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The effects of dietary eicosapentaenoic acid and arachidonic acid on gene expression changes in a mouse model of human inflammatory bowel diseases

A thesis presented in partial fulfilment of the requirements for the degree of

Doctor of Philosophy
in
Nutritional Science

at Massey University
Palmerston North, New Zealand

Bianca Knoch
2010
ABSTRACT

Nutrigenomics studies the genome-wide influence of nutrients to understand the association between nutrition and human health. Studies in animal models and humans have demonstrated that dietary n-3 polyunsaturated fatty acids (PUFA) from fish oil may be beneficial in inflammatory bowel diseases (IBD).

This thesis aimed to test the hypothesis that dietary n-3 PUFA eicosapentaenoic acid (EPA) reduced and n-6 PUFA arachidonic acid (AA) increased colitis in the interleukin-10 gene-deficient (Il10−/−) mouse model of IBD, and that these PUFA altered the intestinal bacteria community during colitis development using genome-wide expression and bacterial profiling.

Using a combined transcriptomic and proteomic approach, the time-course study defined the onset and progression of colitis in Il10−/− mice. Histopathology, transcript and protein changes before and after colitis onset involved in innate and adaptive immune responses suggested delayed remodelling processes in colitic Il10−/− mice and 11 weeks of age as suitable time point to study the effects of dietary PUFA on colitis development. Comparing the transcriptome and proteome profiles associated with colon inflammation of mice fed with the AIN-76A or oleic acid (OA) diet showed that OA was an appropriate control for unsaturated fatty acids in multi-omic studies. The PUFA intervention study indicated that dietary EPA-induced lipid oxidation might have a potential anti-inflammatory effect on inflamed colon tissue partially mediated through activation of peroxisome proliferator-activated receptor alpha (PPARα). Unexpectedly, dietary AA decreased the expression of inflammatory and stress colonic genes in Il10−/− mice. Altered intestinal bacteria community observed in Il10−/− mice before and after colitis onset was associated with the lack of IL10 protein led to changes in intestinal metabolic and signalling processes. Interestingly, dietary EPA and AA seemed to change intestinal bacteria profiles during colitis development. The role of PPARα in the colon was further examined in a concluding study which identified vanin1 as a likely new PPARα-target gene which may also be involved in lipid metabolism.

These findings using a state-of-the-art approach combining transcriptomics, proteomics and physiology provide a basis for future research on molecular mechanisms underlying
the effects of dietary PUFA, and might contribute to the development of fortified foods that improve intestinal health and wellness.
ACKNOWLEDGEMENTS

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I thank my parents, Werner and Birgit Knoch, and brother Kevin Knoch for their moral and emotional support and love throughout my life. The warmest thanks to my husband, Thomas Suesse, for his love, dedication and patience during the years.
To them I dedicate this thesis.

Palmerston North, October 2009
TABLE OF CONTENTS

ABSTRACT I
ACKNOWLEDGEMENTS III
TABLE OF CONTENTS V
LIST OF TABLES IX
LIST OF FIGURES XI
LIST OF ABBREVIATIONS XIII

Chapter 1: General introduction 1
  1.1 Background 2
  1.2 Nutrigenomics 4
    1.2.1 Aspects of nutrition and nutrients 5
    1.2.2 Nutrigenomics approach 6
    1.2.3 Gene-diet interactions and disease associations 9
  1.3 Intestinal inflammation in inflammatory bowel disease 10
    1.3.1 Genetics of inflammatory bowel disease 11
      1.3.1.1 Murine models of intestinal inflammation 12
      1.3.1.1.1 The interleukin-10 gene-deficient mouse model 13
      1.3.1.1.2 Interleukin-10 signalling 16
    1.3.2 Immunology in inflammatory bowel disease 18
      1.3.2.1 Mucosal surface and defence system 18
      1.3.2.2 Mucosal immunity 20
        1.3.2.2.1 Epithelial barrier 20
        1.3.2.2.2 Innate immune response 21
        1.3.2.2.3 Adaptive immune response 23
    1.3.3 Bacteria in inflammatory bowel disease 25
      1.3.3.1 Bacteria of the gastrointestinal tract and their role in intestinal inflammation 25
      1.3.3.2 Qualitative and quantitative analysis of bacteria involved in intestinal inflammation 29
        1.3.3.2.1 Community fingerprinting techniques 31
        1.3.3.2.2 Quantitative techniques 33
  1.4 Study of the effects of dietary polyunsaturated fatty acids: Molecular mechanisms involved in intestinal inflammation 35
    1.4.1 Polyunsaturated fatty acids, inflammation and immune response 35
      1.4.1.1 PUFA-derived mediators 36
      1.4.1.2 Immunomodulatory mechanisms 39
    1.4.2 Polyunsaturated fatty acid regulation of gene expression in IBD 41
      1.4.2.1 Fatty acid metabolism 42
      1.4.2.2 Transcription factors 44
    1.4.3 A molecular approach to study polyunsaturated fatty acid-regulated genes in intestinal inflammation 49
      1.4.3.1 Studies on inflammatory lesions and lipid mediator production 50
      1.4.3.2 Studies on intestinal gene expression 54
  1.5 Conclusions and future perspectives 55
  1.6 Aim, approach and outline of this thesis 57

Chapter 2: General materials and methods 60
  2.1 Animals 61
    2.1.1 Housing 61
    2.1.2 Induction of colitis 61
    2.1.3 Experimental design 62
    2.1.4 Fast-feed period and sampling 64
<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.2 Diet compositions</td>
<td>64</td>
</tr>
<tr>
<td>2.3 Histology</td>
<td>67</td>
</tr>
<tr>
<td>2.3.1 Tissue processing and staining</td>
<td>68</td>
</tr>
<tr>
<td>2.3.2 Histopathological assessment</td>
<td>68</td>
</tr>
<tr>
<td>2.3.3 Serum amyloid A (SAA)</td>
<td>69</td>
</tr>
<tr>
<td>2.4 Transcriptomics</td>
<td>69</td>
</tr>
<tr>
<td>2.4.1 Design of the microarray experiment</td>
<td>70</td>
</tr>
<tr>
<td>2.4.2 RNA isolation</td>
<td>73</td>
</tr>
<tr>
<td>2.4.3 Synthesis of labelled cRNA, microarray hybridisation and scanning</td>
<td>74</td>
</tr>
<tr>
<td>2.4.4 Microarray analysis</td>
<td>75</td>
</tr>
<tr>
<td>2.5 Validation of microarray results by quantitative real-time PCR</td>
<td>75</td>
</tr>
<tr>
<td>2.5.1 Primer sequences</td>
<td>77</td>
</tr>
<tr>
<td>2.6 Proteomics</td>
<td>77</td>
</tr>
<tr>
<td>2.6.1 Protein isolation</td>
<td>79</td>
</tr>
<tr>
<td>2.6.2 LC-MS analysis of peptides</td>
<td>80</td>
</tr>
<tr>
<td>2.6.3 MS/MS data processing and analysis</td>
<td>81</td>
</tr>
<tr>
<td>2.7 Bioinformatics analysis</td>
<td>82</td>
</tr>
<tr>
<td>2.7.1 Ingenuity pathway analysis (IPA)</td>
<td>82</td>
</tr>
<tr>
<td>2.7.2 FUNC analysis</td>
<td>83</td>
</tr>
<tr>
<td>2.7.3 EASE analysis</td>
<td>83</td>
</tr>
<tr>
<td>2.8 Bacterial profiling</td>
<td>84</td>
</tr>
<tr>
<td>2.8.1 DNA isolation</td>
<td>84</td>
</tr>
<tr>
<td>2.8.2 PCR amplification</td>
<td>84</td>
</tr>
<tr>
<td>2.8.3 Denaturing gradient gel electrophoresis (DGGE)</td>
<td>85</td>
</tr>
<tr>
<td>2.8.4 Analysis of DGGE profiles</td>
<td>86</td>
</tr>
<tr>
<td>2.8.5 Cloning and sequencing DNA from DGGE fragments</td>
<td>86</td>
</tr>
<tr>
<td>2.8.6 Real-time PCR quantification of total bacteria and selected bacterial groups</td>
<td>87</td>
</tr>
<tr>
<td>2.9 PPARα-target gene identification and validation</td>
<td>89</td>
</tr>
<tr>
<td>2.9.1 In silico analysis of PPAR response elements of a potential PPARα target gene</td>
<td>89</td>
</tr>
<tr>
<td>2.9.2 Plasmids and DNA constructs</td>
<td>89</td>
</tr>
<tr>
<td>2.9.3 Cell culture and transfections</td>
<td>91</td>
</tr>
</tbody>
</table>

Chapter 3: Transcriptomic and proteomic profiling to characterise onset and progression of colon inflammation in interleukin-10 gene-deficient mice

3.1 Abstract | 94 |
3.2 Introduction | 94 |
3.3 Materials and methods | 96 |
3.3.1 Animals and diet | 96 |
3.3.2 Induction of colitis | 97 |
3.3.3 Experimental design | 97 |
3.3.4 Histology | 97 |
3.3.5 RNA isolation, microarray hybridisation and analysis | 97 |
3.3.6 Bioinformatics analysis of pathways and functions | 98 |
3.3.7 Quantitative real-time PCR | 99 |
3.3.8 Protein isolation and identification by LC-MS/MS data analyses | 99 |
3.3.9 Statistical analysis | 99 |
3.4 Results | 99 |
3.4.1 Animal body weight and dietary intake | 99 |
3.4.2 Development and characterisation of intestinal inflammation | 100 |
3.4.3 Inflammation-induced changes in expression profiles | 100 |
3.4.4 Gene ontology, network/function and pathway analysis of colonic genes and proteins of Il10−/− and C57 mice at 7 weeks of age | 101 |
3.4.5 Gene ontology, network/function and pathway analysis of colonic genes and proteins of Il10−/− and C57 mice at 12 weeks of age | 102 |
3.4.6 Pathway comparison analysis of 7- and 12-week-old Il10−/− mice relative to C57 mice at the same age | 103 |
3.5 Discussion | 119 |
LIST OF TABLES

Chapter 1:

Table 1.1 Genetically manipulated mouse models of intestinal inflammation. 14
Table 1.2 Interaction of the genetic susceptible host with environmental factors such as bacteria. 28
Table 1.3 n-3 and n-6 PUFA-derived lipid mediators and their metabolic effects. 38

Chapter 2:

Table 2.1 Ingredient composition and determined chemical analysis of the experimental diets. 66
Table 2.2 Primer sequences used for qRT-PCR. 78

Chapter 3:

Table 3.1 Body weight and dietary intake of Il10–/– and C57 mice at 7, 8.5, 10, 12 and 14 weeks of age. 104
Table 3.2 Time of onset and incidence of colon inflammation in Il10–/– mice. 106
Table 3.3 Validation of gene expression results from microarray analysis using qRT-PCR. 109
Table 3.4 Proteins significantly differentially expressed in the colon of Il10–/– relative to C57 mice at 7 and 12 weeks of age. 111
Table 3.5 Categories of genes with expression increase of 2-fold or more only in Il10–/– relative to C57 mice at 7 weeks of age using EASE analysis. 113
Table 3.6 Proteome and transcriptome network analysis in the colon of 7- and 12-week-old Il10–/– relative to C57 mice using IPA analysis. 114
Table 3.7 Differentially expressed genes in the colon of Il10–/– relative to C57 mice at 7 and 12 weeks of age. 115
Table 3.8 Categories of genes with expression increase of 2-fold or more in Il10–/– relative to C57 mice at 12 weeks of age using EASE analysis. 118

Chapter 4:

Table 4.1 Histological injury score (HIS) in colon tissue and plasma serum amyloid A (SAA) in Il10–/– and C57 mice fed AIN-76A and OA diets. 134
Table 4.2 Functional analysis for genes differentially expressed in the colon of Il10–/– relative to C57 mice fed the AIN-76A or OA diet using IPA analysis. 136
Table 4.3 Microarray data validation by qRT-PCR. 137
Table 4.4 Proteins differentially expressed in the colon of Il10–/– relative to C57 mice the AIN-76A or OA diet. 140
Table 4.5 Comparison of proteomic and transcriptomic profiles in the colon of Il10–/– relative to C57 mice fed the AIN-76A or OA diet. 144

Chapter 5:

Table 5.1 Inflammation and HIS in colon tissue, and plasma SAA concentrations in C57 and Il10–/– mice fed OA or EPA diets. 162
Table 5.2 Expression levels of selected genes from microarray and qRT-PCR analyses of pooled colon tissue of Il10–/– and C57 mice fed OA or EPA diet. 165
Table 5.3 Significant biological functions and pathways in colon tissues of 1) Il10–/– versus C57 mice fed OA diet, 2) EPA- versus OA-fed Il10–/– mice and 3) EPA- versus OA-fed C57 mice. 167
Table 5.4 Pathways with genes differentially expressed in the 1) genotype comparison (Il10–/– vs. C57 mice fed the OA diet), 2) diet comparison (EPA vs. OA) in Il10–/– mice and 3) diet comparison (EPA vs. OA) in C57 mice, as identified by IPA pathway analysis. 168
Table 5.5 Significantly over-represented gene ontology (GO) terms in the colon of Il10–/– vs. C57 mice on the OA diet using FUNC analysis. 174
Table 5.6 Significantly over-represented gene ontology (GO) terms in the colon of EPA-fed Il10–/– mice compared to OA-fed Il10–/– mice using FUNC analysis. 175
Chapter 6:

Table 6.1 Inflammation and HIS in colon tissue, and plasma SAA concentrations in C57 and \( \text{Il10}^{−/−} \) mice fed OA or AA diets. 196

Table 6.2 Validation of gene expression results from microarray analysis using qRT-PCR. 199

Table 6.3 Pathways associated with gene expression changes in the colon of \( \text{Il10}^{−/−} \) and C57 mice fed AA compared to the OA diet, as identified by IPA analysis. 200

Table 6.4 Significantly over-represented gene ontology (GO) terms in the colon of \( \text{Il10}^{−/−} \) mice fed the AA diet compared to OA-fed \( \text{Il10}^{−/−} \) mice using FUNC analysis. 204

Chapter 7:

Table 7.1 Primers used for bacterial quantification by real-time PCR. 220

Table 7.2 Similarity analysis of DGGE profiles between mice within experimental groups (weeks of age or diets). 223

Table 7.3 Species identified from excised bands in study 1. 224

Table 7.4 Species identified from excised bands in study 2. 228

Chapter 8:

Table 8.1 Primers used for qRT-PCR. 246

Table 8.2 Selected genes and their level of expression in liver and small intestine of WY14643-treated PPAR\( \alpha \) wildtype mice and in colon of EPA-fed \( \text{Il10}^{−/−} \) mice. 247
LIST OF FIGURES

Chapter 1:

Fig. 1.1 The ‘omics’ technologies in nutritional science required to establish a phenotype. 3
Fig. 1.2 Overview of IL10 signalling. 17
Fig. 1.3 Structure of the large intestine (colon) with enlarged inset to show details of its layers mucosa, submucosa, muscularis (muscle layers) and serosa. 19
Fig. 1.4 Extracellular and intracellular pattern recognition receptors (TLR and CARD) of the innate immune response. 22
Fig. 1.5 Intestinal immune system showing features of the adaptive immune response. 24
Fig. 1.6 Compartments of the human gastrointestinal tract, physiological processes and conditions and the major bacterial genera found herein. 26
Fig. 1.7 Workflow for denaturing gradient gel electrophoresis (DGGE), a fingerprinting technique to assess complex bacterial communities. 32
Fig. 1.8 Metabolism of n-3 and n-6 PUFA. 37
Fig. 1.9 Cellular uptake of long chain PUFA. 43
Fig. 1.10 Transcription factors influenced by PUFA. 48
Fig. 1.11 Outline of the thesis and experimental chapters. 59

Chapter 2:

Fig. 2.1 Experimental design of the (a) time-course and (b) PUFA intervention study. 63
Fig. 2.2 Fast-feed regimen prior to tissue sampling. 65
Fig. 2.3 Schematic representation of the steps performed in the microarray experiment. 71
Fig. 2.4 Illustration of reference design used in microarray experiments. 72

Chapter 3:

Fig. 3.1 Total histological injury score of duodenum, jejunum, ileum and colon of Il10–/– and C57 mice at 7, 8.5, 10, 12 and 14 weeks of age. 105
Fig. 3.2 Haematoxylin-eosin stained colon sections of Il10–/– and C57 mice. 107
Fig. 3.3 Genes over-represented in EASE analysis comparing Il10–/– and C57 mice at 7 or 12 weeks of age. 108
Fig. 3.4 2D-DIGE gel representing differentially expressed proteins identified in the colon tissue of Il10–/– mice compared to C57 mice at 7 and 12 weeks of age, respectively. 110

Chapter 4:

Fig. 4.1 Mean body weight (g) and dietary intake (g) of Il10–/– and C57 mice fed AIN-76A and OA diets (n=5-6/diet) for the experimental period. 133
Fig. 4.2 Heatmaps showing the top 5 regulated pathways with genes differentially expressed in the colon of AIN-76A- or OA-fed Il10–/– relative to C57 mice on the same diet. 135
Fig. 4.3 Heatmap showing identified proteins differentially expressed in the colon of Il10–/– compared to C57 mice fed either the AIN-76A- or OA diet. 138
Fig. 4.4 Representative 2D-DIGE gel comparing the protein extracts from the colon of Il10–/– relative to C57 mice fed the AIN-76A or OA diet. 139

Chapter 5:

Fig. 5.1 Mean body weight (g) and dietary intake (g) of Il10–/– and C57 mice fed OA and EPA diets for the experimental period. 161
Fig. 5.2 Histological injury score (HIS) of colon tissue for individual Il10–/– mice on the OA diet (n=5) and the EPA diet (n=4), and C57 mice on the OA diet (n=6) and the EPA diet (n=6). 163
Fig. 5.3 Haematoxylin-eosin stained colon sections of a C57 and an Il10–/– mouse. 164
Fig. 5.4 Generation of biological interaction networks of genes of the most significant pathways in Il10–/– versus C57 mice fed the OA diet. 166
Fig. 5.5 Mechanisms of dietary EPA-induced modulation of colon inflammation in inoculated Il10–/– mice. 176
Chapter 6:

Fig. 6.1 Mean body weight (g) and dietary intake (g) of \( Il10^{-/-} \) and C57 mice fed OA and AA diets for the experimental period.

Fig. 6.2 Haematoxylin-eosin-stained colon sections of a C57 and an \( Il10^{-/-} \) mouse.

Fig. 6.3 Venn diagram showing the numbers of differentially expressed genes unique to or in common in the genotype comparison (\( Il10^{-/-} \) mice vs. C57 mice on the OA diet) and the two diet comparisons (AA vs. OA in \( Il10^{-/-} \) mice and AA vs. OA in C57 mice).

Fig. 6.4 Biological interaction network of genes of the most significantly regulated pathways generated for the AA vs. OA \( Il10^{-/-} \) mice comparison using IPA analysis.

Chapter 7:

Fig. 7.1 Consensus DGGE profiles of caecal bacteria from \( Il10^{-/-} \) and C57 mice at 7 and 12 weeks of age.

Fig. 7.2 DGGE profiles of caecal bacteria from individual \( Il10^{-/-} \) and C57 mice at 7 and 12 weeks of age.

Fig. 7.3 Real-time PCR quantification of cells of (a) total bacteria, (b) Bacteroides-Prevotella-Porphyromonas spp. and (c) Enterococcus spp. in the caecum of \( Il10^{-/-} \) and C57 mice at 7 and 12 weeks of age.

Fig. 7.4 Consensus DGGE profiles of caecal bacteria from \( Il10^{-/-} \) and C57 mice fed EPA, AA, OA or AIN-76A diets.

Fig. 7.5 DGGE profiles of caecal bacteria from individual \( Il10^{-/-} \) and C57 mice fed PUFA diets.

Fig. 7.6 Real-time PCR quantification of cells of (a) total bacteria, (b) Clostridium perfringens spp., (c) E. coli spp., (d) Enterococcus spp., (e) Bacteroides-Prevotella-Porphyromonas spp. and (f) B. vulgatus in the caecum of \( Il10^{-/-} \) and C57 mice fed dietary PUFA (AA, EPA) or control (AIN-76A, OA) diets.

Chapter 8:

Fig. 8.1 Agonist-induced PPAR\( \alpha \)-dependent gene regulation in the colon of PPAR\( \alpha \)–/– (KO) and wildtype (WT) mice.

Fig. 8.2 In silico analysis of mouse vanin1 (\( Vnn1 \)) as selected putative PPAR\( \alpha \) target gene.

Fig. 8.3 PPAR\( \alpha \)-mediated increased expression of mouse \( Vnn1 \) is regulated by PPRE in the \( Vnn1 \) genomic sequence.

Fig. 8.4 PPAR\( \alpha \)–/– (KO) and wildtype (WT) mice with DNBS-induced colitis were exposed to WY14643 or vehicle for 4 days.

Fig. 8.5 Total colon histology score in the colon of WY14643- or vehicle-treated PPAR\( \alpha \)–/– (KO) and wildtype (WT) mice with DNBS-induced colitis.

Fig. 8.6 Haematoxylin-eosin stained colon sections of WY14643- and vehicle-treated PPAR\( \alpha \)–/– and wildtype mice with DNBS-induced colitis.
# LIST OF ABBREVIATIONS

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
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<tr>
<td>AA</td>
<td>arachidonic acid</td>
</tr>
<tr>
<td>ABC</td>
<td>ATP-binding cassette</td>
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<tr>
<td>ACSL</td>
<td>long chain acyl-CoA synthase</td>
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<td>ALA</td>
<td>α-linolenic acid</td>
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<td>ALDH</td>
<td>aldehyde dehydrogenase</td>
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<td>APC</td>
<td>antigen presenting cells</td>
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<td>APOA1</td>
<td>apolipoprotein (apo) A1</td>
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<td>B. vulgatus</td>
<td>Bacteroides vulgatus</td>
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<td>CARD15</td>
<td>caspase activation recruitment domain family member15</td>
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<td>CD</td>
<td>Crohn’s disease</td>
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<td>cDNA</td>
<td>complementary DNA</td>
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<td>CES</td>
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<td>CFU</td>
<td>colony forming units</td>
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<td>C. perfringens</td>
<td>Clostridium perfringens</td>
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<td>DGGE</td>
<td>denaturing gradient gel electrophoresis</td>
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<td>docosahexaenoic acid</td>
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<td>DNA</td>
<td>deoxyribonucleic acid</td>
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<td>dinitrobenzene sulfonic acid</td>
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<td>Helicobacter pullorum</td>
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<td>H&amp;E</td>
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<td>hepatocyte nuclear factor 4</td>
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<td>IBD</td>
<td>inflammatory bowel disease</td>
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<td>ICAM</td>
<td>intercellular adhesion molecule</td>
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</tr>
<tr>
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<td>Ingenuity Pathway Analaysis</td>
</tr>
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<td>LOX</td>
<td>lipoxygenase</td>
</tr>
</tbody>
</table>
lsd  least significant difference
LPS  lipopolysaccharide
LT  leukotriene
LX  lipoxin
LXR  liver X receptor
MAPK  mitogen-activated protein kinase
MHC  major histocompatibility class
MMP  matrix metallopeptidase
mRNA  messenger RNA
MUFA  monounsaturated fatty acid
NCBI  National Centre for Biotechnology Information
NFκB  nuclear factor kappa-light-chain-enhancer of activated B cells
NOD2  nucleotide-binding oligomerisation domain containing 2
OA  oleic acid
PBS  phosphate-buffered saline
PCR  polymerase chain reaction
PG  prostaglandin
PPARα, β/δ, γ  peroxisome proliferator-activated receptor alpha, beta/delta or gamma
PPRE  PPAR response element
PUFA  polyunsaturated fatty acids
qRT-PCR  quantitative real-time reverse transcription PCR
Rho GDIβ  Rho GDP dissociation inhibitor (GDI) beta
RNA  ribonucleic acid
rRNA  ribosomal RNA
ROS  reactive oxygen species
RXR  retinoid X receptor
SAA  serum amyloid A
SEM  standard error of mean
SCID  severe combined immune deficiency
SNP  single nucleotide polymorphism
SOCS  suppressor of cytokine signalling
spp.  species
SREBP  sterol regulatory element binding protein
STAT  signal transducer and activator of transcription
SULT  sulfotransferase
TAE, TBE  tris-acetate-EDTA, Tris-borate-EDTA buffer
TG  triglyceride
Th  T helper
TLR  toll-like receptor
TNBS  trinitrobenzene sulfonyl acid
TNFα  tumour necrosis factor alpha
TSS  transcription start site
TX  thromboxane
UC  ulcerative colitis
VNN1  vanin 1