Dietary titanium dioxide particles and intestinal health

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Abstract

The purpose of this dissertation was to investigate the relationship between food-grade titanium dioxide particles and intestinal health, in particular the development of Crohn’s disease after uptake of titanium dioxide particles in intestinal lymphoid tissues.

Crohn’s disease is a common form of inflammatory bowel disease. It is characterised by chronic inflammation of the gastrointestinal tract and affects approximately 1 in 1,000 people. The aetiology of Crohn’s disease is unclear, but both genetic and environmental factors are involved in the development of the disease.

The gene that is most commonly associated with Crohn’s disease is the nucleotide-binding oligomerisation domain (NOD) 2 gene. The diet is one of the most likely environmental factors that have been proposed to play a role in Crohn’s disease. It has been hypothesised that uptake of titanium dioxide particles, which are used as a whitening agent in processed foods, toothpaste, and pharmaceuticals, by macrophages in intestinal lymphoid tissues negatively affects intestinal health and contributes to the development of Crohn’s disease.

To investigate this hypothesis, immune cell-stimulating properties of titanium dioxide were first assessed in vitro with macrophages derived from wild-type mice and mice with a Crohn’s disease-like Nod2 gene variant. These mouse models were also used to determine particle uptake in intestinal lymphoid tissues in vivo after exposure to titanium dioxide with the diet and effects of this dietary exposure on intestinal health and urine metabolites.

The results from the in vitro studies showed that titanium dioxide induced the release of the pro-inflammatory cytokine interleukin-1β. For the first time, it has been shown that accumulation of particles in intestinal lymphoid tissues was a consequence of titanium dioxide intake with the diet. However, this had no negative effects on growth performance and intestinal health of both wild-type mice and mice with a Crohn’s disease-like Nod2 gene variant. Nevertheless, differences in urine metabolite profiles between wild-type mice exposed to titanium dioxide and unexposed wild-type mice indicated that consumption of a titanium dioxide-containing diet affected the metabolism.

This dissertation forms the foundation for future studies with animal models about the relationship between titanium dioxide and intestinal health.
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All animal experiments that were carried out during the course of this project were in compliance with the New Zealand Animal Welfare Act 1999 and were approved by the Grasslands Ethics Committee (Palmerston North, NZ).
Table of contents

Abstract ................................................................................................................................. iii
Acknowledgements ................................................................................................................ v
Table of contents .................................................................................................................. ix
List of figures ......................................................................................................................... xvii
List of tables ......................................................................................................................... xxiii
List of appendices ................................................................................................................. xxvii
List of abbreviations ............................................................................................................. xxix
Introduction ......................................................................................................................... 1

Chapter 1  Literature review ............................................................................................ 5

1.1 The gastrointestinal tract and Crohn’s disease .............................................................. 6
  1.1.1 Structure and function of the mammalian gastrointestinal tract ......................... 6
  1.1.2 Crohn’s disease as a form of inflammatory bowel disease ................................ 11
1.2 The role of the microbial pattern recognition receptor NOD2 in Crohn’s disease .... 14
  1.2.1 Genetic susceptibility to Crohn’s disease ........................................................... 14
  1.2.2 Structure and function of NOD2 ................................................................. 16
  1.2.3 Mouse models with Nod2 gene modifications .................................................. 17
1.3 Dietary particles in the gastrointestinal tract .............................................................. 19
  1.3.1 Endogenous dietary particles ................................................................. 19
  1.3.2 Exogenous dietary particles ....................................................................... 21
  1.3.3 Particle uptake across the intestinal epithelium ............................................. 28
1.4 Effects of titanium dioxide on cultured cells, animal models, and humans ........... 30
  1.4.1 Effects of titanium dioxide on intestinal and macrophage-like cell lines ......... 31
  1.4.2 Effects of titanium dioxide on animal models after gastrointestinal exposure ... 41
  1.4.3 Effects of titanium dioxide and bacterial lipopolysaccharide co-stimulation on cultured human cells ................................................................................. 47
  1.4.4 Effects of titanium dioxide on humans .......................................................... 52
1.5 Concluding remarks........................................................................................................... 54
  1.5.1 Hypotheses concerning dietary particles ................................................................. 54
  1.5.2 Hypothesis, aims, and structure of this dissertation .............................................. 56

Chapter 2 Exposure of cultured immune cells to titanium dioxide with or
without co-stimulation with bacterial antigens ................................................................. 61
  2.1 Introduction...................................................................................................................... 62
  2.2 Hypothesis and aims..................................................................................................... 63
  2.3 Materials and methods ............................................................................................... 64
    2.3.1 Titanium dioxide particles..................................................................................... 64
    2.3.2 Animals................................................................................................................... 65
    2.3.3 Culture of murine bone marrow-derived macrophages ....................................... 66
    2.3.4 Exposure of bone marrow-derived macrophages to titanium dioxide with or
        without bacterial antigens ...................................................................................... 67
    2.3.5 Metabolic activity analysis .................................................................................. 67
    2.3.6 Flow cytometry analysis ...................................................................................... 68
    2.3.7 Exposure of human peripheral blood mononuclear cells to titanium dioxide
        with or without bacterial antigens ........................................................................... 72
    2.3.8 Cytokine detection in cell culture supernatants .................................................. 73
    2.3.9 Statistical analysis ............................................................................................... 74
  2.4 Results........................................................................................................................... 75
    2.4.1 Characterisation of food-grade titanium dioxide particles .................................... 75
    2.4.2 Cytotoxicity of titanium dioxide ......................................................................... 75
    2.4.3 Phenotype and morphology of bone marrow-derived macrophages after
        exposure to titanium dioxide ................................................................................ 79
    2.4.4 Activation of bone marrow-derived macrophages after exposure to titanium
        dioxide ................................................................................................................. 86
    2.4.5 Cytokine secretion by bone marrow-derived macrophages after exposure to
        titanium dioxide .................................................................................................. 92
2.4.6 Cytokine secretion by human peripheral blood mononuclear cells after exposure to titanium dioxide

2.5 Discussion

2.5.1 Reassessment of the hypothesis

2.5.2 Suitability of bone marrow-derived macrophages as a model for intestinal macrophages

2.5.3 Cytotoxicity of titanium dioxide

2.5.4 Uptake of titanium dioxide by cultured cells

2.5.5 Activation marker expression after exposure to titanium dioxide

2.5.6 Pro-inflammatory cytokine secretion after exposure to titanium dioxide

2.5.7 Conclusion

Chapter 3 Exposure of wild-type mice to dietary titanium dioxide

3.1 Introduction

3.2 Hypothesis and aims

3.3 Materials and methods

3.3.1 Study considerations

3.3.2 Preparation of titanium dioxide-containing mouse diets

3.3.3 Animals and experimental design

3.3.4 Tissue collection

3.3.5 Flow cytometry analysis

3.3.6 Sample preparation for microscopy

3.3.7 Dark field microscopy

3.3.8 Haematoxylin and eosin staining and bright field microscopy

3.3.9 Reflectance confocal microscopy and image analysis

3.3.10 Immunofluorescence staining and confocal microscopy

3.3.11 Statistical analysis

3.4 Results

3.4.1 Detection of titanium dioxide particles in the diet
3.4.2 Performance and titanium dioxide intake.................................................. 132
3.4.3 Effects of dietary titanium dioxide exposure on immune cell populations of intestinal lymphoid tissues................................................................. 136
3.4.4 Observation of titanium dioxide particle uptake in intestinal lymphoid tissues with dark field microscopy ............................................................. 150
3.4.5 Observation of titanium dioxide particle uptake in Peyer’s patches with bright field microscopy ................................................................. 150
3.4.6 Assessment of titanium dioxide particle uptake in Peyer’s patches with reflectance confocal microscopy ............................................................ 150
3.4.7 Titanium dioxide particle uptake by Peyer’s patch dendritic cells .......... 156
3.5 Discussion.................................................................................................... 156
  3.5.1 Reassessment of the hypothesis............................................................. 156
  3.5.2 Titanium dioxide incorporation into the diet........................................ 162
  3.5.3 Effects of oral exposure to titanium dioxide on body weights .......... 162
  3.5.4 Daily titanium dioxide intake in this study .......................................... 163
  3.5.5 Immune cell populations of murine Peyer’s patches ......................... 164
  3.5.6 Titanium dioxide particle uptake in Peyer’s patches ......................... 165
  3.5.7 Conclusion............................................................................................ 167

Chapter 4  Exposure of wild-type mice and mice with a Crohn’s disease-like Nod2 gene variant to dietary titanium dioxide ......................................................... 171
  4.1 Introduction............................................................................................... 172
  4.2 Hypothesis and aims............................................................................... 173
  4.3 Materials and methods ........................................................................... 173
    4.3.1 Study considerations........................................................................... 173
    4.3.2 Experimental design, diets, and animals .......................................... 173
    4.3.3 Tissue collection and sample preparation .......................................... 175
    4.3.4 Histology of ileum and colon.............................................................. 175
    4.3.5 Dark field and reflectance confocal microscopy ................................. 176
Chapter 4  Effects of dietary titanium dioxide on body weight and intestinal characteristics of wild-type mice and mice with a Crohn’s disease-like Nod2 gene variant on a standard diet

4.3.6 Flow cytometry analysis

4.3.7 Statistical analysis

4.4 Results

4.4.1 Body weight and intestinal characteristics of wild-type mice and mice with a Crohn’s disease-like Nod2 gene variant on a standard diet

4.4.2 Performance and titanium dioxide intake

4.4.3 Bright-field microscopy and histology of ileum and colon cross-sections

4.4.4 Assessment of titanium dioxide particle uptake in Peyer’s patches and the ileal mucosa

4.4.5 Monocyte populations of Peyer’s patches from wild-type mice and mice with a Crohn’s disease-like Nod2 gene variant on a standard diet

4.5 Discussion

4.5.1 Reassessment of the hypothesis

4.5.2 Considerations about the performance of mice with a Crohn’s disease-like Nod2 gene variant

4.5.3 Titanium dioxide particle uptake in Peyer’s patches of mice with a Crohn’s disease-like Nod2 gene variant

4.5.4 Titanium dioxide particle uptake across the intestinal epithelium

4.5.5 Conclusion

Chapter 5  Effects of dietary titanium dioxide on urine metabolite profiles of wild-type mice and mice with a Crohn’s disease-like Nod2 gene variant

5.1 Introduction

5.2 Hypothesis and aims

5.3 Materials and methods

5.3.1 Metabolomics analysis workflow

5.3.2 Animals and urine collection

5.3.3 Sample preparation

5.3.4 Liquid chromatography mass spectrometry analysis

5.3.5 Data analysis
List of figures

Figure 1.1 Schematic overview of the structure of the gastrointestinal tract. .............................. 7
Figure 1.2 Schematic overview of the small intestine including lymphoid tissues. ................. 10
Figure 1.3 Overview of the structure of this dissertation. ............................................................. 59
Figure 2.1 Gating strategy for flow cytometry analysis of TiO$_2$-exposed BMDMs. ............... 71
Figure 2.2 TiO$_2$ particle characterisation .................................................................................... 76
Figure 2.3 Metabolic activity of BMDMs after TiO$_2$ exposure with or without MDP/PGN co-stimulation. .................................................................................................................. 77
Figure 2.4 Viability of BMDMs after TiO$_2$ exposure with or without MDP/PGN co-stimulation. ........................................................................................................................................... 81
Figure 2.5 F4/80 expression of BMDMs after TiO$_2$ exposure with or without MDP/PGN co-stimulation. .................................................................................................................. 82
Figure 2.6 FSC versus SSC dot plots of TiO$_2$-exposed BMDMs ...................................................... 83
Figure 2.7 FSC intensity of BMDMs after TiO$_2$ exposure with or without MDP/PGN co-stimulation. ........................................................................................................................................... 85
Figure 2.8 SSC intensity of BMDMs after TiO$_2$ exposure with or without MDP/PGN co-stimulation. ........................................................................................................................................... 87
Figure 2.9 Relative SSC increase of TiO$_2$-exposed BMDMs compared to unstimulated controls with or without MDP/PGN co-stimulation. .............................................................................. 88
Figure 2.10 CD80 expression of BMDMs after TiO$_2$ exposure with or without MDP/PGN co-stimulation. .................................................................................................................. 90
Figure 2.11 CD86 expression of BMDMs after TiO$_2$ exposure with or without MDP/PGN co-stimulation. .................................................................................................................. 91
Figure 2.12 TNF-$\alpha$ secretion by BMDMs after TiO$_2$ exposure with or without MDP/PGN co-stimulation. .................................................................................................................. 94
Figure 2.13 IL-1$\beta$ secretion by BMDMs after TiO$_2$ exposure with or without MDP/PGN co-stimulation. .................................................................................................................. 95
Figure 2.14 IL-1$\beta$ secretion by PBMCs after TiO$_2$ exposure with or without MDP/PGN co-stimulation. .................................................................................................................. 97
Figure 2.15 IL-17 secretion by PBMCs after TiO$_2$ exposure with or without MDP/PGN co-stimulation.

Figure 3.1 Gating strategy to identify lymphocyte subsets in PPs and MLNs with flow cytometry.

Figure 3.2 Gating strategy to identify monocyte subsets and DC subpopulations in PPs and MLNs with flow cytometry.

Figure 3.3 TiO$_2$ particles in AIN-76A diet containing 625 mg TiO$_2$/kg.

Figure 3.4 Body weights of WT mice fed a diet with or without TiO$_2$ for 6 weeks.

Figure 3.5 Body weights of WT mice fed a diet with or without TiO$_2$ for 12 weeks.

Figure 3.6 Body weights of WT mice fed a diet with or without TiO$_2$ for 18 weeks.

Figure 3.7 Frequencies of lymphocytes and monocytes in PPs and MLNs from WT mice fed a diet with or without TiO$_2$.

Figure 3.8 Frequencies of lymphocyte populations in PPs and MLNs from WT mice fed a diet with or without TiO$_2$.

Figure 3.9 Frequencies of monocyte populations in PPs and MLNs from WT mice fed a diet with or without TiO$_2$.

Figure 3.10 Frequencies of DC subsets in PPs and MLNs from WT mice fed a diet with or without TiO$_2$.

Figure 3.11 Dark field microscopy images of PP cross-sections from WT mice fed a diet with or without TiO$_2$.

Figure 3.12 Dark field microscopy images of MLN cross-sections from WT mice fed a diet with or without TiO$_2$.

Figure 3.13 Bright field microscopy images of PP cross-sections from WT mice fed a diet with or without TiO$_2$.

Figure 3.14 Reflectance confocal microscopy images of PP cross-sections from WT mice fed a diet with or without TiO$_2$.

Figure 3.15 Reflectance confocal microscopy images of the SED area from a WT mouse fed a diet with TiO$_2$.

Figure 3.16 TiO$_2$ particle uptake in SED areas from WT mice fed a diet with or without TiO$_2$. 
Figure 3.17 Sizes of SED areas used for TiO$_2$ particle uptake assessment from WT mice fed a diet with or without TiO$_2$. ................................................................. 159

Figure 3.18 Immunofluorescence confocal microscopy image of a PP cross-section from a WT mouse fed a diet with TiO$_2$. ............................................................................. 160

Figure 3.19 Immunofluorescence confocal microscopy image of the SED area from a mouse fed a diet with TiO$_2$. ............................................................................. 161

Figure 4.1 Body weights and numbers of PPs of WT and $Nod2^{m/m}$ mice fed a standard rodent diet. ........................................................................................................... 179

Figure 4.2 Small intestine and colon lengths of WT and $Nod2^{m/m}$ mice fed a standard rodent diet. ........................................................................................................... 180

Figure 4.3 Body weights and weight changes relative to initial body weight of WT and $Nod2^{m/m}$ mice fed a diet with or without TiO$_2$ for 18 weeks. ......................................................... 183

Figure 4.4 Numbers of PPs and small intestine and colon lengths of WT and $Nod2^{m/m}$ mice fed a diet with or without TiO$_2$ for 18 weeks. ......................................................... 186

Figure 4.5 Bright field microscopy images of ileum cross-sections from WT and $Nod2^{m/m}$ mice fed a diet with or without TiO$_2$ for 18 weeks. ......................................................... 188

Figure 4.6 Bright field microscopy images of colon cross-sections from WT and $Nod2^{m/m}$ mice fed a diet with or without TiO$_2$ for 18 weeks. ......................................................... 189

Figure 4.7 Ileum and colon histology scores of WT and $Nod2^{m/m}$ mice fed a diet with or without TiO$_2$ for 18 weeks. .......................................................................................... 190

Figure 4.8 Lengths of crypts and villi in the ileum from WT and $Nod2^{m/m}$ mice fed a diet with or without TiO$_2$ for 18 weeks. .......................................................................................... 191

Figure 4.9 Lengths of crypts in the colon from WT and $Nod2^{m/m}$ mice fed a diet with or without TiO$_2$ for 18 weeks. .......................................................................................... 192

Figure 4.10 Dark field microscopy images of PP cross-sections from WT and $Nod2^{m/m}$ mice fed a diet with or without TiO$_2$ for 18 weeks. ......................................................... 193

Figure 4.11 TiO$_2$ particle uptake in SED areas from WT and $Nod2^{m/m}$ mice fed a diet with or without TiO$_2$ for 18 weeks. .......................................................................................... 194

Figure 4.12 Sizes of SED areas used for TiO$_2$ particle uptake assessment from WT and $Nod2^{m/m}$ mice fed a diet with or without TiO$_2$ for 18 weeks. ......................................................... 195
Figure 4.13 Dark field microscopy images of ileum cross-sections from WT and Nod2<sup>m/m</sup> mice fed a diet with or without TiO<sub>2</sub> for 18 weeks................................................................. 196

Figure 4.14 Frequencies of monocytes and monocyte populations in PPs from WT and Nod2<sup>m/m</sup> mice on a standard rodent diet. .......................................................... 198

Figure 4.15 Frequencies of DC subsets in PPs from WT and Nod2<sup>m/m</sup> mice on a standard rodent diet.......................................................... 199

Figure 5.1 Overview of the workflow for metabolomics studies.............................. 211

Figure 5.2 PLS-DA plots of negative and positive ion profiles detected in urine samples from WT mice fed a diet with or without TiO<sub>2</sub> analysed according to sex. ......................... 219

Figure 5.3 PLS-DA plots of negative and positive ion profiles detected in urine samples from female WT mice fed a diet with or without TiO<sub>2</sub> analysed according to urine collection time point.......................................................... 220

Figure 5.4 PLS-DA plots of negative and positive ion profiles detected in urine samples from WT and Nod2<sup>m/m</sup> mice fed a diet with or without TiO<sub>2</sub> analysed according to Nod2 genotype........................................................................ 223

Figure 5.5 PLS-DA plots of negative and positive ion profiles detected in urine samples from WT and Nod2<sup>m/m</sup> mice fed a diet with or without TiO<sub>2</sub> analysed according to urine collection time point........................................................................ 224

Figure 5.6 PLS-DA plot of positive ion profiles detected in urine samples from WT and Nod2<sup>m/m</sup> mice fed a diet with or without TiO<sub>2</sub> analysed according to diet. ......................... 225

Figure 5.7 PLS-DA plots of negative and positive ion profiles detected in urine samples from WT and Nod2<sup>m/m</sup> mice fed a diet with or without TiO<sub>2</sub> for 18 weeks analysed according to Nod2 genotype and diet. ........................................................................ 226

Figure 5.8 Overview of selected tryptophan catabolism pathways.......................... 233

Figure 5.9 Comparisons of the levels for the negative ion m/z 204.0662 and the positive ion m/z 206.0811 detected in urine samples from WT and Nod2<sup>m/m</sup> mice fed a diet with or without TiO<sub>2</sub>.......................................................... 235

Figure 5.10 Comparisons of the levels for the negative ion m/z 174.0554 and the positive ion m/z 176.0705 detected in urine samples from WT and Nod2<sup>m/m</sup> mice fed a diet with or without TiO<sub>2</sub>.......................................................... 236
Figure 5.11 Comparisons of the levels for the negative ions $m/\tilde{z}$ 204.0297 and $m/\tilde{z}$ 160.0408 and the positive ion $m/\tilde{z}$ 206.0447 detected in urine samples from WT and Nod2$^{+/+}$ mice fed a diet with or without TiO$_2$. ..............................................................238
List of tables

Table 1.1 Confectionery and other foodstuffs with TiO$_2$ available in New Zealand supermarkets. ........................................................................................................................................... 24
Table 1.2 Mayonnaises and white dressings with (highlighted in red) or without TiO$_2$ available in New Zealand supermarkets. ........................................................................................................................................... 25
Table 1.3 Toothpastes with (highlighted in red) or without TiO$_2$ available in New Zealand supermarkets. ........................................................................................................................................... 27
Table 1.4 Summary of studies that investigated effects of TiO$_2$ particles on cultured human cell lines. ........................................................................................................................................... 32
Table 1.5 Summary of studies that investigated effects of TiO$_2$ particles on cultured murine cell lines. ........................................................................................................................................... 35
Table 1.6 Studies that investigated TiO$_2$ particle exposure on intestinal and phagocytic cell lines and their reported effects. ........................................................................................................................................... 40
Table 1.7 Summary of studies that investigated effects of TiO$_2$ particles on animals. ....... 42
Table 1.8 Summary of studies that investigated effects of TiO$_2$ particles with LPS co-stimulation on cultured human cells. ........................................................................................................................................... 48
Table 2.1 Two-way ANOVA results for metabolic activity comparison of murine BMDMs exposed to TiO$_2$ with or without MDP/PGN co-stimulation. ................................................................. 78
Table 2.2 Two-way ANOVA results for viability and F4/80 expression comparison of murine BMDMs exposed to TiO$_2$ with or without MDP/PGN co-stimulation. ................................. 80
Table 2.3 Two-way ANOVA results for FSC and SSC intensities and relative SSC increase comparison of murine BMDMs exposed to TiO$_2$ with or without MDP/PGN co-stimulation. ........................................................................................................................................... 84
Table 2.4 Two-way ANOVA results for CD80 and CD86 expression comparison of murine BMDMs exposed to TiO$_2$ with or without MDP/PGN co-stimulation. ................................. 89
Table 2.5 Two-way ANOVA results for IL-1$\beta$ and TNF-$\alpha$ secretion comparison of murine BMDMs exposed to TiO$_2$ with or without MDP/PGN co-stimulation. ................................. 93
Table 3.1 Diet compositions according to the manufacturer. ................................................. 120
Table 3.2 Means ($\pm$ SD) for age, initial and final body weights, daily weight gain, and daily food intake of female WT mice fed a diet with or without TiO$_2$. ........................................ 134
Table 3.3 Means (± SD) for age, initial and final body weight, daily weight gain, and daily food intake of male WT mice fed a diet with or without TiO$_2$. .............................................. 135

Table 3.4 Means (± SD) for daily TiO$_2$ intake and daily TiO$_2$ dose of female WT mice fed a diet with TiO$_2$. ......................................................................................................................... 140

Table 3.5 Means (± SD) for daily TiO$_2$ intake and daily TiO$_2$ dose of male WT mice fed a diet with TiO$_2$. ......................................................................................................................... 141

Table 3.6 Two-way ANOVA results for comparisons of immune cell population frequencies in PPs and MLNs of WT mice fed a diet with or without TiO$_2$. ................................ 142

Table 3.7 Mean (± SD) frequencies of lymphocytes and lymphocyte populations in PPs and MLNs from WT mice according to sampling time point. ........................................... 144

Table 3.8 Mean (± SD) frequencies of monocytes, monocyte populations, and DC subsets in PPs from WT mice according to sampling time point. ............................................. 145

Table 3.9 P-value results for pairwise group mean comparisons of the number of TiO$_2$ particles in SED areas with Tukey’s HSD test. ........................................................................................................... 158

Table 4.1 Means (± SD) for age, initial and final body weight, body weight change, daily weight gain, and daily food intake of female WT and $Nod^{2/–}$ mice fed a diet with or without TiO$_2$. ......................................................................................................................... 182

Table 4.2 Means (± SD) for daily TiO$_2$ intake and daily TiO$_2$ dose of female WT and $Nod^{2/–}$ mice fed a diet with 625 mg TiO$_2$/kg. ......................................................................................................................... 184

Table 4.3 Two-way ANOVA results for comparisons of intestinal parameters of female WT and $Nod^{2/–}$ mice fed a diet with or without TiO$_2$. ......................................................................................................................... 185

Table 5.1 Number of urine samples per respective group collected from mice for metabolomics studies at different time points. ........................................................................................................... 217

Table 5.2 MANOVA results for comparisons of urine metabolite profiles from WT mice fed a diet with or without TiO$_2$. ......................................................................................................................... 218

Table 5.3 MANOVA results for comparisons of urine metabolite profiles from female WT and $Nod^{2/–}$ mice fed a diet with or without TiO$_2$. ......................................................................................................................... 222

Table 5.4 Significantly different negative discriminant ions in urine samples from female WT and $Nod^{2/–}$ mice fed a diet with or without TiO$_2$ for 18 weeks and pairwise group comparisons. ......................................................................................................................... 228
Table 5.5 Significantly different positive discriminant ions in urine samples from female WT and Nod2<sup>−/−</sup> mice fed a diet with or without TiO<sub>2</sub> for 18 weeks and pairwise group comparisons. .................................................................229

Table 5.6 Results of the METLIN database queries for potential metabolites of negative discriminant ions. .................................................................230

Table 5.7 Results of the METLIN database queries for potential metabolites of positive discriminant ions. .................................................................231

Table 5.8 Significantly different metabolites identified in studies comparing urine samples from CD patients and healthy controls, Il10<sup>−/−</sup> mice with intestinal inflammation and healthy WT control mice, or rats orally exposed to TiO<sub>2</sub> and unexposed rats. .................................................................232
# List of appendices

**Appendix A**  
Normality and variance equality analysis *Chapter 2* ................................................. 301

**Appendix B**  
Average number of bone marrow cells per mouse ......................................................... 305

**Appendix C**  
Normality and variance equality analysis *Chapter 3* ................................................. 307

**Appendix D**  
Normality and variance equality analysis *Chapter 4* ................................................. 313

**Appendix E**  
R code for metabolomics data analysis ........................................................................... 315

**Appendix F**  
R code for statistical analysis of metabolomics data ...................................................... 317
List of abbreviations

[M+H]⁺  Positive molecular ion
[M-H]⁻  Negative molecular ion
1,007fs  Frameshift mutation at amino acid position 1,007
A5      Annexin-V
AIN     American Institute of Nutrition
ANOVA   Analysis of variance
APC     Antigen presenting cell
ASC     Apoptosis-associated speck-like protein containing a CARD
ATG16L1 Autophagy-related 16-like 1 gene
B cell  Bursa-derived cell
BMDC    Bone marrow-derived DC
BMDM    Bone marrow-derived macrophage
CARD    Caspase recruitment domain
CCL     CC chemokine ligand
CD      Crohn’s disease
CD[number]  Cluster of differentiation [number]
CX₃CR  CX₃C chemokine receptor
DAPI    4',6-Diamidino-2-phenylindole
DC      Dendritic cell
DNA     Deoxyribonucleic acid
DSS     Dextran sodium sulphate
EDS     Energy-dispersive X-ray spectroscopy
ELISA   Enzyme-linked immunosorbent assay
FACS    Fluorescence-activated cell sorting
FAE     Follicle-associated epithelium
FBS     Foetal bovine serum
FDR     False discovery rate
FSC     Forward scatter
GALT    Gut-associated lymphoid tissue
GC-MS   Gas chromatography mass spectrometry
H&E     Haematoxylin and eosin
HSD     Honest significant difference
IBD  Inflammatory bowel disease
IFN  Interferon
IFR  Interfollicular region
Ig  Immunoglobulin
IL  Interleukin
I10−/−  I10 gene-deficient
IL12B  IL-12 p40 subunit-encoding gene
IL-1Ra  IL-1 receptor antagonist
ILF  Isolated lymphoid follicle
IzB  Inhibitor of NF-κB
LC-MS  Liquid chromatography mass spectrometry
LP  Lamina propria
LPMC  LP mononuclear cell
LPS  Lipopolysaccharide
M cell  Microfold cell
m/z  Mass-to-charge
MANOVA  Multivariate ANOVA
MCP  Monocyte chemotactic protein
MDP  Muramyl dipeptide
MFI  Median fluorescence intensity
MIP  Macrophage inflammatory protein
MLN  Mesenteric lymph node
mRNA  Messenger ribonucleic acid
NF  Nuclear factor
NLR  NOD-like receptor
NLRP  NOD, leucine-rich repeats domain, and pyrin domain
NMR  Nuclear magnetic resonance spectroscopy
NOD  Nucleotide-binding oligomerisation domain
NOD2  NOD2 gene (human)
NOD2 protein (human)
Nod2  Nod2 gene (mouse)
Nod2 protein (mouse)
Nod2−/−  Nod2 gene-deficient
Nod2+/−  Nod2 gene mutation
<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Full Form</th>
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<tbody>
<tr>
<td>NTA</td>
<td>Nanoparticle tracking analysis</td>
</tr>
<tr>
<td>$p$</td>
<td>Probability</td>
</tr>
<tr>
<td>PBMC</td>
<td>Peripheral blood mononuclear cell</td>
</tr>
<tr>
<td>PBS</td>
<td>Phosphate-buffered saline</td>
</tr>
<tr>
<td>PGN</td>
<td>Peptidoglycan</td>
</tr>
<tr>
<td>PI</td>
<td>Propidium iodide</td>
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<tr>
<td>PLS-DA</td>
<td>Partial least squares discriminant analysis</td>
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<tr>
<td>PMT</td>
<td>Photomultiplier tube</td>
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<tr>
<td>PP</td>
<td>Peyer's patch</td>
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<tr>
<td>PRR</td>
<td>Pattern recognition receptor</td>
</tr>
<tr>
<td>RIP</td>
<td>Receptor-interacting protein</td>
</tr>
<tr>
<td>ROS</td>
<td>Reactive oxygen species</td>
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<tr>
<td>RPMI</td>
<td>Roswell Park Memorial Institute</td>
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<tr>
<td>SD</td>
<td>Standard deviation</td>
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<tr>
<td>SED</td>
<td>Subepithelial dome</td>
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<tr>
<td>SEM</td>
<td>Scanning electron microscopy</td>
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<tr>
<td>SNP</td>
<td>Single nucleotide polymorphism</td>
</tr>
<tr>
<td>SSC</td>
<td>Side scatter</td>
</tr>
<tr>
<td>T cell</td>
<td>Thymus-derived cell</td>
</tr>
<tr>
<td>TCM</td>
<td>Tissue culture medium</td>
</tr>
<tr>
<td>TEM</td>
<td>Transmission electron microscopy</td>
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<tr>
<td>TGF</td>
<td>Transforming growth factor</td>
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<tr>
<td>Th</td>
<td>T helper</td>
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<tr>
<td>TiO$_2$</td>
<td>Titanium dioxide</td>
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<tr>
<td>TLR</td>
<td>Toll-like receptor</td>
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<tr>
<td>TNBS</td>
<td>2,4,6-Trinitrobenzenesulfonic acid</td>
</tr>
<tr>
<td>TNF</td>
<td>Tumour necrosis factor</td>
</tr>
<tr>
<td>UC</td>
<td>Ulcerative colitis</td>
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<tr>
<td>WST</td>
<td>Water-soluble tetrazolium salt</td>
</tr>
<tr>
<td>WT</td>
<td>Wild-type</td>
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</table>