shui A, Hollway JA, et al. Metformin for Treatment of Overweight Induced by Atypical Antipsychotic Medication in Young People With Autism Spectrum Disorder: A Randomized Clinical Trial. JAMA Psychiatry. 2016;73(9):928-37.

178. Morrison JA, Cottingham EM, Barton BA. Metformin for weight loss in pediatric patients taking psychotropic drugs. Am J Psychiatry. 2002;159(4):655-7.
179. Larsen JR, Vedtofte L, Jakobsen MS, Jespersen HR, Jakobsen MI, Svensson CK, et al. Effect of liraglutide treatment on prediabetes and overweight or obesity in clozapine-or olanzapine-treated patients with schizophrenia spectrum disorder: a randomized clinical trial. JAMA psychiatry. 2017;74(7):719-28.
180. Babic I, Gorak A, Engel M, Sellers D, Else P, Osborne AL, et al. Liraglutide prevents metabolic side-effects and improves recognition and working memory during antipsychotic treatment in rats. Journal of Psychopharmacology. 2018;32(5):578-90.
181. Medak KD, Shamshoum H, Peppler WT, Wright DC. GLP1 Receptor Agonism Protects Against Acute Olanzapine Induced Hyperglycemia. American Journal of Physiology-Endocrinology and Metabolism. 2020.
182. Matthews DR, Hosker J, Rudenski A, Naylor B, Treacher D, Turner R. Homeostasis model assessment: insulin resistance and β-cell function from fasting plasma glucose and insulin concentrations in man. diabetologia. 1985;28(7):412-9.
183. Wallace TM, Levy JC, Matthews DR. Use and abuse of HOMA modeling. Diabetes care. 2004;27(6):1487-95.
184. Kurtoğlu S, Hatipoğlu N, Mazıcıoğlu M, Kendirici M, Keskin M, Kondolot M. Insulin resistance in obese children and adolescents: HOMA− IR cut− off levels in the prepubertal and pubertal periods. Journal of clinical research in pediatric endocrinology. 2010;2(3):100.
185. Keskin M, Kurtoglu S, Kendirci M, Atabek ME, Yazici C. Homeostasis model assessment is more reliable than the fasting glucose/insulin ratio and quantitative insulin sensitivity check index for assessing insulin resistance among obese children and adolescents. Pediatrics. 2005;115(4):e500-e3.
186. Taylor DM, Barnes TR, Young AH. The Maudsley prescribing guidelines in psychiatry: John Wiley & Sons; 2018.
187. FULWYLER MJ. Status quo in flow-through cytometry. Journal of Histochemistry & Cytochemistry. 1974;22(7):605-6.

188. Drijvers JM, Awan IM, Perugino CA, Rosenberg IM, Pillai S. The Enzyme-Linked Immunosorbent Assay: The Application of ELISA in Clinical Research. Basic Science Methods for Clinical Researchers: Elsevier; 2017. p. 119-33.
189. Hálfdánarson Ó, Zoëga H, Aagaard L, Bernardo M, Brandt L, Fusté AC, et al. International trends in antipsychotic use: a study in 16 countries, 2005–2014. European Neuropsychopharmacology. 2017;27(10):1064-76.
190. Kalverdijk LJ, Bachmann CJ, Aagaard L, Burcu M, Glaeske G, Hoffmann F, et al. A multi-national comparison of antipsychotic drug use in children and adolescents, 2005–2012. Child and adolescent psychiatry and mental health. 2017;11(1):55.
191. Wilkinson S, Mulder RT. Antipsychotic prescribing in New Zealand between 2008 and 2015. The New Zealand medical journal. 2018;131(1480):61-7.
192. Verdoux H, Pambrun E, Cortaredona S, Tournier M, Verger P. Antipsychotic prescribing in youths: a French community-based study from 2006 to 2013. European child & adolescent psychiatry. 2015;24(10):1181-91.
193. Abbas S, Ihle P, Adler J-B, Engel S, Günster C, Linder R, et al. Psychopharmacological Prescriptions in Children and Adolescents in Germany: A Nationwide Analysis of Over 4 Million Statutorily Insured Individuals From 2004 to 2012. Deutsches Ärzteblatt International. 2016;113(22-23):396.
194. Crystal S, Mackie T, Fenton MC, Amin S, Neese-Todd S, Olfson M, et al. Rapid growth of antipsychotic prescriptions for children who are publicly insured has ceased, but concerns remain. Health affairs. 2016;35(6):974-82.
195. Lake JK, Denton D, Lunsky Y, Shui AM, Veenstra-VanderWeele J, Anagnostou E. Medical conditions and demographic, service and clinical factors associated with atypical antipsychotic medication use among children with an autism spectrum disorder. Journal of autism and developmental disorders. 2017;47(5):1391-402.
196. Conlan K, McGrath J, Teeling M, MacAvin M, Bennett K, Gallagher L. Antipsychotic prescribing in GMS paediatric and young adult population in Ireland 2005–2015: repeated cross-sectional study. Irish Journal of Psychological Medicine. 2021:1-10.
197. Association AD. American Association of Clinical Endocrinologists, North American Association for the Study of Obesity: Consensus development

conference on antipsychotic drugs and obesity and diabetes. Diabetes Care. 2004;27:596-601.

198. Gleason MM, Egger HL, Emslie GJ, Greenhill LL, Kowatch RA, Lieberman AF, et al. Psychopharmacological treatment for very young children: contexts and guidelines. Journal of the American Academy of Child & Adolescent Psychiatry. 2007;46(12):1532-72.

199. Penfold RB, Stewart C, Hunkeler EM, Madden JM, Cummings JR, Owen-Smith AA, et al. Use of antipsychotic medications in pediatric populations: what do the data say? Current psychiatry reports. 2013;15(12):426.

200. Baker JL, Olsen LW, Sørensen TI. Childhood body-mass index and the risk of coronary heart disease in adulthood. New England journal of medicine. 2007;357(23):2329-37.

201. Daviss WB, Barnett E, Neubacher K, Drake RE. Use of antipsychotic medications for nonpsychotic children: risks and implications for mental health services. Am Psychiatric Assoc; 2016.

202. Kimberly MM, Leary ET, Cole TG, Waymack PP, Network CRML. Selection, validation, standardization, and performance of a designated comparison method for HDL-cholesterol for use in the cholesterol reference method laboratory network. Clinical chemistry. 1999;45(10):1803-12.

203. Ling JCY, Mohamed MNA, Jalaludin MY, Rampal S, Zaharan NL, Mohamed Z. Determinants of high fasting insulin and insulin resistance among overweight/obese adolescents. Scientific reports. 2016;6(1):1-10.

204. Tracey M, McHugh S, Buckley C, Canavan R, Fitzgerald A, Kearney P. The prevalence of Type 2 diabetes and related complications in a nationally representative sample of adults aged 50 and over in the Republic of Ireland. Diabetic Medicine. 2016;33(4):441-5.

205. O'Dea M, O'Connell S, O'Grady M. Prevalence and characteristics of paediatric type 2 diabetes in the Republic of Ireland. Diabetic Medicine. 2017;34(11):1603-7.

206. Grimes RT, Bennett K, Hoey H, Tilson L, Henman MC. A retrospective cohort analysis of hypoglycaemic and cardiovascular agent use in young adults in the Irish primary care setting. Irish Journal of Medical Science (1971 -). 2017;186(2):461-70.

207. Franks PW, Hanson RL, Knowler WC, Sievers ML, Bennett PH, Looker HC. Childhood obesity, other cardiovascular risk factors, and premature death. New England Journal of Medicine. 2010;362(6):485-93.
208. Aboraya A, Fullen JE, Ponieman BL, Makela EH, Latocha M. Hyperprolactinemia associated with risperidone: a case report and review of literature. Psychiatry (Edgmont). 2004;1(3):29.
209. Kelly DL, Powell MM, Wehring HJ, Sayer MA, Kearns AM, Hackman AL, et al. Adjunct Aripiprazole Improves Prolactin and Prolactin-Related Side Effects in Premenopausal Women with Psychosis: Results from the DAAMSEL Clinical Trial. Journal of clinical psychopharmacology. 2018;38(4):317.
210. Zheng W, Cai D-B, Yang X-H, Zheng W, Ungvari GS, Ng CH, et al. Adjunctive aripiprazole for antipsychotic-related hyperprolactinaemia in patients with first-episode schizophrenia: a meta-analysis. General psychiatry. 2019;32(5).
211. De Hert M, Detraux J, Stubbs B. Relationship between antipsychotic medication, serum prolactin levels and osteoporosis/osteoporotic fractures in patients with schizophrenia: a critical literature review. Expert Opinion on Drug Safety. 2016;15(6):809-23.
212. O'Dwyer M, Peklar J, McCallion P, McCarron M, Henman MC. Factors associated with polypharmacy and excessive polypharmacy in older people with intellectual disability differ from the general population: a cross-sectional observational nationwide study. BMJ Open. 2016;6(4):e010505.
213. Grigg J, Worsley R, Thew C, Gurvich C, Thomas N, Kulkarni J. Antipsychotic-induced hyperprolactinemia: synthesis of world-wide guidelines and integrated recommendations for assessment, management and future research. Psychopharmacology. 2017;234(22):3279-97.
214. Kritharides L, Chow V, Lambert TJ. Cardiovascular disease in patients with schizophrenia. The Medical journal of Australia. 2017;207(4):179.
215. Hirvikoski T, Mittendorfer-Rutz E, Boman M, Larsson H, Lichtenstein P, Bölte S. Premature mortality in autism spectrum disorder. The British Journal of Psychiatry. 2016;208(3):232-8.
216. Crump C, Winkleby MA, Sundquist K, Sundquist J. Comorbidities and mortality in persons with schizophrenia: a Swedish national cohort study. Am J Psychiatry. 2013;170(3):324-33.

217. Schuster DP. Obesity and the development of type 2 diabetes: the effects of fatty tissue inflammation. Diabetes, metabolic syndrome and obesity: targets and therapy. 2010;3:253.
218. Strissel KJ, Denis GV, Nikolajczyk BS. Immune regulators of inflammation in obesity-associated type 2 diabetes and coronary artery disease. Current opinion in endocrinology, diabetes, and obesity. 2014;21(5):330.
219. Carolan E, Tobin LM, Mangan BA, Corrigan M, Gaoatswe G, Byrne G, et al. Altered distribution and increased IL-17 production by mucosal-associated invariant T cells in adult and childhood obesity. The Journal of Immunology. 2015;194(12):5775-80.
220. Jung U, Choi M-S. Obesity and its metabolic complications: the role of adipokines and the relationship between obesity, inflammation, insulin resistance, dyslipidemia and nonalcoholic fatty liver disease. International journal of molecular sciences. 2014;15(4):6184-223.
221. Tobin LM, Mavinkurve M, Carolan E, Kinlen D, O'Brien EC, Little MA, et al. NK cells in childhood obesity are activated, metabolically stressed, and functionally deficient. JCI insight. 2017;2(24).
222. Boulenouar S, Michelet X, Duquette D, Alvarez D, Hogan AE, Dold C, et al. Adipose type one innate lymphoid cells regulate macrophage homeostasis through targeted cytotoxicity. Immunity. 2017;46(2):273-86.
223. Wensveen FM, Jelenčić V, Valentić S, Šestan M, Wensveen TT, Theurich S, et al. NK cells link obesity-induced adipose stress to inflammation and insulin resistance. Nature immunology. 2015;16(4):376-85.
224. Maurizi G, Della Guardia L, Maurizi A, Poloni A. Adipocytes properties and crosstalk with immune system in obesity-related inflammation. Journal of cellular physiology. 2018;233(1):88-97.
225. Chen M-L, Tsai T-C, Wang L-K, Lin Y-Y, Tsai Y-M, Lee M-C, et al. Risperidone modulates the cytokine and chemokine release of dendritic cells and induces TNF-α-directed cell apoptosis in neutrophils. International immunopharmacology. 2012;12(1):197-204.
226. Chen M-L, Wu S, Tsai T-C, Wang L-K, Tsai F-M. Regulation of macrophage immune responses by antipsychotic drugs. Immunopharmacology and immunotoxicology. 2013;35(5):573-80.

227. Pae CU, Yoon CH, Kim TS, Kim JJ, Park SH, Lee CU, et al. Antipsychotic treatment may alter T-helper (TH) 2 arm cytokines. International immunopharmacology. 2006;6(4):666-71.
228. Tourjman V, Kouassi É, Koué M-È, Rocchetti M, Fortin-Fournier S, Fusar-Poli P, et al. Antipsychotics' effects on blood levels of cytokines in schizophrenia: a meta-analysis. Schizophrenia research. 2013;151(1-3):43-7.
229. Kim Y-K, Myint A-M, Lee B-H, Han C-S, Lee H-J, Kim D-J, et al. Th1, Th2 and Th3 cytokine alteration in schizophrenia. Progress in Neuropsychopharmacology & Biological Psychiatry. 2004;28:1129-34.
230. Kawano M, Takagi R, Saika K, Matsui M, Matsushita S. Dopamine regulates cytokine secretion during innate and adaptive immune responses. Int Immunol. 2018;30(12):591-606.
231. Yan Y, Jiang W, Liu L, Wang X, Ding C, Tian Z, et al. Dopamine controls systemic inflammation through inhibition of NLRP3 inflammasome. Cell. 2015;160(1-2):62-73.
232. Guilliams M, Mildner A, Yona S. Developmental and functional heterogeneity of monocytes. Immunity. 2018;49(4):595-613.
233. Boutens L, Stienstra R. Adipose tissue macrophages: going off track during obesity. Diabetologia. 2016;59(5):879-94.
234. Caligiuri MA. Human natural killer cells. Blood, The Journal of the American Society of Hematology. 2008;112(3):461-9.
235. O'Shea D, Hogan AE. Dysregulation of natural killer cells in obesity. Cancers. 2019;11(4):573.
236. Michelet X, Dyck L, Hogan A, Loftus RM, Duquette D, Wei K, et al. Metabolic reprogramming of natural killer cells in obesity limits antitumor responses. Nature immunology. 2018;19(12):1330-40.
237. Gavin MA, Rasmussen JP, Fontenot JD, Vasta V, Manganiello VC, Beavo JA, et al. Foxp3-dependent programme of regulatory T-cell differentiation. Nature. 2007;445(7129):771-5.
238. Winer S, Paltser G, Chan Y, Tsui H, Engleman E, Winer D, et al. Obesity predisposes to Th17 bias. European journal of immunology. 2009;39(9):2629-35.
239. Yang H, Youm Y-H, Vandanmagsar B, Ravussin A, Gimble JM, Greenway F, et al. Obesity increases the production of proinflammatory mediators from adipose tissue T cells and compromises TCR repertoire diversity: implications for

systemic inflammation and insulin resistance. The Journal of Immunology. 2010;185(3):1836-45.
240. Godfrey DI, Koay H-F, McCluskey J, Gherardin NA. The biology and functional importance of MAIT cells. Nature immunology. 2019;20(9):1110-28.
241. Pisarska MM, Dunne MR, O'Shea D, Hogan AE. Interleukin-17 producing mucosal associated invariant T cells-emerging players in chronic inflammatory diseases? European Journal of Immunology. 2020;50(8):1098-108.
242. Provine NM, Klenerman P. MAIT cells in health and disease. Annual Review of Immunology. 2020;38:203-28.
243. Bertrand L, Lehuen A. MAIT cells in metabolic diseases. Molecular Metabolism. 2019;27:S114-S21.
244. Le Bourhis L, Martin E, Péguillet I, Guihot A, Froux N, Coré M, et al. Antimicrobial activity of mucosal-associated invariant T cells. Nature immunology. 2010;11(8):701-8.
245. Magalhaes I, Pingris K, Poitou C, Bessoles S, Venteclef N, Kiaf B, et al. Mucosal-associated invariant T cell alterations in obese and type 2 diabetic patients. The Journal of clinical investigation. 2015;125(4):1752-62.
246. Park YJ, Park J, Huh JY, Hwang I, Choe SS, Kim JB. Regulatory Roles of Invariant Natural Killer T Cells in Adipose Tissue Inflammation: Defenders Against Obesity-Induced Metabolic Complications. Frontiers in Immunology. 2018;9(1311).
247. Balato A, Unutmaz D, Gaspari AA. Natural killer T cells: an unconventional T-cell subset with diverse effector and regulatory functions. Journal of Investigative Dermatology. 2009;129(7):1628-42.
248. Lynch L, Nowak M, Varghese B, Clark J, Hogan AE, Toxavidis V, et al. Adipose tissue invariant NKT cells protect against diet-induced obesity and metabolic disorder through regulatory cytokine production. Immunity. 2012;37(3):574-87.
249. Schipper HS, Rakhshandehroo M, van de Graaf SF, Venken K, Koppen A, Stienstra R, et al. Natural killer T cells in adipose tissue prevent insulin resistance. The Journal of clinical investigation. 2012;122(9):3343-54.
250. Baumeister D, Ciufolini S, Mondelli V. Effects of psychotropic drugs on inflammation: consequence or mediator of therapeutic effects in psychiatric treatment? Psychopharmacology (Berl). 2016;233(9):1575-89.

251. Coope A, Torsoni AS, Velloso LA. Mechanisms in endocrinology: metabolic and inflammatory pathways on the pathogenesis of type 2 diabetes. European Journal of Endocrinology. 2016;174(5):R175-R87.
252. Reinehr T, Roth CL. Inflammation Markers in Type 2 Diabetes and the Metabolic Syndrome in the Pediatric Population. Curr Diab Rep. 2018;18(12):131.
253. Hotamisligil GS, Shargill NS, Spiegelman BM. Adipose expression of tumor necrosis factor-alpha: direct role in obesity-linked insulin resistance. Science. 1993;259(5091):87-91.
254. Herder C, Dalmas E, Böni-Schnetzler M, Donath MY. The IL-1 Pathway in Type 2 Diabetes and Cardiovascular Complications. Trends Endocrinol Metab. 2015;26(10):551-63.
255. Zatterale F, Longo M, Naderi J, Raciti GA, Desiderio A, Miele C, et al. Chronic adipose tissue inflammation linking obesity to insulin resistance and type 2 diabetes. Frontiers in physiology. 2020:1607.
256. O'Brien A, Loftus RM, Pisarska MM, Tobin LM, Bergin R, Wood NA, et al. Obesity reduces mTORC1 activity in mucosal-associated invariant T cells, driving defective metabolic and functional responses. The Journal of Immunology. 2019;202(12):3404-11.
257. Chehimi M, Vidal H, Eljaafari A. Pathogenic Role of IL-17-Producing Immune Cells in Obesity, and Related Inflammatory Diseases. J Clin Med. 2017;6(7).
258. Li X, Bechara R, Zhao J, McGeachy MJ, Gaffen SL. IL-17 receptor-based signaling and implications for disease. Nat Immunol. 2019;20(12):1594-602.
259. Fabbrini E, Cella M, Mccartney SA, Fuchs A, Abumrad NA, Pietka TA, et al. Association between specific adipose tissue CD4+ T-cell populations and insulin resistance in obese individuals. Gastroenterology. 2013;145(2):366-74. e3.
260. Møller HJ, Frikke-Schmidt R, Moestrup SK, Nordestgaard BG, Tybjærg-Hansen A. Serum soluble CD163 predicts risk of type 2 diabetes in the general population. Clinical chemistry. 2011;57(2):291-7.
261. Parkner T, Sørensen LP, Nielsen AR, Fischer CP, Bibby BM, Nielsen S, et al. Soluble CD163: a biomarker linking macrophages and insulin resistance. Diabetologia. 2012;55(6):1856-62.

262. Grill HJ, Schwartz MW, Kaplan JM, Foxhall JS, Breininger J, Baskin DG. Evidence that the caudal brainstem is a target for the inhibitory effect of leptin on food intake. Endocrinology. 2002;143(1):239-46.
263. Omar S, Lorenz J, Rawas F, Randall-Hlubek D, McGrath K, Brown C, et al. Expression of Memory (CD45RO+) and Naive (CD45RA+) T-cells in Newborn Infants with Sepsis.† 1334. Pediatric Research. 1997;41(4):225-.
264. Toubal A, Kiaf B, Beaudoin L, Cagninacci L, Rhimi M, Fruchet B, et al. Mucosal-associated invariant T cells promote inflammation and intestinal dysbiosis leading to metabolic dysfunction during obesity. Nature communications. 2020;11(1):1-20.
265. Bretler T, Weisberg H, Koren O, Neuman H. The effects of antipsychotic medications on microbiome and weight gain in children and adolescents. BMC Med. 2019;17(1):112.
266. Golubovskaya V, Wu L. Different subsets of T cells, memory, effector functions, and CAR-T immunotherapy. Cancers. 2016;8(3):36.
267. Wagner NM, Brandhorst G, Czepluch F, Lankeit M, Eberle C, Herzberg S, et al. Circulating regulatory T cells are reduced in obesity and may identify subjects at increased metabolic and cardiovascular risk. Obesity. 2013;21(3):461-8.
268. Touch S, Clément K, André S. T Cell Populations and Functions Are Altered in Human Obesity and Type 2 Diabetes. Current Diabetes Reports. 2017;17(9):81.
269. Fay NS, Larson EC, Jameson JM. Chronic Inflammation and γδ T Cells. Frontiers in Immunology. 2016;7(210).
270. Cua DJ, Tato CM. Innate IL-17-producing cells: the sentinels of the immune system. Nat Rev Immunol. 2010;10(7):479-89.
271. Lawand M, Déchanet-Merville J, Dieu-Nosjean M-C. Key Features of Gamma-Delta T-Cell Subsets in Human Diseases and Their Immunotherapeutic Implications. Frontiers in Immunology. 2017;8(761).
272. Costanzo AE, Taylor KR, Dutt S, Han PP, Fujioka K, Jameson JM. Obesity impairs γδ T cell homeostasis and antiviral function in humans. PLoS One. 2015;10(3):e0120918.

273. Peluso I, Palmery M. The relationship between body weight and inflammation: Lesson from anti-TNF-α antibody therapy. Hum Immunol. 2016;77(1):47-53.
274. Sabat R, Grütz G, Warszawska K, Kirsch S, Witte E, Wolk K, et al. Biology of interleukin-10. Cytokine & growth factor reviews. 2010;21(5):331-44.
275. Arango Duque G, Descoteaux A. Macrophage cytokines: involvement in immunity and infectious diseases. Frontiers in immunology. 2014;5:491.
276. O'Shea D, Corrigan M, Dunne MR, Jackson R, Woods C, Gaoatswe G, et al. Changes in human dendritic cell number and function in severe obesity may contribute to increased susceptibility to viral infection. International journal of obesity. 2013;37(11):1510-3.
277. Kolls JK, Lindén A. Interleukin-17 family members and inflammation. Immunity. 2004;21(4):467-76.
278. Ruiz de Morales JMG, Puig L, Daudén E, Cañete JD, Pablos JL, Martín AO, et al. Critical role of interleukin (IL)-17 in inflammatory and immune disorders: An updated review of the evidence focusing in controversies. Autoimmun Rev. 2020;19(1):102429.
279. Goepfert A, Lehmann S, Wirth E, Rondeau J-M. The human IL-17A/F heterodimer: a two-faced cytokine with unique receptor recognition properties. Scientific reports. 2017;7(1):1-13.
280. Zúñiga LA, Shen W-J, Joyce-Shaikh B, Pyatnova EA, Richards AG, Thom C, et al. IL-17 regulates adipogenesis, glucose homeostasis, and obesity. The Journal of Immunology. 2010;185(11):6947-59.
281. Mishra A, Singh S, Shukla S. Physiological and functional basis of dopamine receptors and their role in neurogenesis: possible implication for Parkinson’s disease. Journal of experimental neuroscience. 2018;12:1179069518779829.
282. Levite M. Dopamine and T cells: dopamine receptors and potent effects on T cells, dopamine production in T cells, and abnormalities in the dopaminergic system in T cells in autoimmune, neurological and psychiatric diseases. Acta physiologica. 2016;216(1):42-89.
283. McKenna F, McLaughlin PJ, Lewis BJ, Sibbring GC, Cummerson JA, Bowen-Jones D, et al. Dopamine receptor expression on human T- and B-

lymphocytes, monocytes, neutrophils, eosinophils and NK cells: a flow cytometric study. J Neuroimmunol. 2002;132(1-2):34-40.

284. Pacheco R, Prado CE, Barrientos MJ, Bernales S. Role of dopamine in the physiology of T-cells and dendritic cells. Journal of neuroimmunology. 2009;216(1-2):8-19.

285. Ghosh MC, Mondal AC, Basu S, Banerjee S, Majumder J, Bhattacharya D, et al. Dopamine inhibits cytokine release and expression of tyrosine kinases, Lck and Fyn in activated T cells. International immunopharmacology. 2003;3(7):1019-26.

286. Melnikov M, Belousova O, Murugin V, Pashenkov M, Boyко A. The role of dopamine in modulation of Th-17 immune response in multiple sclerosis. Journal of neuroimmunology. 2016;292:97-101.

287. Melnikov M, Rogovskii V, Boyko A, Pashenkov M. Dopaminergic therapeutics in multiple sclerosis: focus on Th17-cell functions. Journal of Neuroimmune Pharmacology. 2019:1-11.

288. Elgueta D, Contreras F, Prado C, Montoya A, Ugalde V, Chovar O, et al. Dopamine receptor D3 expression is altered in CD4+ T-cells from Parkinson's disease patients and its pharmacologic inhibition attenuates the motor impairment in a mouse model. Frontiers in immunology. 2019;10:981.

289. Obuchowicz E, Bielecka-Wajdman AM, Paul-Samojedny M, Nowacka M. Different influence of antipsychotics on the balance between pro- and anti-inflammatory cytokines depends on glia activation: An in vitro study. Cytokine. 2017;94:37-44.

290. Subbanna M, Shivakumar V, Venugopal D, Narayanaswamy JC, Berk M, Varambally S, et al. Impact of antipsychotic medication on IL-6/stat3 signaling axis in peripheral blood mononuclear cells of drug-naive schizophrenia patients. Psychiatry and clinical neurosciences. 2020;74(1):64-9.

291. O'Connell J, Kieran P, Gorman K, Ahern T, Cawood TJ, O'Shea D. BMI≥ 50 kg/m 2 is associated with a younger age of onset of overweight and a high prevalence of adverse metabolic profiles. Public health nutrition. 2010;13(7):1090-8.

292. Farr OM, Gavrieli A, Mantzoros CS. Leptin applications in 2015: what have we learned about leptin and obesity? Current opinion in endocrinology, diabetes, and obesity. 2015;22(5):353.

293. Patterson E, Ryan PM, Cryan JF, Dinan TG, Ross RP, Fitzgerald GF, et al. Gut microbiota, obesity and diabetes. Postgraduate Medical Journal. 2016;92(1087):286-300.
294. Vedi K, Bernard S. The mental health needs of children and adolescents with learning disabilities. Curr Opin Psychiatry. 2012;25(5):353-8.
295. Chen P, Lin L, Xu X, Zhang Z, Cai W, Shao Z, et al. Liraglutide improved inflammation via mediating IL-23/Th-17 pathway in obese diabetic mice with psoriasiform skin. Journal of Dermatological Treatment. 2019:1-7.
296. Kelly AS, Auerbach P, Barrientos-Perez M, Gies I, Hale PM, Marcus C, et al. A randomized, controlled trial of liraglutide for adolescents with obesity. New England Journal of Medicine. 2020;382(22):2117-28.

APPENDICES

Appendix 1 Parent Information Leaflet

Parent Information Leaflet

Study title: **Inflammatory markers and Antipsychotics study (InflamAP)**

Principal investigator's name:	Prof Jane McGrath (1) Prof Louise Gallagher (2)
Principal investigator's title:	(1) Consultant Child and Adolescent Psychiatrist, Associate Professor of Child and Adolescent Psychiatry (2) Consultant Child and Adolescent Psychiatrist, Director of Research, School of Medicine, Trinity College, Dublin
Telephone number of principal investigator:	Prof McGrath: 01 8962241 Prof Gallagher 01-8962144
Co-investigator's name:	Dr Karen Conlan
Co-investigator's title:	Clinical Research Fellow
Co-investigator's name:	Dr Andrew Hogan

Co-investigator's title: Senior Scientist, Obesity Immunology Group

Data Controller's Identity: Trinity College Dublin

Data Controller's Contact Details: 01-8961000

Data Processor's Identity: Dr Karen Conlan whelank3@tcd.ie

Data Protection Officer's Identity: John Eustace

Data Protection Officer's Contact Details: dataprotection@tcd.ie

You are being invited to take part in a research study to be carried out by the Autism and related Neurodevelopmental Disorders research group at Trinity College Dublin in collaboration with the Obesity Immunology Group in the National Children's Research Centre.

Before you decide whether or not you wish to take part, you should read the information provided below carefully and, if you wish, discuss it with your family, friends or GP (doctor). Take time to ask questions – don't feel rushed and don't feel under pressure to make a quick decision. You should clearly understand the risks and benefits of taking part in this study so that you can make a decision that is right for you. This process is known as 'Informed Consent'.

You don't have to take part in this study. If you decide not to take part, it won't affect your child's future care. You can change your mind about taking part in the study any time you like. Even if the study has started, you can still opt out. You don't have to give us a reason. If you do opt out, rest assured it won't affect the quality of treatment you get in the future.

Why is this study being done?

Treatments for child mental health disorders typically combine a range of approaches, including talking therapy, education about the condition and medication where required. Unfortunately, some medications, particularly antipsychotics used to treat mental health disorders in young people, are associated with significant side-effects,

including weight gain and obesity. Antipsychotic medications are effective in managing some of the more distressing symptoms of child mental health disorders, but some young people, commenced on these medications, will experience rapid weight gain and problems with the body's metabolism, which puts the child at increased risk for chronic diseases such as diabetes and heart disease. It is not possible currently for doctors to predict which of their patients will develop these side effects to antipsychotic medication. Currently the advice given is to monitor weight and diet and engage in exercise. However despite this advice, a significant proportion of patients gain weight rapidly, and develop abnormalities in blood cholesterol.

In this research, we have partnered with experts in obesity and inflammation to try to identify factors that increase risk of weight gain, obesity and metabolic changes in patients in response to antipsychotic medication. We will test if these factors help to predict which young people are most at risk. Ultimately, this will lead to better guidelines for doctors and patients to reduce the risks associated with these medications. Further studies could then be undertaken to find additional approaches to manage weight and obesity for young people on antipsychotic medications in the future.

Who is organising and funding this study?

Dr Karen Conlan, Senior Registrar in Child and Adolescent Psychiatry is carrying out this study as part of a PhD in Psychiatry. This is under the supervision of Prof Jane McGrath, Consultant Child and Adolescent Psychiatrist, Linn Dara CAMHS, Trinity College Dublin and Prof Louise Gallagher, Consultant Child and Adolescent Psychiatrist, Linn Dara CAMHS, Trinity College Dublin. Professor Donal O'Shea, Consultant Endocrinologist, St Vincent's University Hospital, University College Dublin; Dr Andrew Hogan, NCRC Fellow & Senior Scientist, Obesity Immunology Group, National University of Ireland Maynooth are collaborators on this study. The National Children's Research Centre in Crumlin has funded the study. This study has been approved by the St. Patrick's Mental Health Services, Linn Dara CAMHS, North Dublin City and County CAMHS, Tallaght University Hospital, Crumlin Hospital and Temple Street Children's Hospital Research Ethics Committees.

Why am I being asked to take part?

You are being contacted about the study because your child's doctor is planning on prescribing an antipsychotic medication for your child.

What will happen to me or my child if I agree to take part?

If you agree to take part in this research, we will agree a date that is suitable for you and your child to meet the researcher (Dr Conlan) in the hospital or CAMHS clinic that your child attends. They will collect some clinical information – height, weight and blood pressure and you will be asked to complete some questionnaires and rating scales. This first meeting will take less than 1 hour. These clinical measurements will be repeated at 3 months, 6 months and 12 months and Dr Conlan will arrange to do this in your child's CAMHS clinic at a time that is convenient for you.

Blood tests are recommended at baseline, 3 months, 6 months and 12 months for all patients on antipsychotic medication, and blood samples for this study will be collected during the routine blood testing procedures in Crumlin, Temple Street or Tallaght hospital that your clinician will be arranging. Dr Conlan will meet you and your child at the phlebotomy department and get the research blood samples when your child is getting their routine bloods done (so that no extra needle is required). Dr Conlan will then take the samples to the lab in the National Children's Research Centre in Crumlin where she works and will process the samples and store them in the National Children's Research Centre until analysis.

What are the benefits?

Your child will not receive direct benefit from participation in this research project. Through this research, we may learn more about the possible contributors of weight gain due to antipsychotic medications. This may be of benefit to people taking these medications in the future.

What are the risks?

Obtaining a blood sample will involve a blood test with a needle. Blood testing will be ordered by your child's clinician as part of routine clinical care before they start on the antipsychotic medication. It is important to note that this study does not involve an additional blood test as blood samples for the study will be taken during the same procedure arranged by your child's clinician. This is a low risk procedure with limited risks but may include:

- Excessive bleeding (more common in those with risk factors such as a history of coagulation difficulties or bleeding risk factors such as medication therapy which prolongs bleeding time)
- Dizziness or feeling light-headed
- Hematoma (blood accumulating under the skin)
- Infection (a slight risk any time the skin is broken)

Is the study confidential?

All data collected for this study will be treated as confidential according to the Trinity College Dublin and St Patrick's Mental Health Services policies, except in such cases required by law where information emerges that raises a concern for the safety or well-being of any individual.

Access to Medical Records

As part of our ongoing data collection, Dr Conlan or another doctor on the team may review your child's medical records to obtain information on blood results and/or clinical follow up. If you consent, you will allow Dr Conlan or a doctor on our research team to review your child's medical records in the strictest confidence to collect relevant information for the research study. The information will be kept strictly private and confidential. Protecting your privacy is extremely important to us and all the research team members at Trinity College Dublin. All the study information is assigned study ID numbers in a process called 'pseudonymisation'. This process is intended to mask your child's identity. Personal identifiers, such as your child's name are never used to label samples or clinical information. Only Dr Conlan has the necessary information to link your child's personal identifiers with the study ID number. Any information we have on file for your child is entirely confidential. All the details are stored in a locked research room. Only Dr Conlan has access to these details. No details that might identify your child are shared with the researchers outside our group in Trinity College Dublin.

What happens to my child's blood samples?

Blood samples for the research study are collected along with routine bloods when your child attends the phlebotomy department of the hospital for their blood tests that their doctor will organise. Dr Conlan will meet you and your child at the phlebotomy department and she will take the research blood samples directly to the lab in the National Children's Research centre in Crumlin Hospital where she will analyse them. The samples will be labelled with the unique study number and no identifying information will be sent with the samples. Sample analysis will be carried out in Dr

Hogan's lab (Obesity Immunology Group) in the National University of Ireland, Maynooth. The samples will be stored in the lab in the National Children's Research Centre and in the Obesity Immunology lab, National University of Ireland Maynooth.

What results will I get?

We are not planning to routinely feedback the results of the inflammatory marker testing. This is because we will be looking at all of the results of all of the participants together as the individual results will not give us much information. We are hoping to publish the results of the study in medical journals and to present the results at medical conferences. There will be no information capable of identifying your child in any publications or presentations.

Future Research Studies

As part of our ongoing investigations in Trinity College Dublin, we may approve and grant controlled data access to other groups outside of Trinity College Dublin including academic research groups and pharmaceutical/biotechnology research groups in order to conduct health-related research and to increase the chance of important discoveries. If we share data with other researchers we will only send them pseudonymised data that cannot be used to identify your child. Sometimes data from these types of studies are put into databases that researchers everywhere can get access to. They might want access to the information to use in studies that they are doing in their own universities. If we contribute your child's data to a public database it will also be pseudonymised. We will also request permission in advance from the ethics committee for public datasharing.

What do I do if I no longer want to be involved in research?

You and your child can leave the study at any time without giving any reason, and without penalty. If you wish for your child to leave the study, you may contact the Project Investigators, Prof Jane McGrath / Dr Karen Conlan or any member of the research team. We will take the necessary steps to remove your child's information from the database. It is important to know that declining or withdrawing participation will not impact on any service provision and if you decide that you would like your child to participate you can change your mind at any time. When your child reaches 18 years of age they will be contacted and further consent will be obtained for continued participation in this study and use of their pseudonymised data.

Data Protection

Under the new Data Protection Laws we need to let you know what happens to your child's data. We will be using your child's personal information in our research to help us study the effects of antipsychotic medications on inflammation and weight gain. The legal basis under which we are processing the data is exercise of official authority vested in Trinity College and for scientific research purposes. This is covered under Articles 6 and 9 of the General Data Protection Regulation 2016 (GDPR).

Only Dr Conlan and the named data processors and controllers in the research study will have access to your child's information. Data will be given a unique study number (coded) and this coded data will be stored on a secure password protected laptop that travels with the researcher (Dr Conlan) between sites. The key to identify the data will be stored separately in a locked research office in the Dept of Psychiatry in Trinity College. The coded data will be backed up in a password protected file accessed by Dr Conlan and on secure password protected servers in the Dept of Psychiatry, Trinity College Dublin. The data and information that comes from the biological materials will be kept for 5 years in a secure database to support continuing scientific research. This is important for research because as changes and advances emerge over time, the data we collect may gain new further significance. Biological samples will be destroyed by incineration (burning) 5 years after the research study is completed – unless the research team applies to and is given approval by the ethics board to continue with the research. The information that links your child's name with their Study Number is held securely in the National Children's Research Centre. These documents will be destroyed after the research study is completed.

If you agree to your child's data being contributed to a publicly available database but later decide to withdraw, we will take the necessary steps to have the data withdrawn. You should be aware however, that if some researchers have already applied for data that includes your child's information it may not be possible to withdraw this data from their studies that are already completed.

Your rights in relation to Data Protection

You have a number of rights in relation to your data and your child's data:

- The right to withdraw consent at any time;
- The right to lodge a complaint with the Data Protection Commission;
- The right to request access to your data and your child's data and to get a copy of it;
- The right to having any inaccurate information about you or your child deleted from the research database;

- The right to data portability meaning that you can move your data and your child's data from one data controller to another in a readable format (this means we would give you this data if you wanted someone else to be able to use it);
- The right to restrict to or object to processing of your data and your child's data.

Some of the clinical data we gather in the form of questionnaires will also be used by your clinical team to evaluate the effect of the medication on behaviour in order to see if it is having an impact while also looking at side effects experienced on the medication.

You have a right to object to this processing of data if you wish.

Where can I get further information?

If you have any further questions about the study or if you want to opt out of the study, you can rest assured it won't affect the quality of treatment you get in the future.

If you need any further information now or at any time in the future, please contact:

Dr Karen Conlan

Dept of Psychiatry, Trinity Centre for Health Sciences, St James's Hospital, Dublin 8.

01-8962144 (during office hours)

For further information on how personal data is processed as part of the study, please contact:

Data Protection Officer,Trinity College Dublin, Dublin 2, dataprotection@tcd.ie

Appendix 2 Parent Consent Form

Parent Consent Form

Study title: **Inflammatory markers and Antipsychotics study (InflamAP)**

I have read and understood the **Information Leaflet** about this research project. The information has been fully explained to me and I have been able to ask questions, all of which have been answered to my satisfaction.	**Yes** ☐	**No** ☐
I understand that my child does not have to take part in this study and that they can opt out at any time. I understand that my child does not have to give a reason for opting out and I understand that opting out will not affect their future medical care.	**Yes** ☐	**No** ☐
I understand that I can withdraw my child's biological material at any time without any negative repercussions.	**Yes** ☐	**No** ☐
I understand that my child's biological material will be disposed of in a lawful and respectful way.	**Yes** ☐	**No** ☐
I am aware of the potential risks, benefits and alternatives of this research study.	**Yes** ☐	**No** ☐
I give permission for researchers to look at my child's medical records to get information.	**Yes** ☐	**No** ☐
I have been given a copy of the Information Leaflet and this completed consent form for my records.	**Yes** ☐	**No** ☐
I have been assured that information about me and my child will be kept private and confidential. I understand that confidentiality may be breached in circumstances required by law where information emerges that raises a concern for the safety or well-being of any individual.	**Yes** ☐	**No** ☐
I consent for my child to take part in this research study having been fully informed of the risks, benefits and alternatives.	**Yes** ☐	**No** ☐
I give informed consent to have my child's data processed as part of this research study.	**Yes** ☐	**No** ☐
I understand that my child's data will be coded (with a study number) and stored on a secure password protected laptop that is held by the researcher and the key to link the data to my child will be stored in a locked research office.	**Yes** ☐	**No** ☐
I consent for my child to give blood samples for this research project. I understand that giving blood samples for this research is my decision and my child's decision.	**Yes** ☐	**No** ☐

Parent/Guardian Name (Block Capitals) | Parent/Guardian Signature | Date

STORAGE AND FUTURE USE OF INFORMATION		
STORAGE AND USE FOR FUTURE RESEARCH RELATED TO THE CURRENT STUDY		
I give permission for my child's biological material/data be stored for possible future research (that has been approved by a Research Ethics Committee) related to the current study only if consent is obtained at the time of the future research.	Yes ☐	No ☐
I give permission for my child's biological material/data to be stored for possible future research (that has been approved by a Research Ethics Committee) related to the current study without further consent being required.	Yes ☐	No ☐
STORAGE AND USE FOR FUTURE RESEARCH UNRELATED TO THE CURRENT STUDY		
I give permission for my child's biological material/data to be stored for possible future research (that has been approved by a Research Ethics Committee) unrelated to the current study only if consent is obtained at the time of the future research.	Yes ☐	No ☐
I give permission for my child's biological material/data to be stored for possible future research (that has been approved by a Research Ethics Committee) unrelated to the current study without further consent being required.	Yes ☐	No ☐
USE OF DATA RELATED TO BIOLOGICAL MATERIAL		
I request that my child's biological material be destroyed but give permission for data derived from my biological material to be stored for future research (that has been approved by a Research Ethics Committee) related to the current study only if consent is obtained at the time of the future research.	Yes ☐	No ☐
I request that my child's biological material be destroyed but I give permission for my data derived from my biological material to be stored for possible future research (that has been approved by a Research Ethics Committee) related to the current study without further consent being required.	Yes ☐	No ☐
I request that my child's biological material be destroyed but give permission for data derived from my child's biological material to be stored for future research (that has been approved by a Research Ethics Committee) unrelated to the current study only if consent is obtained at the time of the future research.	Yes ☐	No ☐
I request that my child's biological material be destroyed but give permission for data derived from my child's biological material to be stored for future research (that has been approved by a Research Ethics Committee) unrelated to the current study without further consent being required.	Yes ☐	No ☐

OTHER		
I request that all biological material/data collected can no longer be used by researchers and is destroyed.	Yes □	No □

Parent/Guardian Name (Block Capitals) | Parent/Guardian Signature | Date

To be completed by the Principal Investigator or nominee.

I, the undersigned, have taken the time to fully explain to the above patient/service user the nature and purpose of this study in a way that they could understand. I have explained the risks involved as well as the possible benefits. I have invited them to ask questions on any aspect of the study that concerned them.

Name (Block Capitals) | Qualifications | Signature | Date

Appendix 3 Child Information Leaflets broken down by age range

INFORMATION SHEET (< 8 years)

Title of Project:

Inflammatory markers of antipsychotic weight gain and cardiometabolic dysfunction in youth mental health disorders. (InflamAP Study)

We would like you to help us with our project. You can talk about it with your family until you are sure you want to help us. This sheet explains what we are trying to do and what you can do to help. You do not have to help and you can change your mind about this whenever you want.

What are you trying to do?

We are trying to understand why some people get side effects from the medicine your doctor thinks you need to take to help you feel better. Scientists can learn a lot about how people are different by looking at your blood in a special room called a laboratory. By looking at these things, scientists can learn new ways of helping people who might have problems on this medicine in the future.

What will happen if I help?

If you want to help us, we will talk with your parents and we will meet you in your doctor's clinic. During this visit we will:

1. Check some measurements like how tall you are and how much you weigh
2. Do a tracing of your heart with a special machine and stickers
3. Talk to your parents and get them to fill in some forms

This will all be done in one hour and then you can go home.

When you are on this medicine your doctor will need you to get **blood samples** taken in the hospital a few times to see how your body is getting on with the medicine. We will meet you in the hospital at these times and take some of the blood sample for the study. We will take your measurements again in the clinic too.

What will happen when I come in?

Blood Sample

The doctor might tie a strap onto the top of your arm so it is easier to get a sample of your blood. The doctor will clean your arm and use a needle to get the blood safely out. We will take small amounts of blood and put them into little glass tubes. The doctor will then clean your arm and put on a plaster. You will feel a "pinching" feeling during this and your arm might get a small bruise.

Will I get anything for taking part?

You will not get anything for taking part, but we will learn a lot from the blood you give us. This will really help our project. If you do not want to take part that is ok, you will still see your doctor in the clinic and get the medicine if you need it. If you decide to take part you can change your mind at any time.

Will anyone else know about this?

Only people working on the project will know that you helped our project. Some of these people work outside the hospital.

Contact:

If you have any questions, you can call or write to Karen Conlan on (01) 896 2144 / whelank3@tcd.ie

Research Team:

Dr. Karen Conlan, Clinical Research Fellow, Trinity College Dublin

Dr. Jane McGrath, Consultant Child and Adolescent Psychiatrist

Prof. Louise Gallagher, Professor and Consultant in Child and Adolescent Psychiatry, Trinity College Dublin

Dr. Andy Hogan, Senior Scientist, Obesity Immunology Group, National Children's Research Centre

TRINITY COLLEGE DUBLIN

INFORMATION SHEET (8 – 12 years)

Title of Project:

Inflammatory markers of antipsychotic weight gain and cardiometabolic dysfunction in youth mental health disorders. (InflamAP Study)

We would like you to help us with our project. You can talk about it with your family until you are sure you want to help us. This sheet explains what we are trying to do and what you can do to help. You do not have to help and you can change your mind about this whenever you want.

What are you trying to do?

We are trying to understand why some people get side effects from the medicine your doctor thinks you need to take to help you feel better. Scientists can learn a lot about how people are different by looking at your blood in a special room called a laboratory. By looking at these things, scientists can learn new ways of helping people who might have problems on this medicine in the future.

What will happen if I help?

If you want to help us, we will talk with your parents and we will meet you in your doctor's clinic. During this visit we will:

1. Check some measurements like how tall you are and how much you weigh
2. Do a tracing of your heart with a special machine and stickers
3. Talk to your parents and get them to fill in some forms

This will all be done in one hour and then you can go home.

When you are on this medicine your doctor will need you to get **blood samples** taken in the hospital a few times to see how your body is getting on with the medicine. We will meet you in the hospital at these times and take some of the blood sample for the study. We will take your measurements again in the clinic too.

What will happen when I come in?

Blood Sample

The doctor might tie a strap onto the top of your arm so it is easier to get a sample of your blood. The doctor will clean your arm and use a needle to get the blood safely out. We will take small amounts of blood and put them into little glass tubes. The doctor will then clean your arm and put on a plaster. You will feel a "pinching" feeling during this and your arm might get a small bruise.

Will I get anything for taking part?

You will not get anything for taking part, but we will learn a lot from the blood you give us. This will really help our project. If you do not want to take part that is ok, you will still see your doctor in the clinic and get the medicine if you need it. If you decide to take part you can change your mind at any time.

Will anyone else know about this?

Only people working on the project will know that you helped our project. Some of these people work outside the hospital.

Contact:

If you have any questions, you can call or write to Karen Conlan on (01) 896 2144 / whelank3@tcd.ie

Research Team:

Dr. Karen Conlan, Clinical Research Fellow, Trinity College Dublin

Dr. Jane McGrath, Consultant Child and Adolescent Psychiatrist

Prof. Louise Gallagher, Professor and Consultant in Child and Adolescent Psychiatry, Trinity College Dublin

Dr. Andy Hogan, Senior Scientist, Obesity Immunology Group, National Children's Research Centre

TRINITY COLLEGE DUBLIN

INFORMATION SHEET (12 – 14 years)

Title of Project:

Inflammatory markers of antipsychotic weight gain and cardiometabolic dysfunction in youth mental health disorders. (InflamAP Study)

We would like you to help us with our project. You can talk about it with your family until you are sure you want to help us. This sheet explains what we are trying to do and what you can do to help. You do not have to help and you can change your mind about this whenever you want.

What are you trying to do?

We are trying to understand why some people get side effects from the medicine your doctor thinks you need to take to help you feel better. Scientists can learn a lot about how people are different by looking at your blood in a special room called a laboratory. By looking at these things, scientists can learn new ways of helping people who might have problems on this medicine in the future.

What will happen if I help?

1. Check some measurements like how tall you are and how much you weigh
2. Do a tracing of your heart with a special machine and stickers
3. Talk to your parents and get them to fill in some forms

This will all be done in one hour and then you can go home.

When you are on this medicine your doctor will need you to get **blood samples** taken in the hospital a few times to see how your body is getting on with the medicine. We will meet you in the hospital at these times and take some of the blood sample for the study. We will take your measurements again in the clinic too.

What will happen when I come in?

Blood Sample

On your arm, the doctor will look for a **vein** (this moves blood around the body). The doctor might tie a strap onto the top of your arm so the vein is easy to find. The doctor will clean your arm and use a needle to get the blood safely out. We will take small amounts of blood and put them into little glass tubes. The doctor will then clean your arm and put on a plaster. You will feel a "pinching" feeling during this and your arm might get a small bruise.

Will I get anything for taking part?

You will not get anything for taking part, but we will learn a lot from the blood you give us. This will really help our project. If you do not want to take part that is ok, you will still see your doctor in the clinic and get the medicine if you need it. If you decide to take part you can change your mind at any time.

Will anyone else know about this?

Only scientists working on the project will have access to any information in our files – some of these people work outside of the hospital and your information will be shared with only them. We use a special system where we use numbers to identify people in the project. Your information will be completely secret.

Contact:

If you have any questions, you can call or write to Karen Conlan on (01) 896 2144 / whelank3@tcd.ie

Research Team:

Dr. Karen Conlan, Clinical Research Fellow, Trinity College Dublin

Dr. Jane McGrath, Consultant Child and Adolescent Psychiatrist

Prof. Louise Gallagher, Professor and Consultant in Child and Adolescent Psychiatry, Trinity College Dublin

Dr. Andy Hogan, Senior Scientist, Obesity Immunology Group, National Children's Research Centre

TRINITY COLLEGE DUBLIN

INFORMATION SHEET (14 – 16 years)

Title of Project:

Inflammatory markers of antipsychotic weight gain and cardiometabolic dysfunction in youth mental health disorders. (InflamAP Study)

We would like you to help us with our research project. You can talk about it with your family, friends or doctor until you are sure you want to help us. Please read this sheet carefully, as it explains what we are trying to do and what you can do to help. You do not have to help and you can change your mind about this whenever you want, at any point. Once you understand the benefits and risks of the study you can then make a decision if you want to take part – this is called **informed assent**.

What we are trying to do:

We are trying to understand why some people get side effects from the medicine your doctor thinks you need to take to help you feel better. Scientists can learn a lot about how people are different by looking at your blood in a special room called a laboratory. By looking at these things, scientists can learn new ways of helping people who might have problems on this medicine in the future.

Scientists can examine your blood when you are taking this new medicine to see if there are any changes in any of the cells over time.

What happens if you decide to take part:

If you want to help us, we will talk with your parents and we will meet you in your doctor's clinic. During this visit we will:

1. Check some measurements like how tall you are and how much you weigh
2. Do a tracing of your heart with a special machine and stickers
3. Talk to your parents and get them to fill in some forms

This will all be done in one hour and then you can go home.

When you are on this medicine your doctor will need you to get **blood samples** taken in the hospital a few times to see how your body is getting on with the medicine. We will meet you in the hospital at these times and

take some of the blood sample for the study. We will take your measurements again in the clinic too.

What happens when you come in:

Blood Sample

On your arm, the doctor will look for a **vein** (this moves blood around the body). The doctor might tie a strap onto the top of your arm so the vein is easy to find. The doctor will clean your arm and use a needle to get the blood safely out. We will take small amounts of blood and put them into little glass tubes. The doctor will then clean your arm and put on a plaster. You will feel a "pinching" feeling during this and your arm might get a small bruise.

What are the benefits of you taking part?

You will not get anything for taking part, but we will learn a lot from the blood you give us. This will really help our project and help us to understand more about how the medicine effects young people who need to take it. If you do not want to take part that is ok, you will still see your doctor in the clinic and get the medicine if you need it. If you decide to take part you can change your mind at any time.

Will anyone else know about you taking part?

Only scientists working on the project will have access to any information in our files – some of these people work outside of the hospital and your information will be shared with only them. We use a special system where we use numbers to identify people in the project. Your information will be completely confidential.

Contact: If you have any queries or concerns that you wish to discuss please do not hesitate to contact Karen Conlan on (01) 896 2144 / whelank3@tcd.ie

Research Team:

Dr. Karen Conlan, Clinical Research Fellow, Trinity College Dublin

Dr. Jane McGrath, Consultant Child and Adolescent Psychiatrist

Prof. Louise Gallagher, Professor and Consultant in Child and Adolescent Psychiatry, Trinity College Dublin

Dr. Andy Hogan, Senior Scientist, Obesity Immunology Group, National Children's Research Centre

TRINITY COLLEGE DUBLIN

INFORMATION SHEET (> 16 years)

Title of Project:

Inflammatory markers of antipsychotic weight gain and cardiometabolic dysfunction in youth mental health disorders. (InflamAP Study)

We would like you to help us with our research project. You can talk about it with your family, friends or doctor until you are sure you want to help us. Please read this sheet carefully, as it explains what we are trying to do and what you can do to help. You do not have to help and you can change your mind about this whenever you want, at any point. Once you understand the benefits and risks of the study you can then make a decision if you want to take part – this is called **informed consent**.

What we are trying to do:

We are trying to understand why some people get side effects from the medicine your doctor thinks you need to take to help with the unpleasant symptoms you have been experiencing. Scientists can learn a lot about how people are different by looking at your blood in the laboratory. By looking at these things, scientists can learn new ways of helping people who might have problems on this medicine in the future. Scientists can examine your blood when you are taking this new medicine to see if there are any changes in any of the cells over time.

What happens if you decide to take part:

If you want to help us, we will meet you in your doctor's clinic and talk to you and your parents about it. During this visit we will:

1. Check some measurements such as your weight and height
2. Do a tracing of your heart with a special machine called an ECG machine
3. Talk to you and your parents and get you to fill in some forms

This will all be done in one hour and then you can go home.

As part of the treatment when you are starting this new medicine, your doctor will be sending you for some blood tests (this is called a **blood sample**).

When you are on this medicine your doctor will need you to get blood samples done after 3 months and 6 months, and then again after 12 months to see how your body is getting on with the new medicine. If you take part in the research we will meet you again at these times and get the blood samples like before and we will take your measurements again.

What happens when you come in:

Blood Sample

On your arm, the doctor will look for a **vein** (this moves blood around the body). The doctor might tie a strap onto the top of your arm so the vein is easy to find. The doctor will clean your arm and use a needle to get the blood safely out. We will take small amounts of blood and put them into little glass tubes. The doctor will then clean your arm and put on a plaster. You will feel a "pinching" feeling during this and your arm might get a small bruise.

If you decide to help us with the research then we will get the nurse to take some extra blood samples at the same time so that you won't have to get an extra needle in your arm.

What are the benefits of you taking part?

You will not get anything for taking part, but we will learn a lot from the blood you give us. This will really help our project and help us to understand why some people might get side effects on the medicine. If we know more about this then doctors might be able to help stop these side effects from happening in the future. If you do not want to take part that is ok, you will still see your doctor in the clinic and get the medicine if you need it. If you decide to take part you can change your mind and leave the study at any time.

Will anyone else know about you taking part?

Only scientists working on the project will have access to any information in our files – some of these people work outside of the hospital and your information will be shared with only them. We use a special system where we use numbers to identify people in the project. Your information will be completely confidential. Information associated with the study might be presented at research conferences or published in scientific journals in the future, but your identity will always be protected. The information you give us will be stored for a minimum of 5 years after our project finishes. If you would like this destroyed at any time, you can ask a parent to contact us about this, or you may contact us yourself.

Contact:

If you have any queries or concerns that you wish to discuss please do not hesitate to contact Karen Conlan on (01) 896 2144 / whelank3@tcd.ie

Research Team:

Dr. Karen Conlan, Clinical Research Fellow, Trinity College Dublin

Dr. Jane McGrath, Consultant Child and Adolescent Psychiatrist

Prof. Louise Gallagher, Professor and Consultant in Child and Adolescent Psychiatry, Trinity College Dublin

Dr. Andy Hogan, Senior Scientist, Obesity Immunology Group, National Children's Research Centre

Appendix 4 Child Assent Form

Inflammatory markers and Antipsychotics study - Child Assent Form

Please sign your names below to show that you would like to take part in this project and that you understand what that means.

- I have read (or been read to) the information about the Inflammatory markers and Antipsychotics study project and understand what I have read.
-
- I agree to take part in the project. I know that I don't have to if I don't want to.

- I know that the researchers at Trinity will not tell anyone my name or where I live.

- I know that if the researchers find out something that affects my health they may need to tell my parents and I might need to have some tests done with the doctor.

- I know that I can leave the study at any time. I can tell my parents to tell the researchers and I will be taken out of the study.

- I know that this study will include getting and using blood samples for a study.

- I know that I will not receive any money or benefits from this study, and others might.

- I know that scientists are going to research my blood samples, and this may be in a university, in a company, here in Ireland, in Europe and outside of Europe.

Child's Name: _____

Signed: _____ **Date:** _____

Parent's Name: _____

Signed: _____ **Date:** _____

Researcher's Name: _____

Signed: _____ **Date:** _____

Appendix 5 Child Information Leaflet and Assent Form (picture based)

Picture Based Information Sheet and Consent Form

Hello! My name is Karen ☺

I work in a research group in Trinity College. We are doing a research study. We are looking for young people who are going to be taking the medicine that your doctor has recommended you take.

This study is being funded by the National Children's Research Centre in Crumlin Hospital

	I would like to meet you in the clinic and check to see how tall you are and how much you are growing.
	I would like also like to check your blood pressure and see how healthy you are.
	I would like to take a blood sample from you so I can learn about how the medicine in working in your body.
	At the end of this research, I will write a report. We may use some of your blood results in the report. We will not use your name in the report.

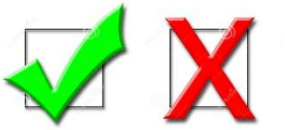
	Information you give us during the research is called data. All data must be kept safely and in line with the law. Trinity College Dublin is responsible for overseeing the collection, studying and storing the data.

	The people taking part in this research will be given a code number. This is so we can avoid using their names. The codes will be used by the research team to manage the data.

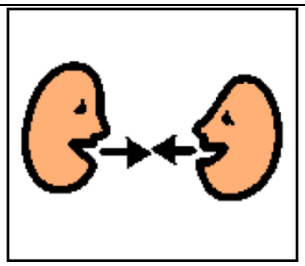
	The people taking part can ask to see their data. They can ask for their data to be removed or can stop the research team from studying it. When the final report is published, all personal data will be destroyed. This means all names will be taken off the data.

	My research group can use this information to see if we can find out about how these medicines affect the body.

	Would you like to take part in this study?

	You can say yes or no. It's ok to say no. It is your choice.

	If you say no that is no problem at all. If you want to leave the study that is ok too. You can change your mind at any time.

	Do you want to take part in this study?

YES ☐ ✓ NO ☐

✗

Signed: _____ Date: _____

Appendix 6 Study Assessment Form

InflamAP Clinical Assessment Form (T0) - Baseline

Date:

Study ID number:

Age:

Name of antipsychotic: Dose:

Diagnosis (if any):

Reason for starting antipsychotic:

Previous antipsychotic (if any) & dose: when stopped:

Current health status:

Recent infections/ antibiotic courses:

Past medical history/ chronic illnesses:

Allergies:

Neonatal period- gestation, birth weight:

Family history (if significant):

Pubertal status (if male) – fill in pubertal stage questionnaire to determine:

Menstrual cycle (if female) - fill in pubertal stage questionnaire to determine:

CHECKLIST FOR ASSESSMENT OF CHILD OR ADOLESCENT

Consent Forms x3 ☐

(Parent, Medical Chart, Folder)

Assent Form x1 ☐

Measurements

Weight (kg): - also plot on centile chart

Height (cm): - also plot on centile chart

BMI (kg/m2): - also plot on centile chart

Height velocity:

Waist circumference:

Pubertal stage:

Blood Pressure:

Heart Rate:

ECG ☐

Clinical and Metabolic Bloods ☐

Research Blood ☐

InflamAP Clinical Assessment Form (T1) – 3 months

Date:

Study ID number:

Age:

Name of antipsychotic: Dose:

Diagnosis (if any):

Reason for starting antipsychotic:

Current health status:

Recent infections/ antibiotic courses:

CHECKLIST FOR ASSESSMENT OF CHILD OR ADOLESCENT

Measurements

Weight (kg): - also plot on centile chart

Height (cm): - also plot on centile chart

BMI (kg/m2): - also plot on centile chart

Height velocity:

Waist circumference:

Pubertal stage:

Blood Pressure:

Heart Rate:

ECG ☐

Clinical and Metabolic Bloods ☐

Research Blood ☐

InflamAP Clinical Assessment Form (T2) - 6 months

Date:

Study ID number:

Age:

Name of antipsychotic: Dose:

Diagnosis (if any):

Reason for starting antipsychotic:

Current health status:

Recent infections/ antibiotic courses:

CHECKLIST FOR ASSESSMENT OF CHILD OR ADOLESCENT

Measurements

Weight (kg) : - also plot on centile chart

Height (cm): - also plot on centile chart

BMI (kg/m2): - also plot on centile chart

Height velocity:

Waist circumference:

Pubertal stage:

Blood Pressure:

Heart Rate:

ECG ☐

Clinical and Metabolic Bloods ☐

Research Blood ☐

InflamAP Clinical Assessment Form (T3) – 12 months

Date:

Study ID number:

Age:

Name of antipsychotic: Dose:

Diagnosis (if any):

Reason for starting antipsychotic:

Current health status:

Recent infections/ antibiotic courses:

CHECKLIST FOR ASSESSMENT OF CHILD OR ADOLESCENT

Measurements

Weight (kg): - also plot in centile chart

Height (cm): - also plot in centile chart

BMI (kg/m2): - also plot in centile chart

Height velocity:

Waist circumference:

Pubertal stage:

Blood Pressure :

Heart Rate:

ECG ☐

Clinical and Metabolic Bloods ☐

Research Blood ☐