DOI 10.1002/mnfr.200900543 *Mol. Nutr. Food Res.* 2010, *54*, S62–S74

RESEARCH ARTICLE

Dietary arachidonic acid-mediated effects on colon inflammation using transcriptome analysis

Bianca Knoch[1,2], Matthew P. G. Barnett[1]*, Warren C. McNabb[1]*, Shuotun Zhu[3]*, Zaneta A. Park[4]*, Anar Khan[4] and Nicole C. Roy[1]**

[1] Food, Metabolism and Microbiology Section, Food and Textiles Group, AgResearch Limited, Palmerston North, New Zealand
[2] Institute of Food, Nutrition and Human Health, Massey University, Palmerston North, New Zealand
[3] Faculty of Medical and Health Sciences, The University of Auckland, Auckland, New Zealand
[4] Bioinformatics, Mathematics and Statistics Section, AgResearch Limited, Palmerston North and Mosgiel, New Zealand

Increased levels of n-6 arachidonic acid (AA), a precursor of pro-inflammatory eicosanoids, have been found in the colon mucosa of inflammatory bowel disease patients when compared with healthy subjects. The hypothesis was that dietary AA would aggravate colon inflammation by changing expression of genes in inflammatory signaling pathways. AA-enriched diet was fed to IL10 gene-deficient ($Il10^{-/-}$) mice, model of a inflammatory bowel disease, and compared with $Il10^{-/-}$ mice fed an oleic acid control diet. Effects of AA on gene expression profiles during colitis were examined using whole genome microarray analysis. Dietary AA decreased the expression levels of some colonic genes in ER stress, complement system, nuclear respiratory factor 2-mediated oxidative stress and positive acute phase response pathways compared with $Il10^{-/-}$ mice fed an oleic acid diet. AA increased the expression levels of fatty acid catabolism genes, but decreased that of lipid synthesis genes during colitis, likely by sterol regulatory element binding transcription factor 1 and target gene regulation. A link has been suggested between AA and reduction of intestinal fibrosis by down-regulating the expression levels of pro-inflammatory and fibrotic marker genes. Contrary to the hypothesis, these findings suggest that dietary AA, in the present experimental conditions, is not pro-inflammatory, reduces ER stress and protects colonocytes from oxidative stress in $Il10^{-/-}$ mice.

Received: November 11, 2009
Revised: March 16, 2010
Accepted: March 22, 2010

Keywords:
Colitis / Gene expression / IL10 gene-deficient mice / n-6 Polyunsaturated fatty acid

1 Introduction

Long chain PUFA are involved in cellular processes such as membrane biosynthesis, energy metabolism, eicosanoid production, signal transduction and regulating the expression of genes that modulate inflammatory processes by altering fatty acid metabolism in intestinal epithelial cells [1, 2]. Metabolism of *n-3* and *n-6* PUFA is interlinked. For

Correspondence: Dr. Nicole C. Roy, Food Nutrition Genomics, Food, Metabolism and Microbiology Section, AgResearch Limited, Grasslands Research Centre, Private Bag 11008, Palmerston North 4442, New Zealand
E-mail: nicole.roy@agresearch.co.nz
Fax: +64-6-351-8003

Abbreviations: AA, arachidonic acid; **Aldh1a1,** aldehyde dehydrogenase 1 family member A1; **CANX,** calnexin; **CAR,** constitutive androstane receptor; **CES,** carboxylesterase; **COX,** cyclooxygenase; **CYP,** cytochrome P450 family members; **EIF2AK3,** eukaryotic translation initiation factor 2-alpha kinase 3; **EPA,** eicosapentaenoic acid; **FC,** fold change; **GO,** gene ontology; **HETE,** hydroxyeicosatetraenoic acid; **HIS,** histological injury score; **IBD,** inflammatory bowel disease; **IGFBP5,** insulin-like growth factor binding protein 5; ***Il10*$^{-/-}$,** IL10 gene-deficient; **IL1B,** IL1 beta; **IPA,** Ingenuity Pathway Analysis; **KEAP1,** Kelch-like ECH associated protein 1; **Limma,** linear models for microarray analysis; **LXR,** liver X receptor; **MMP,** matrix metalloproteinase; **NFκB,** nuclear factor-κB; **NRF2,** nuclear respiratory factor 2; **OA,** oleic acid; **PPARα,** peroxisome proliferator-activated receptor alpha; **RXR,** retinoid X receptor; **SAA,** serum amyloid A; **SREBF1,** sterol regulatory element binding transcription factor 1; **SULT1,** sulfotransferase family 1 member 1; **TNF,** tumor necrosis factor; **TRAF2,** TNF receptor associated factor 2

*These authors are part of Nutrigenomics New Zealand (www.nutrigenomics.org.nz).

example, the *n*–3 PUFA eicosapentaenoic acid (EPA) competes with arachidonic acid (AA), the most abundant n-6 PUFA in animal cell membranes, for incorporation into membrane phospholipids, replacing it and blocking the production of pro-inflammatory eicosanoids [3]. Higher levels of AA are also found in the colon mucosa of inflammatory bowel disease (IBD) patients compared with that of healthy subjects [4].

Epidemiological studies have associated increased intake of dietary *n*-6 PUFA and decreased intake of *n*-3 PUFA with a higher incidence of IBD [5, 6]. This is supported by a study with IL10 gene-deficient ($Il10^{-/-}$) mice (a model for a T-helper type 1-mediated colitis), which found that *n*-6 PUFA-rich corn oil increased colon inflammation compared with *n*-3 PUFA-rich fish oil [7]. Although AA has been postulated to have pro-inflammatory effects compared with EPA [2], another study has reported that feeding an AA ethyl ester oil-enriched diet to mice with dextran sodium sulfate-induced colitis increased AA content in colon tissue without increasing colon inflammation. It also reduced weight loss and diarrhea scores compared with the diets containing *n*-3 PUFA-rich fish oil and *n*-9 oleic acid (OA)-rich sunflower seed oil [8].

Dietary PUFA can regulate the expression levels of several genes crucial for immuno-regulatory reactions [9] by interacting with transcription factors that play an important role in IBD [10, 11]. Both *n*-6 and *n*-3 PUFA and their metabolites are natural ligands for peroxisome proliferator-activated receptors (PPARs) such as PPARα [11, 12]. PPARα induces changes in the expression levels of genes involved in fatty acid oxidation and lipoprotein metabolism and inhibits that of genes coding for pro-inflammatory proteins. PUFA can inhibit lipogenic (cholesterol, triglyceride and fatty acid synthesis) gene expression by suppression of the transcription factor sterol regulatory element binding protein (SREBP) [11, 12]. PUFA also regulate another transcription factor, nuclear factor-κB (NFκB), which controls the expression of pro-inflammatory genes (*e.g.* cyclooxygenase 2 (COX2) and IL6). AA can induce NFκB translocation and subsequent activation of target gene transcription leading to inflammation, whereas EPA inhibits NFκB translocation by PPARα-mediated activation, resulting in decreased pro-inflammatory and immuno-regulatory genes and proteins [12]. The interaction of PUFA with the transcription factors SREBP and NFκB in the regulation of fatty acid metabolism and immune and inflammatory responses is still poorly defined. Rodent studies in which AA-enriched diets were fed showed AA present at inflammatory sites, and acting as an important regulator of inflammatory responses within tissues [13], but AA also has protective effects in mice with experimental colitis [8].

The present study was designed to test whether dietary AA aggravates colon inflammation in $Il10^{-/-}$ mice, particularly the immune and inflammatory pathways. $Il10^{-/-}$ and C57 mice were inoculated with a mix of ''complex intestinal microflora'' and pure cultures of *Enterococcus faecalis* [14, 15], and genome-wide expression profiling of colon tissues was used to identify gene regulatory networks by which dietary AA affected colon inflammation. Dietary AA supplementation was also studied in non-inflamed colon tissue of C57 mice to determine levels of gene expression compared with inflamed colon. The genotype comparison ($Il10^{-/-}$ *versus* C57 mice) on OA diet reflects the changes in gene expression levels due to colon inflammation alone.

2 Materials and methods

2.1 Animals, treatment and tissue collection

This study was approved by the AgResearch Ruakura Animal Ethics Committee in Hamilton, New Zealand according to the Animal Protection Act and Animal Protection Regulations and amendments, animal ethics approval no. 10587. Twelve male $Il10^{-/-}$ (C57BL/6J background, formal designation B6.129P2-*Il10*<tm1Cgn>/J) and twelve male C57 control (C57BL/6J) mice were obtained from The Jackson Laboratory (Bar Harbor, ME, USA). All mice were 35 days or 5 wk of age at the start of the trial. The animals were individually housed under conventional conditions in standard shoebox size cages containing untreated wood shavings. Water was provided *ad libitum*. After 4 days, all mice were inoculated orally with a mixture of commensal bacteria, as previously described [14, 15], to obtain a more consistent and reproducible intestinal inflammation.

Mice were randomly assigned to one of two AIN-76A-based diets made in-house containing equivalent amounts of OA or AA ethyl esters (Table 1), with six $Il10^{-/-}$ and C57 mice on each diet. Ethyl esters of AA, OA, linoleic acid and α-linolenic acid were 99% pure (NuCheck, Elysian, MN, USA). The OA ethyl ester was chosen as a control fatty acid because it has no effect on tissue AA content or on eicosanoid biosynthesis [16]. Both diets contained 1% corn oil, and this was supplemented with purified linoleic acid and α-linolenic acid to meet the nutritional requirements for mice [17]. The OA and AA diets were isocaloric, isoproteinic and varied only in lipid composition, both containing 5% w/w fat in total. Analysis of the fatty acid content of the diets was as described previously [14] (Table 1) and was completed before the study began to confirm fatty acid composition. To reduce lipid oxidation, diets were stored at −80°C and supplied fresh daily (3.5–4.0 g/mouse). Throughout the experimental period, dietary intake was estimated and adjusted daily to equal the mean amount of food consumed by $Il10^{-/-}$ mice on the previous day to ensure similar intakes between C57 and $Il10^{-/-}$ mice. All mice were weighed three times a week and carefully monitored for disease symptoms (weight loss, soft faeces, inactivity, *etc.*).

Table 1. Composition and analysis of key fatty acids of the two experimental diets

	OA diet	AA diet
Ingredient (%)		
Casein[a)]	20	20
DL-methionine[b)]	0.3	0.3
Sucrose[c)]	50	50
Dextrin[d)]	15	15
Arbocel, non-nutritive bulk[e)]	5	5
Corn oil[f)]	1	1
C18:2, 99%+[g)]	0.2	0.2
C18:3, 99%+[h)]	0.06	0.06
OA, 99%+	3.74	0
AA, 99%+	0	3.74
Choline bitartrate[b)]	0.2	0.2
AIN-76A mineral mix[i)]	3.5	3.5
AIN-76A vitamin mix[i)]	1	1
TBHQ[j)]	0.001	0.001
Analysis		
Gross energy (MJ/kg)	18.14	18.16
Nitrogen (%)	3.27	3.01
Lipid (%)	5.15	5.18
Fatty acids (%)[k)]		
Palmitic acid (C16:0)	0.14	0.13
OA (C18:1)	3.03	0.26
AA (C20:4)	0	2.75
Linoleic acid (C18:2n-6)	0.53	0.50
Linolenic acid (C18:3n-3)	0.05	0.05

Both diets were prepared in-house using ingredients from the suppliers listed, and according to the compositions shown.

a) Alacid, lactic casein 30 mesh, NZMP Ltd., Wellington, New Zealand.
b) Sigma, Sigma-Aldrich, St. Louis, MO, USA.
c) Caster sugar, Chelsea, New Zealand Sugar Company, Auckland, New Zealand.
d) Wheaten cornstarch, Golden Harves, Primary Foods, Auckland, New Zealand.
e) Arbocell B600, J. Rettenmaier & Sohne, Rosenberg, Germany.
f) Tradewinds, Davis Trading, Palmerston North, New Zealand.
g) C18:2, linoleic acid.
h) C18:3, α-linolenic acid.
i) Prepared in-house based on the AIN-76A diet formulation [45].
j) TBHQ, tertiary butylhydroquinone as antioxidant.
k) Those analysed.

At 77 days of age, all mice were randomly divided into sampling groups with 12 mice euthanized each day. A previous study showed that by 77 days of age, inoculated *Il10*$^{-/-}$ mice had an acceptable and consistent level of colon inflammation [18]. On the last day, mice were fasted overnight (14 h), then food was reoffered for 2 h and again removed for the 2 h prior to sampling [14]. This was done to minimize the variation in time between the last food intake and tissue sampling [19]. Mice were euthanized by CO_2 asphyxiation and cervical dislocation, and cardiac puncture was then performed. Plasma was separated from the blood, frozen in liquid nitrogen and stored at −80°C for analysis of serum amyloid A (SAA). The intestine was quickly removed, cut open lengthwise, flushed with 0.9% sodium chloride to remove digesta and divided into duodenum, jejunum, ileum and colon. The proximal part of the colon was divided in half. The epithelial layer of the proximal first half of the colon was scraped off and the mucus frozen at −80°C. The distal half of the colon was divided in half again. The first piece was stored in phosphate buffered 10% formaldehyde at room temperature for histological analysis, and the second piece was frozen in liquid nitrogen and stored at −80°C for gene expression profiling (microarrays, quantitative RT-PCR).

2.2 Histology and SAA

Formaldehyde-fixed colon samples were embedded in a paraffin block, cut into 5 μm thick sections and stained with haematoxylin and eosin. The stained sections were evaluated by a pathologist (who had no knowledge of the experimental protocol) for indications of inflammation, including scoring inflammatory cell infiltration (monocytes, neutrophils, fibrin exudation and lymphangiectasis), tissue destruction (enterocyte loss, ballooning degeneration, oedema and mucosal atrophy) and tissue repair (hyperplasia, angiogenesis, granulomas and fibrosis). A rating score between 0 (no change from normal tissue section) and 3 (lesions involving most areas and all layers of the tissue section including mucosa, muscle and omental fat) was given for each aspect of inflammatory lesions, tissue destruction and tissue repair. The histological injury score (HIS) was calculated as: HIS = (inflammatory lesions score × 2)+tissue destruction score+tissue repair score. Monocyte and neutrophil infiltration represented the main characteristic of the observed colon inflammation; therefore the sum of inflammatory lesions was multiplied by 2 to give more weight to this value. A total colon HIS score from 0–3 was regarded as no inflammation, from 4–6 as moderate inflammation and ≥7 as severe inflammation.

Inflammation was also assessed by measuring SAA levels in plasma using a murine-specific SAA kit (Tridelta Development, Maynooth, County Kildare, Ireland), according to the manufacturer's instructions and as described previously [14].

2.3 RNA isolation

Total RNA from colon tissue was isolated by homogenizing the samples in TRIzol (Invitrogen, Auckland, New Zealand), a modification of the acid guanidinium thiocyanate-phenol-chloroform extraction method, according to the manufacturer's instructions. RNA levels and sub-unit ratios were quantified with a Nanodrop ND-1000 spectrophotometer (NanoDrop Technologies, Wilmington, DE, USA), and RNA integrity assessed with an RNA 6000 Nano Labchip kit using the Agilent 2100 Bioanalyzer (Agilent Technologies, Santa

Clara, CA, USA). Extracted RNA was purified using the Qiagen RNeasy Mini kit (Qiagen, San Diego, CA, USA). Only total RNA with an OD 260/280 ratio >2.0, a Bioanalyzer 28 s/18 s peak ratio >1.2 and an RNA integrity number >8 was used for microarray hybridization. An equimolar pool of colon RNA extracts from two to three mice *per* treatment was made to minimize individual variation. For the reference microarray design, the reference RNA was prepared using an equal amount of total purified RNA extracted from small intestine, colon, kidney, liver and fetuses of normal, healthy growing Swiss mice.

2.4 Microarrays

The Low RNA Input Linear Amplification kit (Agilent Technologies) was used according to the manufacturer's instructions to synthesize and label cRNA with cyanine 3 (samples) and cyanine 5 (reference) dye (10 mM; Perkin-Elmer/NEN Life Science (NEL 580, 581), Boston, MA, USA). Dye incorporation was monitored spectrophotometrically using a Nanodrop ND-1000. The labeled cRNA was hybridized onto 44 k mouse oligonucleotide arrays (Agilent Technologies, G4122-60510) using the *in situ* Hybridization plus kit (Agilent Technologies) and following the manufacturer's protocols. These arrays comprise 44 290 probe sets representing 33 994 unique genes. After hybridization, the slides were washed in solutions I, II and III (Agilent Technologies), air-dried and scanned immediately with a GenePix Professional 4200A scanner (Molecular Devices, Sunnyvale, CA, USA). Spots were identified and quantified using GenePix 6.0 software (Molecular Devices). Irregular features and poor quality spots were eliminated before analysis. Array data have been submitted to the Gene Expression Omnibus, accession number GSE12028.

2.5 Analysis of microarray data

Linear models for microarray analysis (Limma) from the Bioconductor project was used for statistical analysis of the microarray data [20]. Data quality was assessed on diagnostic plots (boxplots and density plots) and spatial images generated from the raw (non-processed) data. All arrays passed the quality control and were included in the analyses. Intensity ratios for all microarray spots were normalized using a global loess smoothing procedure to remove the effect of systematic variation in the microarrays and no background correction was necessary due to homogeneous hybridization [21]. The normalized data from the arrays of each dietary group were $\log_2$-transformed, as is typical for microarray data, and averaged. Three experimental comparisons were performed: (i) diet comparison (AA *versus* OA) in *Il10*$^{-/-}$ mice; (ii) diet comparison (AA *versus* OA) in C57 mice; and (iii) genotype comparison of inflammation on the OA control diet, *Il10*$^{-/-}$ mice (inflamed) *versus* C57 mice (not inflamed). For each comparison, a list of differentially expressed probe sets was generated by calculating moderated *t*-statistics for each probe set using the Limma package, which implements an empirical Bayes approach to assign differential gene expression.

2.6 Quantitative RT-PCR

Total RNA (0.6 μg) of all sample pools used for microarrays and all individual animals was reverse transcribed using the Transcriptor First Strand cDNA Synthesis kit (Roche Diagnostics, Mannheim, Germany). Quantitative RT-PCR was performed on a LightCycler 480 Instrument (Roche Diagnostics). The LightCycler 480 SYBR Green I Master kit (Roche Diagnostics) was used according to the manufacturer's protocol to quantify ten differentially expressed genes, *i.e.* ATP-binding cassette, sub-family B member 1, aldehyde dehydrogenase 1 family member A1 (*Aldh1a1*), carboxylesterase 2 (*Ces2*), fatty acid binding protein 2, insulin-like growth factor binding protein 5 (*Igfbp5*), IL1 beta (*Il1b*), matrix metalloproteinase 13 (*Mmp13*), *Ppara,* sterol regulatory element binding transcription factor 1 (*Srebf1*) and sulfotransferase family 1A member 1 (*Sult1a1*) and the results expressed as mRNA level normalized to calnexin (*Canx*). Primer sequences used for quantitative RT-PCR are available in Supporting Information Table S1. These genes were chosen based on pathway analysis, as they seemed to be important in the effect of AA on colon inflammation. *Canx* was chosen as a suitable normalization reference gene after evaluating a panel of candidate control genes for similar expression level amongst all samples. LightCycler 480 Relative Quantification Software was used to calculate mRNA concentrations based on the appropriate standard curves and normalized ratios (target/reference).

2.7 Bioinformatics analysis of pathways, networks and functions

Ingenuity Pathway Analysis software (IPA, Version 7.0, Ingenuity Systems, Redwood City, CA, USA; www.ingenuity.com) was used for pathway and network analyses of differentially expressed probes in the microarray data set. The data files containing probe identifier (gene accession numbers) and the corresponding changes in expression values (fold change (FC) and *p*-value) were uploaded into IPA. Probes from the microarray data set that satisfied the cut-off criteria of 1.3 FC in expression levels (up- or down-regulated) and a $p < 0.01$ were considered for the pathway and network analysis. Recent studies have shown that a FC $\geq$ 1.6 might underestimate the number of differentially

expressed genes from a dietary treatment [22, 23]. Each probe was mapped to its corresponding gene object in the Ingenuity Pathway Knowledge Base. Metabolic and regulatory processes were assigned to each gene and the pathways ranked according to the significance of the data. Pathways were considered significantly affected by dietary AA when $p<0.01$ (calculated by the Fisher's exact test), where at least 10% of the genes from a particular pathway were differentially expressed in the microarray data set. Biological interaction networks were generated by IPA to highlight regulatory relationships between genes from the data set and other gene objects stored in the Ingenuity Pathway Knowledge Base.

FUNC, a tool for gene ontology (GO) enrichment analysis, was applied as a complementary method to IPA to relate changes in gene expression levels to specific biological functions and pathways. For the FUNC approach, differentially expressed genes ($p<0.01$, FC$\geq$1.3) from each comparison were analysed for over-represented GO terms using the hypergeometric test and subsequent refinement provided by the FUNC package [24]. GenBank accession numbers were used as the basis for the analysis, and duplicate accessions were consolidated into one count. Only GO terms annotated to at least ten genes were considered. GO terms with false discovery rate <0.05 according to the hypergeometric test were passed to the refinement step, and GO terms with $p<0.05$ after refinement were considered over-represented in the list of differentially expressed genes.

2.8 Statistics

SAS (9.1.3 Service Pack 4, SAS Institute, Cary, NC, USA) was used for statistical analyses of body weight and dietary intakes, and GenStat (10th edition, VSN International, Hemel Hempstead, UK) used to analyze HIS, SAA and quantitative RT-PCR data. Analysis of repeated measurements with ante-dependence and autoregressive variance-covariance structures was used to fit the body weight and dietary intake data and to test for treatment differences in each strain over time. Linear regression and ANOVA was applied to estimate whether the rate of body weight gain differed between AA and OA diets for *Il10*$^{-/-}$ and C57 mice. For ANOVA analyses, log-transformations of raw data were performed in cases of unequal variances. Differences in HIS between AA and OA diets for each mouse strain were analyzed using an ANOVA with pooled variance. A constant was added to the SAA data prior to log-transformation to avoid negative values found in C57 mice. Differences were considered significant at $p<0.05$. A trend was declared when $0.05<p<0.10$. One *Il10*$^{-/-}$ mouse from the OA diet group died during the experiment (for unknown reasons) and no samples could be obtained from this animal for histological examination, scoring and statistics.

3 Results

3.1 Body weight and dietary intake

The average body weight and dietary intake of mice from 35 to 75 days of age are shown in Fig. 1. Average body weight of *Il10*$^{-/-}$ and C57 mice increased over time in both diet groups. From days 47 to 75 of age, C57 mice fed AA gained less weight than C57 mice fed OA ($p<0.05$). The body weight of *Il10*$^{-/-}$ mice fed the AA diet compared with *Il10*$^{-/-}$ mice fed the OA diet was reduced only at day 54 ($p=0.02$). There was a significant decrease ($p<0.05$) in dietary intake from day 61 to 65 and from day 70 to 72 between AA- and OA-treated *Il10*$^{-/-}$ mice.

3.2 Histology and SAA

There was a trend ($0.05<p<0.10$) to reduce colon HIS in AA-fed *Il10*$^{-/-}$ mice compared with OA-fed *Il10*$^{-/-}$ mice, but no difference between C57 mice fed the OA and AA diets (Table 2). *Il10*$^{-/-}$ mice on the OA diet showed severe (HIS$\geq$7) colon inflammation, AA-fed *Il10*$^{-/-}$ mice showed moderate (HIS 4–6) inflammation, and C57 animals no sign of colon inflammation (Fig. 2). Figure 2 shows that colon HIS was mainly characterized by inflammatory cell infiltration (mostly monocytes) and to a lesser extent by tissue destruction and repair. The inflammatory lesions only occurred in some areas of the colon, but involved most layers of the colon. There was no difference in inflammatory cell infiltration or tissue destruction and repair in the colon of *Il10*$^{-/-}$ mice in the AA compared with the OA group. There was a 22-fold difference in plasma SAA concentration between *Il10*$^{-/-}$ and C57 mice on the OA diet ($p<0.05$; Table 2). There was also a trend ($0.05<p<0.10$) to reduced plasma SAA in AA-fed *Il10*$^{-/-}$ mice compared with OA-fed *Il10*$^{-/-}$ mice.

3.3 Effects of dietary AA on gene expression during colon inflammation

The number of shared and unique differentially expressed genes for each comparison is summarized in Fig. 3. The following comparisons were made: (i) diet comparison (AA *versus* OA in *Il10*$^{-/-}$ mice); (ii) diet comparison (AA *versus* OA in C57 mice) and (iii) genotype comparison (inflammation; *Il10*$^{-/-}$ *versus* C57 mice on the OA diet). In total, 426 genes were differentially expressed ($p<0.01$; FC$\geq$1.3) in the colon of *Il10*$^{-/-}$ mice fed the AA compared with the OA diet (213 up- and 213 down-regulated). Of these, 208 of the 426 differentially expressed genes were common to *Il10*$^{-/-}$ mice fed OA (compared with C57 on the same diet) and *Il10*$^{-/-}$ mice fed AA (compared with *Il10*$^{-/-}$ mice fed OA), and 202 genes were related to the effect of dietary AA on colon inflammation. In C57 mice, the AA diet resulted in differential expression of only 217 genes, and of those, 7 genes were common to those found in AA-fed *Il10*$^{-/-}$ mice.

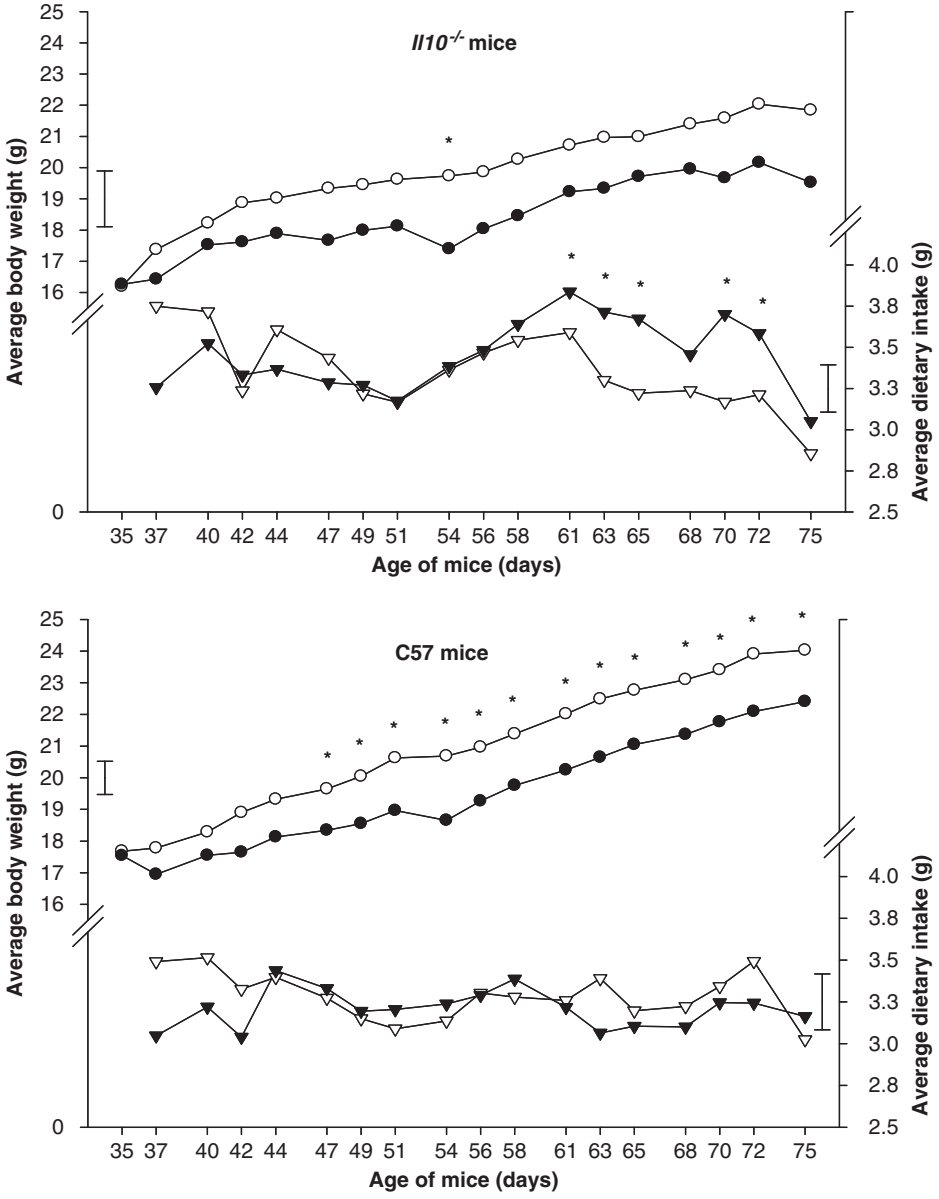

Figure 1. Average body weight (g) and dietary intake (g) of *Il10*$^{-/-}$ and C57 mice fed OA and AA diets for the experimental period. Error bars represent the least significant difference (lsd, 5% level) over the time points within each strain. *indicate significant daily difference ($p < 0.05$). ○ = body weight OA diet; ● = body weight AA diet; Δ = dietary intake OA diet; ▲ = dietary intake AA diet.

Microarray results were confirmed for selected genes by quantitative RT-PCR for the pooled colon samples (Table 3). The *Srebf1* gene showed a tendency but not a significant expression ($p = 0.06$) by quantitative RT-PCR in *Il10*$^{-/-}$ mice (unlike the array result) comparing AA with OA diet, due to higher variability in the AA-fed *Il10*$^{-/-}$ mice.

3.4 Pathway analysis of the dietary AA effects on colon inflammation

The metabolic and regulatory pathways most affected by dietary AA during colon inflammation in *Il10*$^{-/-}$ mice (compared with OA-fed *Il10*$^{-/-}$ mice), in which at least 10% of the genes were differentially expressed according to IPA, are discussed (Supporting Information Table S2). These pathways involved stress and immune/inflammatory response, retinoid X receptor (RXR) activation pathways, lipid metabolism and fibrosis development.

The expression levels of genes involved in stress and immune/inflammatory response pathways (ER stress, complement system, nuclear respiratory factor 2 (NRF2)-mediated oxidative stress response and acute phase response signaling) were down-regulated in the colon of AA-fed *Il10*$^{-/-}$ mice. This includes genes coding for complement component 1 q or s subcomponent (*C1qa*, *C1qb*, *C1s*), eukaryotic translation initiation factor 2-alpha kinase 3 (*Eif2ak3*), *Il1b*, IL1 receptor type 2 (*Il1r2*), Kelch-like ECH

Table 2. Inflammation and HIS in colon tissue, and plasma SAA concentrations in C57 and *Il10*$^{-/-}$ mice fed OA or AA diets

	C57 mice		*Il10*$^{-/-}$ mice		
	OA diet	AA diet	OA diet[a)]	AA diet	lsd[b)]
Colon HIS	0.13	0.34	7.00	5.00	3.00
Inflammatory cell infiltration (×2)	0.13	0.34	5.30	3.58	1.95
Tissue destruction	0	0	1.30	1.17	1.17
Tissue repair	0	0	0.40	0.25	0.47
SAA (μg/mL)[c)]	23.8 (4.28)	82.2 (4.87)	538 (6.38)	161 (5.35)	1.26

A trend ($0.05 < p < 0.10$) has been observed for colon HIS and plasma SAA.
a) One *Il10*$^{-/-}$ mouse from the OA group died during the experiment and could not be taken into account for statistical analysis. The scores for total colon HIS and inflammation type (inflammatory cell infiltration, tissue destruction and repair) are shown as average of all mice in the treatment group and lsd (statistical analysis within *Il10*$^{-/-}$ mice, mostly zero scores for C57 mice).
b) lsd, least significant difference.
c) SAA is shown as average with log-transformed values in parentheses, and lsd of the log-transformed data.

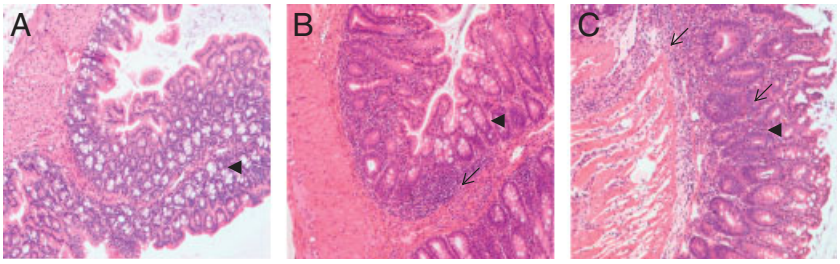

Figure 2. Haematoxylin and eosin stained colon sections of a C57 and an *Il10*$^{-/-}$ mouse. (A) Colon section (× 100) from a normal, non-inflamed C57 mouse fed AA diet. (B) Moderate inflamed colon section (× 100) from an *Il10*$^{-/-}$ mouse fed AA diet. (C) Severe inflamed colon section (× 100) from an *Il10*$^{-/-}$ mouse fed OA diet. Lesions are mainly caused by inflammatory cell infiltration with a high number of monocytes and neutrophils (←), loss of crypt cells through crypt abscesses and loss of goblet cell (◀) in the lamina propria.

associated protein 1 (*Keap1*), tumor necrosis factor (*Tnf*) and TNF receptor associated factor 2 (*Traf2*).

Genes from pathways related to RXR activation were also affected in the colon of AA- compared with OA-fed *Il10*$^{-/-}$ mice. These include constitutive androstane receptor (CAR)/RXR activation, liver X receptor (LXR)/RXR activation, LPS/IL1-mediated inhibition of RXR function and co-factor metabolism. RXR, as a major heterodimeric partner for nuclear receptors such as CAR and LXR, regulates the expression of several genes of xenobiotic metabolism and immune/inflammatory response (*Il1a*, *Il1b*, *Il1r2* and *Tnf*). Analysis of biological networks by IPA showed interaction of genes involved in xenobiotic metabolism with those involved in stress and immune/inflammatory response (Fig. 4). The expression levels of xenobiotic metabolism genes up-regulated in AA-fed *Il10*$^{-/-}$ mice included *Aldh1a1*, cytochrome P450 family members (CYP, *e.g. Cyp3a5*), glutathione *S*-transferase A4 (*Gsta4*), flavin containing monooxygenase2 (*Fmo2*), sulfotransferase family 1 members (*Sult1a1*, *Sult1c2*) and uridine diphosphate glucuronosyltransferase family 1 member (*Ugt1a1*). ABC transporter (Phase III) enzyme (*e.g. Abcb1*), and carboxylesterase (*e.g. Ces2*) gene expression levels, were significantly increased in the colon of AA-fed *Il10*$^{-/-}$ mice when examined with the more sensitive quantitative RT-PCR. The mRNA abundance of retinol dehydrogenase 5 (*Rdh5*), a gene involved in retinoic acid synthesis, was increased in the colon of AA-fed *Il10*$^{-/-}$ mice.

The AA-fed *Il10*$^{-/-}$ mice had decreased colonic expression levels of the *Srebf1* gene, which is also regulated by RXR. Gene networks show that the decrease in *Srebf1* gene expression levels might be due to a reduction in *Tnf* mRNA abundance and indirectly due to a reduction in *Il1b* gene expression levels. Expression of the *Srebf1* gene is further linked to decreased expression levels of the cholesterol biosynthesis genes 3-hydroxy-3-methylglutaryl-Coenzyme A (CoA) reductase (*Hmgcr*) and farnesyl diphosphate synthase (*Fdps*). The expression levels of other lipogenic transcription factors such as *Ppara* and members of the nuclear receptor superfamily *Nr1h3* (LXRα), *Nr1i3* (CAR) and *Rxra* (RXRα) were unchanged in the AA-fed *Il10*$^{-/-}$ mice. Dietary AA up-regulated the expression levels of genes of fatty acid catabolism including acetyl-Co A acyltransferase 2 (*Acaa2*) and acyl-CoA thioesterase 5 (*Acot5*), whereas that of genes of fatty acid synthesis, *e.g.*

acetyl-CoA carboxylase alpha (*Acaca*) were down-regulated in the colon of *Il10*$^{-/-}$ mice.

Genes of the fibrosis pathway (such as collagen type 1 alpha 2 (*Col1a2*), fibroblast growth factor 7 (*Fgf7*) and *Igfbp5*), had decreased mRNA levels in the colon of AA- compared with OA-fed *Il10*$^{-/-}$ mice. Decreased expression levels of fibrosis development genes was also associated with decreased expression levels of genes involved in tissue degradation (*e.g. Mmp13*) and immune/inflammatory response in the colon of AA-compared with OA-fed *Il10*$^{-/-}$ mice. In contrast, the levels of these genes were more highly expressed in *Il10*$^{-/-}$ mice on the OA diet *versus* C57 mice on OA diet.

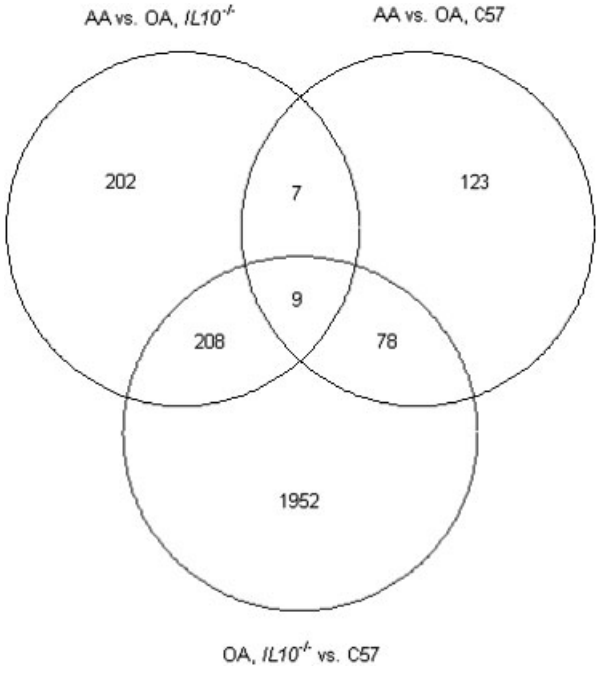

Figure 3. Venn diagram showing the numbers of differentially expressed genes unique to or in common with the genotype comparison (*Il10*$^{-/-}$ mice *versus* C57 mice on OA diet) and the two diet comparisons (AA *versus* OA in *Il10*$^{-/-}$ mice and AA *versus* OA in C57 mice). Numbers represent genes that satisfied the criteria of $p < 0.01$ and FC ≥ 1.3 in each comparison.

3.5 Functional analysis of dietary AA effects on colon inflammation

GO enrichment analysis was performed on the differentially expressed gene list to identify biological processes and functions affected by dietary AA (compared with OA-fed *Il10*$^{-/-}$ mice). Table 4 shows 15 terms most strongly over-represented in the colon of *Il10*$^{-/-}$ mice fed AA diet compared with *Il10*$^{-/-}$ mice fed OA diet. These included GO terms such as extracellular space (complement component, insulin-like growth factor binding protein, and immunoglobulin-complexes) and collagen (fibrillar and transmembrane collagen), which can be linked to immune response and fibrosis processes, respectively. Other over-represented GO terms were antigen binding and mono-oxygenase activity, which are descriptors for immune response and lipid metabolism. The FUNC analysis agreed well with the IPA analysis, so dietary AA can be confidently linked with those processes involved in colon inflammation.

4 Discussion

Contrary to the hypothesis, this study showed beneficial effects of dietary AA on colon inflammation in *Il10*$^{-/-}$ mice,

Table 3. Validation of gene expression results from microarray analysis using quantitative RT-PCR

Gene symbol	Fold Change microarray		Fold Change quantitative RT-PCR *Canx*[a)]	
	Il10$^{-/-}$ *versus* C57 OA diet	AA *versus* OA *Il10*$^{-/-}$ mice	*Il10*$^{-/-}$ *versus* C57 OA diet	AA *versus* OA *Il10*$^{-/-}$ mice
Abcb1a	−3.3	1.6[b)]	−9.1	2.2
Aldh1a1	−2.1	1.6	−5.3	2.7
Ces2	−3.6	1.6[b)]	−7.7	3.7
Fabp2	−2.4	1.4[b)]	−14.3	2.2
Igfbp5	2.0	−2.3	3.0	−1.8
Il1b	8.5	−2.6	16.9	−3.3
Mmp13	3.2	−2.2	9.0	−3.6
Ppara	−2.5	1.4[b)]	−5.0	2.5[c)]
Srebf1	1.1[b)]	−1.7	1.8[c)]	−1.4[c)]
Sult1a1	−2.1	2.2	−8.1	2.9

a) *Canx* reference gene used to normalize the data.
b) Microarray result was not significantly different using moderated *t*-statistics in Limma. Probe sets that satisfied the criterion of ≥ 1.3 FC with moderated $p < 0.01$ considered to be significantly different.
c) Quantitative RT-PCR result showed a tendency to significance using ANOVA ($p = 0.06$).

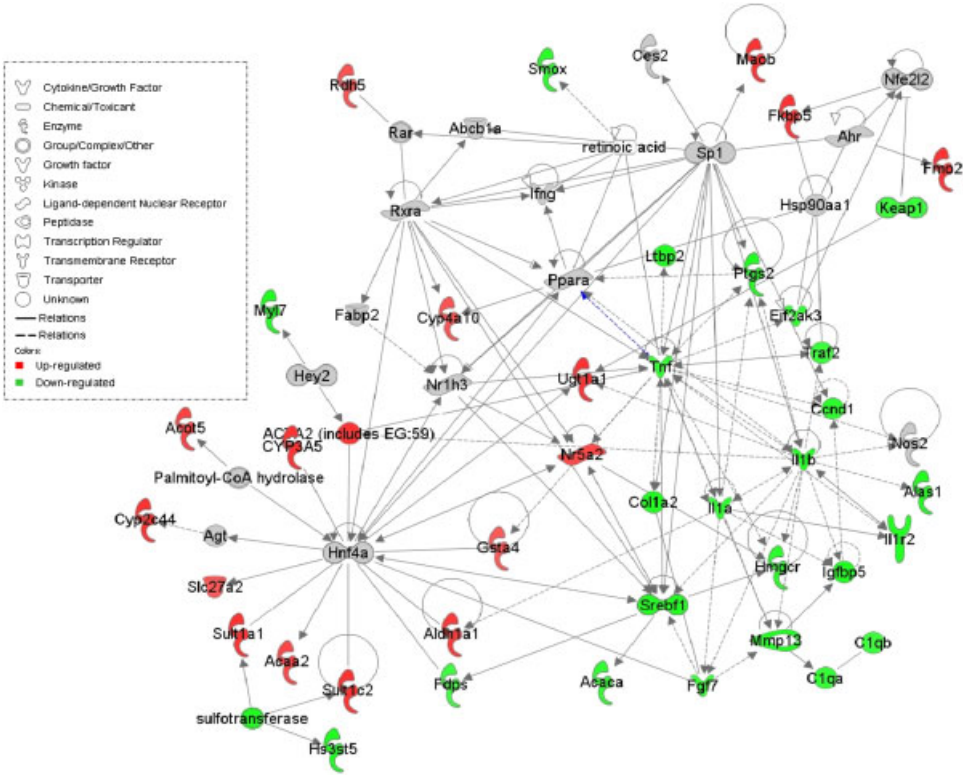

Figure 4. Biological interaction network of genes of the most significantly regulated pathways generated for the AA *versus* OA *Il10*$^{-/-}$ mice comparison using IPA. Genes or gene products are represented as nodes, and the biological relationship between two nodes is represented as an edge. All edges are supported by at least one reference from the literature. Red and green colored nodes indicate genes with up- and down-regulated expression, respectively, in *Il10*$^{-/-}$ mice fed AA diet compared with *Il10*$^{-/-}$ mice fed OA diet. The intensity of the colors specifies the degree of up- or down-regulation. Greater intensity represents a higher expression level. Nodes and edges are displayed with various shapes and labels that present the functional class of genes and the nature of the relationships between the nodes.

Table 4. GO terms over-represented in the colon of *Il10*$^{-/-}$ mice fed AA diet compared with OA-fed *Il10*$^{-/-}$ mice

GO ID	GO term	Number of genes	*p* Over-representation after data refinement
GO:0005615	Extracellular space	185	2.48E–12
GO:0005792	Microsome	23	8.68E–07
GO:0016616	Oxidoreductase activity, acting on the CH-OH group of donors, NAD or NADP as acceptor	15	6.13E–05
GO:0030020	Extracellular matrix structural constituent conferring tensile strength	8	1.28E–04
GO:0005581	Collagen	8	3.74E–04
GO:0016705	Oxidoreductase activity, acting on paired donors, with incorporation or reduction of molecular oxygen	14	3.99E–04
GO:0031672	A band	5	1.04E–03
GO:0005859	Muscle myosin complex	5	1.34E–03
GO:0003823	Antigen binding	6	1.35E–03
GO:0003924	GTPase activity	16	2.07E–03
GO:0003774	Motor activity	18	2.24E–03
GO:0004497	Monooxygenase activity	13	3.64E–03
GO:0008483	Transaminase activity	6	3.70E–03
GO:0005737	Cytoplasm	221	8.59E–03
GO:0017111	Nucleoside-triphosphatase activity	42	2.26E–02

FUNC analysis was used to identify significantly over-represented GO terms in the colon of AA-fed *Il10*$^{-/-}$ mice compared with OA-fed *Il10*$^{-/-}$ mice. GO terms annotated to at least ten genes and with false discovery rate <0.05 (according to the hypergeometric test) were passed to data refinement, and those with $p<0.05$ after refinement were considered over-represented in the list of differentially expressed genes.

using a combination of statistical, functional, pathway and network analysis of the whole murine transcriptome. Dietary AA tended to decrease colon HIS and plasma SAA in *Il10*$^{-/-}$ mice when compared with OA-fed *Il10*$^{-/-}$ mice. Genome-wide expression analysis revealed a complex network of transcript changes distinctive to the AA-fed *Il10*$^{-/-}$ mice, characterized by changes in stress and immune/inflammatory responses, fibrosis development,

lipid metabolism, RXR activation pathways and LPS/IL1-mediated inhibition of RXR function. These pathways were shown to be affected by inflammation (*Il10*$^{-/-}$ *versus* C57 mice, both fed OA, reported by Knoch *et al.* [14]); dietary AA counteracted some of these effects in *Il10*$^{-/-}$ mice (compared with OA-fed *Il10*$^{-/-}$ mice). In most cases, dietary AA fed to C57 mice did not affect these pathways (compared with OA-fed C57 mice). The following paragraphs discuss the pathways most significantly affected by dietary AA, and explain how the various transcript changes are interconnected and modulate adaptive cellular responses at the transcriptional level during the course of colon inflammation.

4.1 Dietary AA reduced colonocyte stress

Compared with dietary OA, dietary AA reduced the expression levels of several genes of ER stress (*Eif2ak3* and *Traf2*), complement system (*C1qa* and *C1qb*) and positive acute phase response (*C1s*, *Il1b* and *Tnf*) pathways in the colon of *Il10*$^{-/-}$ mice. Inhibition of complement components (*e.g.* C5) has been shown to attenuate the activity of several mediators of intestinal tissue injury including neutrophil trafficking, cytokine production and adhesion molecule expression [25]. Activation of the complement system has been linked with mediation of tissue damage, and increase in intestinal inducible nitric oxide synthase (iNOS) protein activity seems to play a role in this activation [26]. iNOS is induced by the cytokines TNF, IL1, IFNγ, and by LPS in a variety of cell types [27]. The gene network built here using IPA indicates that the reduced *Tnf* and *Il1b* cytokine gene expression levels observed in the colon of AA-fed *Il10*$^{-/-}$ mice were not linked to a change in mRNA abundance of the *Nos2* gene.

Dietary AA reduced the abundance of the *Eif2ak3 (Perk)* gene, which codes for an ER transmembrane protein kinase that phosphorylates the eukaryotic translation initiation factor eIF2α and suppresses protein synthesis in response to ER stress [28]. Decreased *Eif2ak3* gene expression levels suggest reduced ER stress in response to dietary AA, which is supported by reduced mRNA abundance of *Keap1*. Upon ER stress, NRF2 is released from its repressor KEAP1, which leads to accumulation of NRF2 in the nucleus. NRF2 binds to the antioxidant response element and activates transcription of genes coding for detoxifying and antioxidant enzymes, glutathione *S*-transferase (GST) and uridine diphosphate glucuronosyltransferase (UGT) [29]. Reduced ER stress in the colon of *Il10*$^{-/-}$ mice fed an AA diet was not associated with change in expression levels of NRF2 (*Nfe2l2*) gene, but linked to increased mRNA abundance of its target enzyme genes, *e.g. Gsta4* and *Ugt1a1*. This implies an increase in detoxification activities in the colon of those mice and likely an effect of dietary AA on NRF2 protein phosphorylation, which is not measured in this study, leading to increased GST and UGT activities. Dietary AA also increased expression levels of the *Aldh1a1* gene (which is involved in oxidative stress and prevents lipid damage by destruction of reactive aldehydes [30]) in *Il10*$^{-/-}$ mice, and decreased expression levels of the *Traf2* gene, which codes for a protein that can elicit (when activated during stress response) *c*-Jun N-terminal kinase phosphorylation and activation [31]. The findings suggest mechanisms by which dietary AA could reduce ER stress response and protect colonocytes from oxidative stress in *Il10*$^{-/-}$ mice.

4.2 Dietary AA and lipid metabolism genes

As expected, dietary supplementation with AA ethyl ester affected several genes critical to lipid metabolism in inflamed colon. Dietary AA increased fatty acid transport (*Slc27a2* or *Fatp2*), mitochondrial (*Acaa2*), peroxisomal (*Acot5*) and microsomal (*Cyp4a11*) fatty acid oxidation genes in the colon of *Il10*$^{-/-}$ mice. It decreased the expression levels of cholesterol and fatty acid (*Acaca*) synthesis genes likely *via* down-regulation of *Srebf1* and its target genes, for example, the rate-limiting enzyme of sterol biosynthesis *Hmgcr* and signaling co-factors such as *Tnf* in the colon of *Il10*$^{-/-}$ mice. These transcript changes are in agreement with existing knowledge about fatty acid transport, synthesis and metabolism; fatty acids can be β-oxidized in mitochondria or peroxisomes or ω-oxidized in microsomes for energy production and cell signaling [32, 33].

In this study, COX-dependent AA metabolism was reduced in the AA-fed *Il10*$^{-/-}$ mouse colon, because expression levels of the *Cox2* (*Ptgs2*) gene, and subsequently inflammatory response genes, were decreased, suggesting a protective effect of dietary AA in the colon of *Il10*$^{-/-}$ mice. This finding does not agree with a study where supplementation of purified AA (1.5% AA ethyl ester) to an EPA diet (10% w/w fat total) was tumorigenic by reducing the anti-tumorigenic effect of purified EPA on colorectal cancer using the *Apc*$^{Min/+}$ mouse model [34]. AA can be metabolized by different enzyme systems: COX, lipoxygenases (LOX) and CYP [35]. COX (*e.g.* prostaglandins, thromboxane and prostacyclin) and LOX (*e.g.* leukotrienes and hydroxyeicosatetraenoic acids) products are differentially distributed in the intestine with effects on intestinal motility, secretion and blood flow [35]. CYP in intestinal microsomal fractions metabolize AA to several oxygenated metabolites (epoxyeicosatrienoic acids or hydroxyeicosa-tetraenoic acids, HETE) [36]. Expression levels of the *Cyp2c40* gene were highly reduced in inflamed colon of *Il10*$^{-/-}$ mice, and showed a tendency to be increased by dietary AA. CYP2C40, the main CYP2C in the intestinal tract, has also been identified in murine colon and metabolizes AA to 16-HETE [36]. The anti-inflammatory mediator 16-HETE can inhibit adhesion and aggregation of neutrophils, suggesting a role in resolution of inflammation [36]. The exact roles of the CYP2C differentially expressed in various extrahepatic

tissues remains unknown, but CYP2C40 may play an important role in intestinal inflammation and in metabolizing AA, and may contribute to specific AA metabolite production in those tissues to reduce or promote inflammation.

However, similar to the present study, Ramakers *et al.* [8] reported a protective effect of dietary AA in dextran sodium sulfate-induced colitis mice (colon mostly affected) fed AA-enriched diet (1.1% AA ethyl ester oil, 6% w/w total fat; approximately one-third of the AA amount used here); the animals showed decreased weight loss and less diarrhea compared with the OA-enriched diet. Sustained increased delivery of dietary AA during the onset of colon inflammation might have resulted in increased transport of AA from the peripheral circulation into the colonocytes, increased storage in the colonocyte membrane and esterification of AA with glycerol in membrane phospholipids. Therefore, the concentration of free AA in the intracellular compartment might have remained at a level insufficient to initiate inflammation.

4.3 Dietary AA reduced colonocyte fibrosis

Several genes implicated in the development of intestinal fibrosis were expressed at lower levels in the colon of AA-fed *Il10*$^{-/-}$ mice. Supporting this observation, colonic shortening and deformation appeared to be reduced in the colon tissue of *Il10*$^{-/-}$ mice fed AA diet compared with that of OA-fed *Il10*$^{-/-}$ mice (visual observation noted at tissue sampling, data not shown), although fibrotic features were not scored in detail in the histological examination. Crohn's disease-associated fibrosis is the result of a complex interplay among fibroblasts, smooth muscle cells, cytokines, inflammatory cells and chronic transmural inflammation.

Increase in collagen synthesis by smooth muscle cells, fibroblasts, and an increase in muscle layer thickness have also been observed in Crohn's disease patients with intestinal fibrosis [37]. In AA-fed *Il10*$^{-/-}$ mice, decreased expression levels of pro-inflammatory cytokines were linked to lower *Mmp13* gene expression levels. MMP can degrade extracellular proteins (*e.g.* collagen, laminin and fibronectin) and their expression is activated by pro-inflammatory cytokines (*e.g.* TNF and IL1A), with over-expression leading to dysregulated extracellular matrix turnover and intestinal inflammation [38].

Reduction of intestinal fibrosis by dietary AA is also suggested by down-regulation of the expression levels of *Col1a2* gene involved in collagen synthesis. Reduced mRNA abundance of *Igfbp5* gene by dietary AA in the colon of *Il10*$^{-/-}$ mice is in agreement with observations that higher expression levels of *Igfbp5* are related to extracellular matrix remodeling by increasing collagen synthesis and cell proliferation, and thus, to fibrosis development in inflamed colon [37]. The colonic expression levels of *Fgf7* gene, which is involved in epithelial tissue homeostasis, repair and disease and exerts some of its cytoprotective effects through activation of NRF2 under stress conditions [39], was also decreased in AA-treated *Il10*$^{-/-}$ mice. It has been reported that an AA-enriched oil can decrease the expression levels of genes involved in collagen synthesis and cell adhesion in mice [30], but the link between dietary AA and intestinal fibrosis has not been made previously.

While the changes in immune and inflammatory responses to dietary AA may be specific to the present experimental conditions, the impact of the *IL10* mutation on colitis development varies as a function of the inbred strain background when maintained under the same conditions. The colitis in *Il10*$^{-/-}$ mice on the C57BL/6J background for example is mild [40], compared with when the *Il10*^{tm1Cgn} allele is bred into the 129/SvEv, C3H/HeJBir and C3H.SW background strains, where colitis is severe and progressive [41–43]. This suggests that other genes or gene interactions particular to the genetic background of each strain modify the development of colitis and probably the response to a dietary intervention. For example, the study by Andrikopoulos *et al.* [44] has shown that the effect of a high fat diet on insulin secretory function was dependent on the mouse strain. Future investigations of the effects of dietary AA on whole genome gene expression profiles of *Il10*$^{-/-}$ mice on different genetic backgrounds may unravel gene interactions that lead to phenotypic differences.

In conclusion, it was found that in *Il10*$^{-/-}$ mice, dietary AA reduced the expression levels of some genes associated with cellular stress, inflammatory and immune response and intestinal fibrosis development, compared with dietary OA. This suggests that the colonocytes were able to deal with a sustained exposure to AA as used here, a recognized pro-inflammatory fatty acid during colitis development. Dietary AA could reach the colonocytes from the peripheral circulation and remain at a level that did not initiate inflammatory processes resulting from reduced COX-dependent metabolism. Since sampling was performed at only one time point in this disease model, further investigation is warranted to study the effect of AA on changes in the gene expression profile at several time points, including postprandial intervention and after clinical manifestation of colitis. The different bioavailability of AA and OA ethyl esters and triglycerides containing these fatty acids is also important to explain their bioactive role in the process of inflammation.

This study is part of Nutrigenomics New Zealand, a collaboration between AgResearch Limited, Plant and Food Research, and The University of Auckland, and is primarily funded by the New Zealand Foundation for Research, Science and Technology (FRST). The authors acknowledge the important contribution of: Yvonne Dommels for assistance in diet preparation; Michael Agnew for fatty acid analysis; Kate Broadley, Ric Broadhurst, Philippa Dryland, Kim Oden for technical support; Dongwen Luo for statistical support; Alan McCulloch for microarray submission to GEO; Katia Nones and Scott Knowles (a PhD

supervisor of Bianca Knoch) for valuable discussions; Bruce Sinclair and Denise Martin for manuscript reviewing. Bianca Knoch's PhD Fellowship is funded by AgResearch Limited within the Nutrigenomics New Zealand partnership; Matthew Barnett was funded by FRST Postdoctoral Fellowship AGRX0504 during the course of this study.

The authors have declared no conflict of interest.

5 References

[1] Mutch, D. M., Grigorov, M., Berger, A., Fay, L. B. *et al.*, An integrative metabolism approach identifies stearoyl-CoA desaturase as a target for an arachidonate-enriched diet. *FASEB J.* 2005, *19*, 599–601.

[2] Belluzzi, A., N-3 fatty acids for the treatment of inflammatory bowel disease. *Proc. Nutr. Soc.* 2002, *61*, 391–395.

[3] Serhan, C. N., Chiang, N., Endogenous pro-resolving and anti-inflammatory lipid mediators: a new pharmacologic genus. *Br. J. Pharmacol.* 2008, *153*, S200–S215.

[4] Pacheco, S., Hillier, K., Smith, C., Increased arachidonic acid levels in phospholipids of human colonic mucosa in inflammatory bowel disease. *Clin. Sci. (Lond).* 1987, *73*, 361–364.

[5] Kroman, N., Green, A., Epidemiological studies in the Upernauik district, Greenland. *Acta Med. Scand.* 1980, *208*, 401–406.

[6] Shoda, R. M. K., Yamato, S., Umeda, N., Epidemiologic analysis of Crohn disease in Japan: increased dietary intake of n-6 polyunsaturated fatty acids and animal protein relates to the increased incidence of Crohn disease in Japan. *Am. J. Clin. Nutr.* 1996, *63*, 741–745.

[7] Chapkin, R. S., Davidson, L. A., Ly, L., Weeks, B. R. *et al.*, Immunomodulatory effects of (n–3) fatty acids: Putative link to inflammation and colon cancer. *J. Nutr.* 2007, *137*, 200S–204S.

[8] Ramakers, J. D., Mensink, R. P., Verstege, M. I., Te Velde, A. A., Plat, J., An arachidonic acid-enriched diet does not result in more colonic inflammation as compared with fish oil- or oleic acid-enriched diets in mice with experimental colitis. *Br. J. Nutr.* 2008, *100*, 347–354.

[9] Camandola, S., Leonarduzzi, G., Musso, T., Varesio, L. *et al.*, Nuclear factor kB is activated by arachidonic acid but not by eicosapentaenoic acid. *Biochem. Biophys. Res. Commun.* 1996, *229*, 643–647.

[10] Heimerl, S., Moehle, C., Zahn, A., Boettcher, A. *et al.*, Alterations in intestinal fatty acid metabolism in inflammatory bowel disease. *Biochim. Biophys. Acta. Mol. Basis Dis.* 2006, *1762*, 341–350.

[11] Salter, A., Tarling, E., Regulation of gene transcription by fatty acids. *Animal* 2007, *1*, 1314–1320.

[12] Sampath, H., Ntambi, J. M., Polyunsaturated fatty acid regulation of genes of lipid metabolism. *Ann. Rev. Nutr.* 2005, *25*, 317–340.

[13] Mariotto, S., Suzuki, Y., Persichini, T., Colasanti, M. *et al.*, Cross-talk between NO and arachidonic acid in inflammation. *Curr. Med. Chem.* 2007, *14*, 1940–1944.

[14] Knoch, B., Barnett, M. P. G., Zhu, S., Park, Z. A. *et al.*, Genome-wide analysis of dietary eicosapentaenoic acid- and oleic acid-induced modulation in colon inflammation of interleukin-10 gene-deficient mice. *J. Nutrigenet. Nutrigenom.* 2009, *2*, 9–28.

[15] Roy, N., Barnett, M., Knoch, B., Dommels, Y., McNabb, W., Nutrigenomics applied to an animal model of inflammatory bowel diseases: Transcriptomic analysis of the effects of eicosapentaenoic acid and arachidonic acid-enriched diets. *Mutat. Res. Fund. Mol. Mech. Mutagen.* 2007, *622*, 103–116.

[16] Whelan, J., McEntee, M. F., Dietary (n-6) PUFA and intestinal tumorigenesis. *J. Nutr.* 2004, *134*, 3421S–3426S.

[17] No authors listed. Report of the American Institute of Nutrition ad hoc Committee on Standards for Nutritional Studies. *J. Nutr.* 1977, *107*, 1340–1348.

[18] Knoch, B., Barnett, M. P. G., Zhu, S. T., Broadhurst, R. *et al.*, Establishment of the optimal tissue sampling time for the interleukin-10 knockout mouse inoculated with endogenous intestinal bacteria, a model of inflammatory bowel disease. *Reprod. Nutr. Dev.* 2006, *46*, S71–S72.

[19] Park, E., Paisley, E. A., Mangian, H. J., Swartz, D. A. *et al.*, Lipid level and type alter stearoyl CoA desaturase mRNA abundance differently in mice with distinct susceptibilities to diet-influenced diseases. *J. Nutr.* 1997, *127*, 566–573.

[20] Smyth, G. K., in: Gentleman, R., Carey, V., Dudoit, S., Irizarry, R., Huber, W., (Eds.), *Bioinformatics and Computational Biology Solutions Using R and Bioconductor,* Springer, New York 2005, pp. 397–420.

[21] Smyth, G. K., Speed, T., Normalization of cDNA microarray data. *Methods* 2003, *31*, 265–273.

[22] Dieck, T. H., Döring, F., Roth, H.-P., Daniel, H., Changes in rat hepatic gene expression in response to zinc deficiency as assessed by DNA array. *J. Nutr.* 2003, *133*, 1004–1010.

[23] Sülzle, A., Hirche, F., Eder, K., Thermally oxidized dietary fat upregulates the expression of target genes of PPARα in rat liver. *J. Nutr.* 2004, *134*, 1375–1383.

[24] Prüfer, K., Muetzel, B., Do, H. H., Weiss, G. *et al.*, FUNC: a package for detecting significant associations between gene sets and ontological annotations. *BMC Bioinformatics* 2007, *8*, 41.

[25] Wada, K., Montalto, M. C., Stahl, G. L., Inhibition of complement C5 reduces local and remote organ injury after intestinal ischemia/reperfusion in the rat. *Gastroenterology* 2001, *120*, 126–133.

[26] Montalto, M. C., Hart, M. L., Jordan, J. E., Wada, K., Stahl, G. L., Role for complement in mediating intestinal nitric oxide synthase-2 and superoxide dismutase expression. *Am. J. Physiol. Gastrointest. Liver Physiol.* 2003, *285*, G197–G206.

[27] Dijkstra, G., Zandvoort, A. J. H., Kobold, A. C. M., de Jager-Krikken, A. *et al.*, Increased expression of inducible nitric oxide synthase in circulating monocytes from patients with active inflammatory bowel disease. *Scand. J. Gastroenterol.* 2002, *37*, 546–554.

[28] Harding, H. P., Zhang, Y. H., Ron, D., Protein translation and folding are coupled by an endoplasmic-reticulum-resident kinase. *Nature* 1999, *397*, 271–274.

[29] Itoh, K., Wakabayashi, N., Katoh, Y., Ishii, T. *et al.*, Keap1 regulates both cytoplasmic-nuclear shuttling and degradation of Nrf2 in response to electrophiles. *Genes Cells* 2003, *8*, 379–391.

[30] Berger, A., Roberts, M. A., Hoff, B., How dietary arachidonic- and docosahexaenoic-acid rich oils differentially affect the murine hepatic transcriptome. *Lipids Health Dis.* 2006, *5*, 10.

[31] Malhotra, J. D., Kaufman, R. J., Endoplasmic reticulum stress and oxidative stress: A vicious cycle or a double-edged sword? *Antioxid. Redox Signal.* 2007, *9*, 2277–2293.

[32] Dulpus, E., Glorian, M., Forest, C., Fatty acid regulation of gene transcription. *J. Biol. Chem.* 2000, *275*, 30749–30752.

[33] Salter, A., Tarling, E., Regulation of gene transcription by fatty acids. *Animal* 2007, *1*, 1314–1320.

[34] Hansen Petrik, M. B., McEntee, M. F., Chiu, C. H., Whelan, J., Antagonism of arachidonic acid is linked to the anti-tumorigenic effect of dietary eicosapentaenoic acid in $Apc^{Min/+}$ mice. *J. Nutr.* 2000, *130*, 1153–1158.

[35] Zeldin, D. C., Foley, J., Goldsworthy, S. M., Cook, M. E. *et al.*, CYP2J subfamily cytochrome P450s in the gastrointestinal tract: expression, localization, and potential functional significance. *Mol. Pharmacol.* 1997, *51*, 931–943.

[36] Tsao, C. C., Coulter, S. J., Chien, A., Luo, G. *et al.*, Identification and localization of five CYP2Cs in murine extrahepatic tissues and their metabolism of arachidonic acid to regio- and stereoselective products. *J. Pharmacol. Exp. Ther.* 2001, *299*, 39–47.

[37] Martinez-Augustin, O., Merlos, M., Zarzuelo, A., Suarez, M. D., de Medina, F. S., Disturbances in metabolic, transport and structural genes in experimental colonic inflammation in the rat: a longitudinal genomic analysis. *BMC Genomics* 2008, *9*, 490.

[38] Burke, J. P., Mulsow, J. J., O'Keane, C., Docherty, N. G. *et al.*, Fibrogenesis in Crohn's disease. *Am. J. Gastroenterol.* 2007, *102*, 439–448.

[39] Beer, H.-D., Bittner, M., Niklaus, G., Munding, C. *et al.*, The fibroblast growth factor (FGF) binding protein is a novel interaction partner of FGF-7, FGF-10 and FGF-22 and regulates FGF activity: Implications for epithelial repair. *Oncogene* 2005, *24*, 5269–5271.

[40] Berg, D. J., Davidson, N., Kuhn, R., Muller, W. *et al.*, Enterocolitis and colon cancer in interleukin-10-deficient mice are associated with aberrant cytokine production and CD4(+) TH1-like responses. *J. Clin. Invest.* 1996, *98*, 1010–1020.

[41] Takeda, K., Clausen, B. E., Kaisho, T., Tsujimura, T. *et al.*, Enhanced Th1 activity and development of chronic enterocolitis in mice devoid of Stat3 in macrophages and neutrophils. *Immunity* 1999, *10*, 39–49.

[42] Bristol, I. J., Farmer, M. A., Cong, Y. Z., Zheng, X. X. *et al.*, Heritable susceptibility for colitis in mice induced by IL-10 deficiency. *Inflamm. Bowel Dis.* 2000, *6*, 290–302.

[43] Mahler, M., Leiter, E. H., Genetic and environmental context determines the course of colitis developing in IL-10-deficient mice. *Inflamm. Bowel Dis.* 2002, *8*, 347–355.

[44] Andrikopoulos, S., Massa, C. M., Aston-Mourney, K., Funkat, A., Differential effect of inbred mouse strain (C57BL/6, DBA/2, 129T2) on insulin secretory function in response to a high fat diet. *J. Endocrininol.* 2005, *187*, 45–53.

[45] Reeves, P. G., Nielsen, F. H., Fahey, G. C., Jr., AIN-93 purified diets for laboratory rodents: Final report of the American Institute of Nutrition *ad hoc* Writing Committee on the reformulation of the AIN-76A rodent diet. *J. Nutr.* 1993, *123*, 1939–1951.

