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Identification of biomarkers of colitis to monitor effects of dietary omega-3 polyunsaturated fatty acids in the interleukin-10 gene-deficient mouse model of inflammatory bowel diseases

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ABSTRACT

Inflammatory bowel diseases (IBD) are characterised by chronic inflammation of the gastrointestinal tract including the colon (colitis). Increased dietary intake of salmon, which is rich in eicosapentaenoic acid (EPA), was well tolerated by IBD patients, leading to a perceived decrease in symptoms. However, better knowledge of the mechanisms by which EPA-rich diets affect IBD severity, and appropriate biomarkers for assessing these effects, are needed for potential targeted nutritional interventions.

This dissertation aimed to determine the temporal effects (early (9 weeks of age) vs. established (12 weeks)) of a diet containing 3.7% EPA, and the dose-dependent effects (15% to 45%) of a salmon diet at 12 weeks of age, on the severity of colitis. Molecular responses in colon and/or liver of the interleukin-10 gene-deficient (Il10−/−) mouse model of IBD and healthy mice were assessed. Caecum digesta, urine and blood were mined to identify biomarkers (microbiota, metabolites and genes) of these responses.

The EPA diet reduced the severity of colitis only in 12-week-old Il10−/− mice. This response was associated with changes in gene expression associated with lymphocyte function, eicosanoid signalling and peroxisome proliferator-activated receptor gamma signalling. The blood immune cell gene expression profile did not correlate with reduced colitis in these mice, but the urine metabolite profile was related to changes in colonic tryptophan metabolism.

The effects of the salmon diets on colitis were dose-dependent in 12-week-old Il10−/− mice. The intermediate amount of salmon (30%) reduced the severity of colitis and lymphocyte-related gene expression, while enhancing genes in metabolic pathways. Tryptophan metabolism was not affected in these mice, but the urinary metabolite profile correlated with effects on hepatic tocopherol metabolism, as shown by reduced abundance of gamma-carboxyethylhydroxychroman glucoside. The abundances of V. akkermansia, Eubacterium spp., and an unclassified Rikenellaceae were further affected in these mice.

This is the first report describing molecular responses in the colon and liver of Il10−/− mice fed a salmon diet associated with reduced colitis. Ultimately these responses could be validated for use in humans, and potentially enable management of IBD with diet.
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# TABLE OF CONTENTS

Abstract i
Acknowledgements iii
Table of Contents v
List of Figures xi
List of Tables xiv
List of Abbreviations xvii
List of Appendices xvi
Introduction xix

## Review of literature

1.1 Inflammatory bowel diseases 2
  1.1.1 Genetic susceptibility 4
  1.1.2 Mucosal immune-regulation 4
    1.1.2.1 Intestinal epithelium 5
    1.1.2.2 Intestinal inflammation 8
    1.1.2.3 The commensal microbiota 10
  1.1.3 The role of the environment 14
  1.1.4 Animal models of IBD 15
    1.1.4.1 The interleukin-10 gene-deficient mouse model 18
1.2 The concept of systems biology 19
  1.2.1 Nutrigenomics 21
  1.2.2 Exploration of biomarkers of intestinal inflammation 21
1.3 Dietary salmon and intestinal inflammation 23
1.4 Anti-inflammatory effects of specific salmon components 24
  1.4.1 Micronutrients 24
  1.4.2 Peptides 26
  1.4.3 Lipids 26
  1.4.4 Putative mechanisms of action of lipids 31
    1.4.4.1 Modulation of cell membrane lipid rafts 31
    1.4.4.2 Formation of lipid mediators 33
    1.4.4.3 Modulation of gene expression 33
    1.4.4.4 Modulation of protein expression 35
    1.4.4.5 Modulation of the intestinal microbiota 36
1.5 Conclusion and outlook 37
1.6 Hypothesis and aims of the dissertation 38
1.7 Approach and structure of the dissertation 38

## Materials and Methods

2.1 Introduction 42
2.2 EPA time-course experiment 43
  2.2.1 Experimental design 43
  2.2.2 Mouse model and induction of colitis 43
  2.2.3 Experimental diets 45
  2.2.4 Sampling procedure and tissue collection 46
  2.2.5 Statistical evaluation of growth performance 48
2.3 Salmon diet experiment

2.3.1 Experimental design
2.3.2 Mouse model and induction of colitis
2.3.3 Experimental diets
2.3.4 Sampling procedure and tissue collection
2.3.5 Statistical evaluation of growth performance

2.4 Histopathological assessment

2.4.1 Tissue preparation and haematoxylin and eosin stain
2.4.2 Histological injury score
2.4.3 Statistical evaluation of histopathological changes

2.5 Isolation of peripheral blood mononuclear cells and RNA extraction

2.6 Extraction of RNA and protein from colon and liver

2.7 Transcriptomic analysis of colon, liver and peripheral blood mononuclear cells

2.7.1 Method overview
2.7.2 Sample preparation
2.7.3 CyDye-labelling and microarray hybridisation
2.7.4 Microarray data processing

2.8 Proteomic analysis of colon tissue

2.8.1 Method overview
2.8.2 Sample preparation
2.8.3 CyDye-labelling
2.8.4 Separation in the first dimension
2.8.5 Equilibration
2.8.6 Separation in the second dimension
2.8.7 In-gel protein digestion
2.8.8 Protein identification

2.9 Metabolomic analysis of urine

2.9.1 Method overview
2.9.2 Sample preparation and LC-MS analysis
2.9.3 MS data processing and statistical analysis

2.10 Microbiomic analysis of caecum digesta

2.10.1 Method overview
2.10.2 Extraction of metagenomic DNA and 454 pyrosequencing
2.10.3 Data analysis

2.11 Bioinformatic analysis

2.11.1 Ingenuity Pathway Analysis
2.11.2 Gene Set Enrichment Analysis
2.11.3 Integration of ‘Omics’ data

2.12 Conclusion and outlook

Effects of eicosapentaenoic acid-based diets on early and established colitis in the interleukin-10 gene-deficient mouse

3.1 Introduction
3.2 Hypothesis and aim
3.3 Methods
3.4 Results

3.4.1 Growth performance
3.4.2 Severity of intestinal inflammation at 9 and 12 weeks of age
3.4.3 Colon gene expression
Dose-response of salmon-based diets on established colitis and associated colonic gene expression in the interleukin-10 gene-deficient mouse

4.1 Introduction
4.2 Hypothesis and aim
4.3 Methods
4.4 Results
  4.4.1 Experimental diet composition
  4.4.2 Growth performance
  4.4.3 Severity of intestinal inflammation
  4.4.4 Colon gene expression
    4.4.4.1 Colon gene expression between mouse genotypes fed the AIN-76A diet
    4.4.4.2 Colon gene expression in mice fed the 15% salmon and 15% control diets
      4.4.4.2.1 Colon gene expression between mouse genotypes fed the 15% control diet
4.4.4.2 Effect of the 15% salmon diet (vs. 15% control diet) on colon gene expression in Il10−/− mice

4.4.4.3 Colon gene expression in mice fed the 30% salmon and 30% control diets

4.4.4.3.1 Colon gene expression between mouse genotypes fed the 30% control diet

4.4.4.3.2 Effect of the 30% salmon diet (vs. 30% control diet) on colon gene expression in Il10−/− mice

4.4.4.4 Colon gene expression in mice fed the 45% salmon and 45% control diets

4.4.4.4.1 Colon gene expression between mouse genotypes fed the 45% control diet

4.4.4.4.2 Effect of the 45% salmon diet (vs. 45% control diet) on colon gene expression irrespective of genotype

4.5 Discussion

4.5.1 Levels of LC n-3 PUFA

4.5.2 Severity of colitis in Il10−/− mice

4.5.3 Transcriptomic profiling of colon tissue

4.5.3.1 Pro-inflammatory gene expression in Il10−/− mice fed the 15% salmon diet (vs. 15% control diet)

4.5.3.2 Enhanced metabolic pathways in Il10−/− mice fed the 30% salmon diet (vs. 30% control diet)

4.5.3.3 Effect of the 45% salmon diet (vs. 45% control diet) dependent on genotype

4.6 Conclusion and outlook

Multi-'Omics’ approach to investigate the effects of a diet containing 30% salmon on the microbial community in the caecum and immune and metabolic pathways in colon and liver in the interleukin-10 gene-deficient mouse

5.1 Introduction

5.2 Hypothesis and aim

5.3 Methods

5.4 Results

5.4.1 Colon protein expression

5.4.1.1 Identification of proteins

5.4.1.2 Colon protein expression between mouse genotypes fed the 30% control diet

5.4.1.3 Effect of the 30% salmon diet (vs. 30% control diet) on colon protein expression in Il10−/− mice

5.4.2 Liver gene expression

5.4.3.1 Liver gene expression between mouse genotypes fed the 30% control diet

5.4.3.2 Effect of the 30% salmon diet (vs. 30% control diet) on liver gene expression in Il10−/− mice

5.4.4 Urinary metabolites

5.4.4.1 Metabolite identification

5.4.4.2 Metabolomic fingerprinting

5.4.4.2.1 Urinary metabolites between mouse genotypes fed the 30% control diet

5.4.4.2.2 Effect of the 30% salmon diet (vs. 30% control diet) on urinary metabolites in Il10−/− mice

5.4.5 Analysis of microbiota from caecum digesta

5.4.5.1 Species diversity estimate
5.4.5.2 Taxonomic differences
5.4.5.3 Beta diversity

5.5 Discussion

5.5.1 Proteomic profiling of colon tissue
5.5.1.1 Colon proteomic profile during colitis
5.5.1.2 Colon proteomic profile in response to the 30% salmon diet (vs. 30% control diet) in \(\text{Il10}^{-/-}\) mice

5.5.2 Transcriptomic profiling of liver tissue
5.5.2.1 Liver transcriptomic profile during colitis
5.5.2.2 Liver transcriptomic profile in response to the 30% salmon diet (vs. 30% control diet) irrespective of genotype
5.5.2.3 Liver transcriptomic profile in response to the 30% salmon diet (vs. 30% control diet) in \(\text{Il10}^{-/-}\) mice

5.5.3 Urine metabolomics
5.5.3.1 Urine metabolomic profile during colitis
5.5.3.2 Urine metabolomic profile in response to the 30% salmon diet (vs. 30% control diet) irrespective of genotype
5.5.3.3 Urine metabolomic profile in response to the 30% salmon diet (vs. 30% control diet) in \(\text{Il10}^{-/-}\) mice

5.5.4 Microbiomic analysis of caecum digesta
5.5.4.1 Microbial community profile during colitis
5.5.4.2 Microbial community profile in response to the 30% salmon diet (vs. 30% control diet) irrespective of genotype
5.5.4.3 Microbial community profile in response to the 30% salmon diet (vs. 30% control diet) in \(\text{Il10}^{-/-}\) mice

5.6 Conclusion and outlook

Integration of ‘Omics’ data to characterise the systemic responses to a diet containing 30% salmon in the interleukin-10 gene-deficient mouse

6.1 Introduction
6.2 Hypothesis and aim
6.3 Methods
6.4 Results
6.4.1 Tryptophan metabolism between mouse genotypes fed the 30% control diet
6.4.2 Tocopherol metabolism between mouse genotypes fed the 30% control diet
6.4.3 Microbial community profile between mouse genotypes fed the 30% control diet
6.4.4 Colon and hepatic gene expression and microbial community profile and their relationship to the urinary metabolite profile in \(\text{Il10}^{-/-}\) mice fed the 30% salmon diet (vs. 30% control diet)

6.5 Discussion
6.5.1 Novel insights into tryptophan metabolism during colitis
6.5.2 Novel insights into tocopherol metabolism during colitis
6.5.3 Novel insights into tocopherol metabolism in response to the 30% salmon diet (vs. 30% control diet) in \(\text{Il10}^{-/-}\) mice

6.6 Conclusion and outlook

General discussion

7.1 Background
7.2 Summary of results
7.3 General discussion 321
7.4 Future perspectives 325
7.5 Conclusion 326

Appendices 329
References 348
**LIST OF FIGURES**

| Figure 1.1 | Susceptibility loci for the disease phenotypes Crohn’s disease (CD) or ulcerative colitis (UC) | 6 |
| Figure 1.2 | Structure of the colonic mucosa | 7 |
| Figure 1.3 | Structure of tight junctions and adherens junctions | 9 |
| Figure 1.4 | Overview of regulatory pathways involved in the cell-mediated immune response and the characteristic defects in the disease phenotypes Crohn’s disease (CD) and ulcerative colitis (UC) | 12 |
| Figure 1.5 | From genes to metabolites in biological systems and the influence of microbial metabolism | 20 |
| Figure 1.6 | Metabolism of omega-6 and omega-3 polyunsaturated fatty acids (n-6 and n-3 PUFA) from precursor fatty acids | 28 |
| Figure 1.7 | Intestinal digestion and absorption of dietary lipids | 30 |
| Figure 1.8 | Putative mechanism of action of omega-3 polyunsaturated fatty acids (n-3 PUFA) on immune cell functions | 32 |
| Figure 1.9 | Overview of chapters in this dissertation. | 39 |
| Figure 2.1 | Design for the “EPA time-course experiment” | 44 |
| Figure 2.2 | Design for the “salmon diet experiment” | 49 |
| Figure 2.3 | Transcriptomic analysis workflow | 58 |
| Figure 2.4 | Principle of 2-dimensional (2D) gel electrophoresis | 63 |
| Figure 2.5 | Proteomic analysis workflow | 64 |
| Figure 2.6 | Metabolomic analysis workflow | 72 |
| Figure 2.7 | Microbiomic analysis workflow | 78 |
| Figure 3.1 | Design for the “EPA time-course experiment” | 92 |
| Figure 3.2 | Mean body weights (g) for C57BL/6J and Il10-/− mice over the experimental period | 94 |
| Figure 3.3 | Histological injury scores (HIS) obtained from the colon of Il10−/− mice at 9 and 12 weeks of age | 97 |
| Figure 3.4 | Heatmap of colon gene expression profiles | 100 |
| Figure 3.5 | Heatmap showing the set of genes comprising the KEGG pathway Tryptophan metabolism | 109 |
| Figure 3.6 | Metabolism of tryptophan to xanthurenic acid (via kynurenine), tryptamine and serotonin | 110 |
| Figure 3.7 | Colon gene expression changes in response to the eicosapentaenoic acid (EPA) diet (vs. oleic acid (OA) diet) in Il10−/− mice at early stages of colitis | 113 |
| Figure 3.8 | Colon gene expression changes in response to the eicosapentaenoic acid (EPA) diet (vs. oleic acid (OA) diet) in Il10−/− mice with established colitis | 114 |
| Figure 3.9 | Heatmap of peripheral blood mononuclear cell (PBMCs) gene expression profiles | 117 |
| Figure 3.10 | 15 highest p-value-ranked canonical pathways in peripheral blood mononuclear cells (PBMCs) from Il10−/− mice compared to C57BL/6J mice both fed the oleic acid (OA) diet | 123 |
| Figure 3.11 | Ingenuity pathway for Primary immunodeficiency signalling | 124 |
| Figure 3.12 | PBMC gene expression changes in response to the eicosapentaenoic acid (EPA) diet (vs. oleic acid (OA) diet) in Il10−/− mice at early stages of colitis | 128 |
| Figure 3.13 | PBMC gene expression changes in response to the eicosapentaenoic acid (EPA) diet (vs. oleic acid (OA) diet) in Il10−/− mice with established colitis | 129 |
| Figure 3.14 | Colon gene expression associated with early stages of colitis in Il10−/− mice | 131 |
| Figure 3.15 | Peripheral blood mononuclear cell (PBMC) gene expression associated with early stages of colitis in Il10−/− mice | 132 |
| Figure 3.16 | Colon gene expression associated with established colitis in Il10−/− mice | 133 |
| Figure 3.17 | Peripheral blood mononuclear cell (PBMC) gene expression associated with established colitis in Il10−/− mice | 134 |
| Figure 3.18 | Partial Least Squares-Discriminant Analysis (PLS-DA) of metabolite fingerprint from urine in positive and negative ionisation mode at 7.1 (T1), 9 (T2), 10.1 (T3) and 12 (T4) weeks of age | 138 |
| Figure 3.19 | Venn diagram indicating numbers of significantly different ionisation products in the urine of Il10−/− mice compared to C57BL/6J mice | 140 |
| Figure 3.20 | Venn diagram indicating numbers of significantly different ionisation products in the | 140 |
urine of mice fed the eicosapentaenoic acid (EPA) diet compared to those fed the oleic acid (OA) diet

Figure 3.21 Partial Least Squares-Discriminant Analysis (PLS-DA) of metabolite fingerprint from urine of Il10−/− mice and C57BL/6J mice in positive and negative ionisation mode

Figure 3.22 Partial Least Squares-Discriminant Analysis (PLS-DA) of metabolite fingerprint from urine in positive and negative ionisation mode

Figure 3.23 Urinary metabolites associated with metabolism of tryptophan via kynurenine and indoleacetaldehyde

Figure 3.24 Urinary metabolites associated with metabolism of tryptophan via serotonin

Figure 4.1 Design for the “salmon diet experiment”

Figure 4.2 Mean body weights (g) for Il10−/− and C57BL/6J mice over the experimental period

Figure 4.3 Representative images of colon sections stained with haematoxylin and eosin

Figure 4.4 Scores of individual histological features in colon tissue of Il10−/− mice fed the 30% salmon diet and 30% control diet

Figure 4.5 KEGG pathway gene sets affected in the colon of Il10−/− mice compared to C57BL/6J mice (both fed the AIN-76A diet)

Figure 4.6 KEGG pathway gene sets affected in the colon of Il10−/− mice compared to C57BL/6J mice (both fed the 15% control diet)

Figure 4.7 Biological interaction network of differentially expressed genes associated with the ten most significant biological functions in Il10−/− mice fed the 15% salmon diet relative to those fed the 15% control diet

Figure 4.8 KEGG pathway gene sets affected in the colon of Il10−/− mice compared to C57BL/6J mice (both fed the 30% control diet)

Figure 4.9 Biological interaction network of differentially expressed genes associated with the ten most significant biological functions in the colon of Il10−/− mice fed the 30% salmon diet compared to those fed the 30% control diet

Figure 4.10 Biological interaction network of transcription factors

Figure 4.11 KEGG pathway gene sets affected in the colon of Il10−/− mice fed the 30% salmon diet compared to those fed the 30% control diet

Figure 4.12 Ingenuity pathway for Production of Nitric Oxide and Reactive Oxygen Species in macrophages

Figure 4.13 KEGG pathway gene sets affected in the colon of Il10−/− mice compared to C57BL/6J mice (both fed the 45% salmon diet)

Figure 4.14 Biological interaction network of differentially expressed genes associated with the ten most significant biological functions in the colon of C57BL/6J mice fed the 45% salmon diet

Figure 5.1 Representative gel image with selected spot-features corresponding to proteins for identification

Figure 5.2 Ingenuity pathway for Antigen presentation indicating molecular events that lead to the presentation of antigens to CD4+ and CD8+ T cells during colitis

Figure 5.3 Ingenuity pathway for Antigen presentation indicating molecular events that lead to the presentation of antigens to CD4+ and CD8+ T cells in Il10−/− mice fed the 30% salmon diet

Figure 5.4 Biological interaction network of differentially expressed genes associated with the ten most significant biological functions in the liver of Il10−/− mice compared to C57BL/6J mice both fed the 30% control diet

Figure 5.5 KEGG pathway gene sets differentially expressed in the liver of Il10−/− mice compared to C57BL/6J mice fed the 30% control diet

Figure 5.6 KEGG pathway gene sets differentially expressed in the liver of Il10−/− mice fed the 30% salmon diet compared to those fed the 30% control diet

Figure 5.7 Structural elucidation of xanthurenic acid

Figure 5.8 Structural elucidation of xanthurenic acid glucoside

Figure 5.9 Structural elucidation of γ-CEHC glucoside

Figure 5.10 Structural elucidation of α-CEHC glucuronide

Figure 5.11 Partial Least Squares-Discriminant Analysis (PLS-DA) of mouse urine in negative and positive ionisation mode at 6.2, 9 and 11.5 weeks of age

Figure 5.12 Peak intensities of the molecular ions corresponding to the urinary metabolites xanthurenic acid glucoside (negative ionisation mode) and xanthurenic acid (positive and negative)

Figure 5.13 Chao1 index [325] of caecal microbiota
Figure 5.14 Dominant phyla in the caeca of C57BL/6J mice and Il10−/− mice fed the 30% control diet and the 30% salmon diet.

Figure 5.15 Principal Coordinate Analysis (PCoA) using (A) an unweighted UniFrac method and (B) a weighted UniFrac method.

Figure 6.1 Biological pathway for Tryptophan metabolism indicating molecular events that lead to the biosynthesis of melatonin from tryptophan (via serotonin), or xanthurenic acid (via kynurenine) in Il10−/− mice fed the 30% control diet compared to C57BL/6J mice fed the same diet.

Figure 6.2 Correlation of urinary α-CEHC glucuronide (“M453.1T348” and “M472.1T347”) and γ-CEHC glucoside (“M425.1T334” and “M444.1T334”) abundance with hepatic expression of genes.

Figure 6.3 Relevance network indicating correlation of (A) negative and (B) positive ions and the caecal microbiota, with expression values of Il10−/− mice fed a 30% control diet (vs. C57BL/6J mice) overlaid.

Figure 6.4 Biological pathway for Tryptophan metabolism indicating molecular events that lead to the biosynthesis of melatonin from tryptophan (via serotonin), or xanthurenic acid (via kynurenine) in Il10−/− mice fed the 30% salmon diet compared to those fed the 30% control diet.

Figure 6.5 Correlation network of urinary α-CEHC glucuronide (“M453.1T348” and “M472.1T347”) and γ-CEHC glucoside (“M425.1T334” and “M444.1T334”) abundance with hepatic expression of genes.

Figure 6.6 Relevance network indicating correlation of (A) negative and (B) positive ions and the caecal microbiota, with expression values of Il10−/− mice fed the 30% salmon diet compared to those fed the 30% control diet overlaid.

Figure 6.7 Proposed hepatic molecular mechanisms that lead to the elimination of α-tocopherol in the form of α-CEHC glucuronide.
**LIST OF TABLES**

| Table 1.1 | Overview of factors that contribute to the pathogenesis of inflammatory bowel diseases (IBD) and the disease phenotypes ulcerative colitis (UC) and Crohn’s disease (CD) | 3 |
| Table 1.2 | Characteristic changes in microbial community composition in inflammatory bowel diseases (IBD) | 13 |
| Table 1.3 | Most commonly used mouse models of inflammatory bowel diseases (IBD) | 16 |
| Table 1.4 | Nutritional profile of farmed New Zealand Chinook salmon fillets (*Oncorhynchus tshawytscha*). | 25 |
| Table 2.1 | Formulation of the unmodified AIN-76A diet and AIN-76A-based eicosapentaenoic acid (EPA) and oleic acid (OA) diets for the “EPA time-course experiment” | 47 |
| Table 2.2 | Formulation of salmon and control diets for the “salmon diet experiment” | 52 |
| Table 2.3 | Preparation of solutions and buffers used for colon protein analysis | 66 |
| Table 2.4 | Numbers of urine samples obtained from C57BL/6J mice and *Il10^-/-* mice fed AIN-76A diets, either unmodified, or enriched with oleic acid (OA) or eicosapentaenoic acid (EPA) | 75 |
| Table 2.5 | Numbers of urine samples obtained from C57BL/6J mice and *Il10^-/-* mice fed 30% salmon or 30% control diets | 76 |
| Table 2.6 | Overview of samples collected and measurements performed in the “EPA time-course experiment” and “salmon diet experiment” including contributions by the PhD candidate | 84 |
| Table 2.7 | Analysis of growth performance for “EPA time-course experiment” | 93 |
| Table 2.8 | Estimated mean histological injury scores (HIS) obtained from intestinal sections of *Il10^-/-* mice fed AIN-76A diets, either unmodified, or enriched with 3.7% oleic acid (OA) or 3.7% eicosapentaenoic acid (EPA) | 96 |
| Table 2.9 | Numbers of differentially expressed genes in the colon of *Il10^-/-* mice and C57BL/6J mice fed AIN-76A diets, either unmodified, or enriched with oleic acid (OA) or eicosapentaenoic acid (EPA) | 99 |
| Table 3.1 | Genes with the highest fold-changes (FC) between the colon from *Il10^-/-* mice and C57BL/6J mice fed the oleic acid (OA) diet (9 and 12 weeks of age) | 102 |
| Table 3.2 | Most significantly affected biological functions in the colon of *Il10^-/-* mice compared to C57BL/6J mice fed the oleic acid (OA) diet (9 and 12 weeks of age) | 103 |
| Table 3.3 | KEGG pathway gene sets affected in the colon of *Il10^-/-* mice compared to C57BL/6J mice sampled at 9 or 12 weeks of age | 106 |
| Table 3.4 | Numbers of differentially expressed genes in peripheral blood mononuclear cells (PBMCs) of *Il10^-/-* mice and C57BL/6J mice fed the oleic acid (OA) diet | 112 |
| Table 3.5 | Differentially expressed genes in peripheral blood mononuclear cells (PBMCs) from *Il10^-/-* mice compared to C57BL/6J mice fed the oleic acid (OA) diet that were in common at 9 and 12 weeks of age | 116 |
| Table 3.6 | Most significantly affected biological functions in peripheral blood mononuclear cells (PBMCs) from *Il10^-/-* mice relative to C57BL/6J mice fed the oleic acid (OA) diet (9 and 12 weeks of age) | 120 |
| Table 3.7 | Differentially expressed genes in peripheral blood mononuclear cells (PBMCs) from *Il10^-/-* mice | 126 |
| Table 3.8 | Most significantly affected biological functions in peripheral blood mononuclear cells (PBMCs) from *Il10^-/-* mice | 130 |
| Table 3.9 | Summary of gene expression changes in the colon and peripheral blood mononuclear cells (PBMCs) from *Il10^-/-* and C57BL/6J mice | 135 |
| Table 3.10 | Permutation MANOVA of the urinary metabolite fingerprints | 137 |
| Table 3.11 | Positive ionisation products significantly different in the urine from *Il10^-/-* mice compared to C57BL/6J mice | 144 |
Table 3.17  Positive ionisation products with differential abundance in the urine of mice fed the eicosapentaenoic acid (EPA) diet

Table 3.18  Negative ionisation products with differential abundance in the urine of mice fed the eicosapentaenoic acid (EPA) diet

Table 3.19  Summary of urinary metabolites detected in Il10-/- and C57BL/6J mice fed AIN-76A-based diets, either enriched with eicosapentaenoic acid (EPA) or oleic acid (OA)

Table 4.1  Nutritional composition of diets salmon and control diets

Table 4.2  Analysis of growth performance for “salmon diet experiment”

Table 4.3  Numbers of differentially expressed genes in the colon of C57BL/6J and Il10-/- mice

Table 4.4  Most significantly affected biological functions in the colon of Il10-/- mice relative to C57BL/6J mice (both fed the AIN-76A diet)

Table 4.5  Most significantly affected biological functions in the colon of Il10-/- mice relative to C57BL/6J mice (both fed the 15% control diet)

Table 4.6  Most significantly affected biological functions in the colon of Il10-/- mice fed the 15% salmon diet relative to those fed the 15% control diet

Table 4.7  Most significantly affected biological functions in the colon of Il10-/- mice fed the 30% salmon diet relative to those fed the 30% control diet

Table 4.8  Most significantly affected biological functions in the colon of Il10-/- mice relative to C57BL/6J mice (both fed the 45% control diet)

Table 4.9  Most significantly affected biological functions in the colon of Il10-/- mice fed the 45% salmon diet relative to those fed the 45% control diet

Table 5.1  List of differentially expressed proteins in the colon of Il10-/- and C57BL/6J mice fed the 30% control or 30% salmon diet

Table 5.2  Numbers of differentially expressed genes in the liver of C57BL/6J and Il10-/- mice

Table 5.3  Most significantly affected biological functions in the liver of Il10-/- relative to C57BL/6J mice (both fed the 30% control diet)

Table 5.4  Differentially expressed genes associated with Xenobiotic metabolism in the liver of Il10-/- mice

Table 5.5  Differentially expressed genes associated with Lipid metabolism and Xenobiotics metabolism in the liver of C57BL/6J mice or Il10-/- mice fed the 30% salmon diet compared to those fed the 30% control diet

Table 5.6  Permutation MANOVA of the urinary metabolite fingerprints

Table 5.7  Ions with the ten highest Partial Least Squares-Discriminant Analysis (PLS-DA) loadings in Il10-/- and C57BL/6J mice fed 30% control or 30% salmon diets

Table 5.8  Average proportions of dominant genera in caecal digesta

Table 7.1  Summary of the main biological functions and microbiota affected in Il10-/- mice fed diets containing either 3.7% eicosapentaenoic acid (EPA) (“EPA time-course experiment”) or 30% salmon (“salmon diet experiment”)
### LIST OF APPENDICES

<table>
<thead>
<tr>
<th>Appendix</th>
<th>Description</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Appendix I</td>
<td>Analysis of lyophilised salmon fillets</td>
<td>329</td>
</tr>
<tr>
<td>Appendix II</td>
<td>R codes applied for pre-processing of metabolomics data</td>
<td>330</td>
</tr>
<tr>
<td>Appendix III</td>
<td>Histological injury scores (HIS) obtained from the duodenum of C57BL/6J and II10−/− mice at 9 and 12 weeks of age</td>
<td>331</td>
</tr>
<tr>
<td>Appendix IV</td>
<td>Differentially expressed genes in the colon of II10−/− mice fed the oleic acid (OA) diet compared to those fed the AIN-76A diet at 12 weeks of age</td>
<td>332</td>
</tr>
<tr>
<td>Appendix V</td>
<td>Molecule shapes and relationship types used by Ingenuity Pathway Analysis</td>
<td>333</td>
</tr>
<tr>
<td>Appendix VI</td>
<td>Positive ionisation products with differential abundance in the urine of mice fed the eicosapentaenoic acid (EPA) diet compared to those fed the oleic acid (OA) diet</td>
<td>334</td>
</tr>
<tr>
<td>Appendix VII</td>
<td>Negative ionisation products with differential abundance in the urine of mice fed the eicosapentaenoic acid (EPA) diet compared to those fed the oleic acid (OA) diet</td>
<td>336</td>
</tr>
<tr>
<td>Appendix VIII</td>
<td>Biological pathway for Tryptophan metabolism indicating molecular events that lead to the biosynthesis of melatonin from tryptophan (via serotonin), or xanthurenic acid (via kynurenine) in II10−/− mice fed the oleic acid (OA) diet compared to C57BL/6J mice fed the same diet at 12 weeks of age.</td>
<td>338</td>
</tr>
<tr>
<td>Appendix IX</td>
<td>Biological pathway for Tryptophan metabolism indicating molecular events that lead to the biosynthesis of melatonin from tryptophan (via serotonin), or xanthurenic acid (via kynurenine) in II10−/− mice fed the eicosapentaenoic acid (EPA) diet compared to those fed the oleic acid (OA) diet.</td>
<td>340</td>
</tr>
<tr>
<td>Appendix X</td>
<td>Histology scores from (A) C57BL/6J mice and (B) II10−/− mice fed diets supplemented with 15%, 30% or 45% salmon and corresponding macronutrient-matched control diets</td>
<td>342</td>
</tr>
<tr>
<td>Appendix XI</td>
<td>qPCR validation of microarray results</td>
<td>343</td>
</tr>
<tr>
<td>Appendix XII</td>
<td>Unidentified ions with the ten highest Partial Least Squares-Discriminant Analysis (PLS-DA) loadings (“discriminant ions”) in II10−/− and C57BL/6J mice fed 30% control or 30% salmon diets</td>
<td>345</td>
</tr>
<tr>
<td>Appendix XIII</td>
<td>Proteins identified from previous experiments.</td>
<td>347</td>
</tr>
</tbody>
</table>
**LIST OF ABBREVIATIONS**

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Full Form</th>
</tr>
</thead>
<tbody>
<tr>
<td>AA</td>
<td>Arachidonic acid</td>
</tr>
<tr>
<td>ALA</td>
<td>Alpha-linolenic acid</td>
</tr>
<tr>
<td>AMP</td>
<td>Antimicrobial protein</td>
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<tr>
<td>ANOVA</td>
<td>Analysis of variance</td>
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<tr>
<td>CAM</td>
<td>Cell adhesion molecule</td>
</tr>
<tr>
<td>CD</td>
<td>Crohn’s disease</td>
</tr>
<tr>
<td>CEBPB, CEBPD, CEBPE</td>
<td>CCAAT/enhancer binding protein (alpha, beta, delta)</td>
</tr>
<tr>
<td>CEHC</td>
<td>Carboxyethylhydroxychroman</td>
</tr>
<tr>
<td>CFU</td>
<td>Colony-forming units</td>
</tr>
<tr>
<td>CIF</td>
<td>Complex intestinal microbiota</td>
</tr>
<tr>
<td>DHA</td>
<td>Docosahexaenoic acid</td>
</tr>
<tr>
<td>DIGE</td>
<td>Difference gel electrophoresis</td>
</tr>
<tr>
<td>DPA</td>
<td>Docosapentaenoic acid</td>
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<tr>
<td>DSS</td>
<td>Dextran Sodium Sulfate</td>
</tr>
<tr>
<td>EF</td>
<td>Enterococcus faecalis and E. faecium</td>
</tr>
<tr>
<td>EF×CIF</td>
<td>Solution for bacterial inoculation</td>
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<tr>
<td>EPA</td>
<td>Eicosapentaenoic acid</td>
</tr>
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<td>ESI</td>
<td>Electrospray ionisation</td>
</tr>
<tr>
<td>FC</td>
<td>Fold-change</td>
</tr>
<tr>
<td>FDR</td>
<td>False discovery rate</td>
</tr>
<tr>
<td>GIT</td>
<td>Gastrointestinal tract</td>
</tr>
<tr>
<td>GLA</td>
<td>Gamma-linolenic acid</td>
</tr>
<tr>
<td>GSEA</td>
<td>Gene Set Enrichment Analysis</td>
</tr>
<tr>
<td>GWAS</td>
<td>Genome-Wide Association Study</td>
</tr>
<tr>
<td>HE</td>
<td>Haematoxylin and eosin</td>
</tr>
<tr>
<td>HIS</td>
<td>Histological injury score</td>
</tr>
<tr>
<td>HMDB</td>
<td>Human Metabolome Database</td>
</tr>
<tr>
<td>IBD</td>
<td>Inflammatory bowel diseases</td>
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<td>IEF</td>
<td>Isoelectric focussing</td>
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<tr>
<td>IL[number]</td>
<td>Interleukin [number]</td>
</tr>
<tr>
<td>II10⁻/⁻</td>
<td>Interleukin-10 gene-deficient</td>
</tr>
<tr>
<td>IPA</td>
<td>Ingenuity Pathway Analysis</td>
</tr>
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<td>KEGG</td>
<td>Kyoto Encyclopedia of Genes and Genomes</td>
</tr>
<tr>
<td>LA</td>
<td>Linoleic acid</td>
</tr>
<tr>
<td>LC</td>
<td>Long-chain</td>
</tr>
<tr>
<td>LPS</td>
<td>Lipopolysaccharide</td>
</tr>
<tr>
<td>LysoPC</td>
<td>Lysophosphatidylcholine</td>
</tr>
</tbody>
</table>
LysoPE  Lysophosphatidylethanolamine

m/z  Mass-per-charge ratio

MANOVA  Multivariate ANOVA

MDT  Marine-derived tocopherol

MS  Mass spectrometry

n-3 PUFA  Omega-3 polyunsaturated fatty acid

n-6 PUFA  Omega-6 polyunsaturated fatty acid

NCBI  National Center for Biotechnology Information

NKT cell  Natural killer T cell

NMR  Nuclear magnetic resonance

OA  Oleic acid

OTU  Operational taxonomic unit

PAMP  Pathogen-associated molecular pattern

PBMC  Peripheral blood mononuclear cell

PBS  Phosphate-buffered saline

PC  Principal coordinate

PCA  Principal Component Analysis

PCoA  Principal Coordinate Analysis

pI  Isoelectric point

PLS-DA  Partial Least Squares-Discriminant Analysis

PPAR  Peroxisome proliferator-activated receptor

PRR  Pattern recognition receptor

QIIME  Quantitative Insights Into Microbial Ecology

qRT-PCR  Real-time reverse transcription polymerase chain reaction

REML  Restricted maximum likelihood

REST  Relative Expression Software Tool

RIN  RNA integrity number

ROS  Reactive Oxygen Species

RT  Retention time

SCFA  Short-chain fatty acid

SDS  Sodium dodecyl sulphate

SDS-PAGE  Sodium dodecyl sulphate polyacrylamide gel electrophoresis

SNP  Single nucleotide polymorphisms

TG  Triacylglycerol

Th cell  T helper cell

TMAO  Trimethylamine N-oxide

TNBS  Trinitrobenzenesulfonic acid

Treg  Regulatory T helper cell

UC  Ulcerative colitis