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**Statistical analysis of human microarray data shows that dietary intervention with omega-3, flavonoids and resveratrol enriches for immune response and disease pathways**

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**Running title:** Dietary intervention on biological pathways

**Keywords:**

Flavonoids, functional food, GWAS, omega-3, resveratrol

**Abstract**

Omega-3, flavonoids and resveratrol are well publicised for their beneficial effects on human health and wellbeing. Identifying common, underlying biological mechanisms targeted by these functional foods would therefore be informative for the public health sector for advising on nutritional health and disease, food and drug product development and consumer interest. The aim of this study was to explore the potential effects of gene expression changes associated with omega-3 eicosapentaenoic and docosahexaenoic fatty acids (EPA/DHA), flavonoids and resveratrol on modifying biological systems and disease pathways. To test this, publicly available human microarray data for significant gene expression changes associated with dietary intervention with EPA/DHA, flavonoids and resveratrol was subjected to pathway analysis and significance testing for overlap with signals from genome-wide association studies (GWAS) for common non-communicable diseases and biological functions. There was an enrichment of genes implicated in immune responses and disease pathways which was common to all of the treatment conditions tested. Analysis of biological functions and disease pathways indicated anti-tumorigenic properties for EPA/DHA. In line with this, significance testing of the intersection of genes associated with these functional foods and GWAS hits for common biological functions (ageing and cognition) and non-communicable diseases (breast cancer, CVD, diabesity, neurodegeneration and psychiatric disorders) identified significant overlap between the EPA/DHA and breast cancer gene sets. Dietary intervention with EPA/DHA, flavonoids and resveratrol can target important biological and disease pathways suggesting a potentially important role for these bioactive compounds in the prevention and treatment of dietary-related diseases.

**Introduction**

Diet is an important factor in promoting health and preventing chronic diseases such as cardiovascular disease (CVD), diabetes, obesity and cancer. The rapid growth of the global ageing population and the limited efficacy of available pharmacological therapies for age-related cognitive decline and neurodegenerative diseases means that there is an increasing interest and public demand for so called ‘functional foods’ that promote cognitive wellbeing and longevity [14](#_ENREF_14); [3](#_ENREF_3); [28](#_ENREF_28). At the other end of the scale a growing body of evidence supports that early nutrition during prenatal development, infancy and childhood affects both cognitive development and cognitive function and behaviour in later life [4](#_ENREF_4); [6](#_ENREF_6); [31](#_ENREF_31); [52](#_ENREF_52). The beneficial effects of certain food groups including plant foods and oily fish which provide high sources of plant bioactives (e.g. polyphenols, glucosinolates and antioxidant vitamins) and omega-3 fatty acids, respectively, are well documented [67](#_ENREF_67); [27](#_ENREF_27); [48](#_ENREF_48); [28](#_ENREF_28). Many modern diets and food supplements are enriched with these bioactive compounds and claim to be beneficial to health through boosting the immune system, supporting cardiovascular function, protecting cells against oxidative stress and promoting healthy skin, teeth and bones [39](#_ENREF_39); [25](#_ENREF_25); [57](#_ENREF_57); [15](#_ENREF_15); [18](#_ENREF_18); [21](#_ENREF_21); [38](#_ENREF_38).

Addressing the synergistic effects of certain functional foods and supplements consumed in combination on health and wellbeing is of growing interest in the field of nutrition [33](#_ENREF_33); [32](#_ENREF_32); [3](#_ENREF_3). The purpose of this communication was to address potential common biological effects of the functional foods omega-3 fatty acids eicosapentaenoic acid and docosahexaenoic acid (EPA/DHA), flavonoids and resveratrol on different regulatory mechanisms including development, immune responses, metabolic processing and disease using a pathway analysis approach. Gene sets associated with these functional foods were also overlaid with GWAS (genome-wide association studies) hits for common diseases and biological functions. The findings of this analysis will have implications for public health, disease intervention, therapeutic management, nutrition-related behaviours, marketing strategy, economic growth and food development.

**Experimental methods**

**Gene expression array database**

Human microarray data for dietary interventions with omega-3 fatty acids (EPA and/or DHA), flavonoids and resveratrol was downloaded from the ArrayExpressdatabase (<https://www.ebi.ac.uk/arrayexpress/>). Study descriptions for the microarray datasets are detailed in Table 1. Studies included in the analysis were selected on the following criteria: 1) performed in healthy human subjects or subjects with a dietary-related disease but otherwise healthy, 2) double-blinded randomised controlled trials, 3) microarray-based gene expression data available at baseline and following treatment intervention for each subject. Gene expression changes between baseline measurements or placebo for crossover studies (control group) and following treatment intervention (treatment group) within the same individuals were analysed using GEO2R [19](#_ENREF_19), based on the Linear Models for Microarray Analysis (limma) R/ Bioconductor software package [56](#_ENREF_56), available at <http://www.ncbi.nlm.nih.gov/geo/info/geo2r.html>. Only significantly regulated genes (p<0.05) were considered for pathway analysis. Unadjusted p-values were used in this study in order to address extended biological networks associated with the dietary interventions. For gene replicates within individual studies or duplicates across different studies for the same treatment group, data points with the least significant p-value were excluded. If replicates within individual studies had fold changes that were opposing (i.e. positive and negative values for the same gene) both data points were excluded from the analysis.

**GWAS database**

Human GWAS data was downloaded from The National Human Genome Research Institute (NHGRI)-European Bioinformatics Institute (EBI) GWAS catalog (<http://www.ebi.ac.uk/gwas/docs/downloads>, accessed August 2015), a publicly available collection of published GWAS for more than 100,000 single-nucleotide polymorphisms (SNPs) with SNP-trait associations of p<1×10-5 [69](#_ENREF_69). Reported genome-wide associated genes for ageing, breast cancer, cognitive function, CVD, diabesity, neurodegeneration and psychiatric disorders were compiled and duplicates removed. GWAS included in this analysis are detailed in *Appendix 1* (Tables A1-A8).

**Significance testing of overlapping gene sets**

The hypergeometric distribution test *phyper* available through the R package (<https://www.r-project.org/>) was used to compute statistical significance between the overlap of two gene sets using the command: *> phyper(q, m, n, k, lower.tail=FALSE)*;where, q = overlap between list 1 and list 2, m = size of list 1, n = population size – list 1, and k = size of list 2. If ‘phyper(q, m, n, k)’ gives the probability of getting *x* overlap or fewer, ‘*lower.tail=FALSE’* gives the probability of getting *x* + 1 or more. A p-value of <0.05 was considered significant. The reference gene set in the PANTHER database (20,814 genes) was selected as the population size based on subsequent pathway analysis methods using this reference list. Significant overlap of multiple gene sets was addressed using the GeneOverlap package in R, downloaded from [https://www.bioconductor.org/packages/release/bioc/html/GeneOverlap. html](https://www.bioconductor.org/packages/release/bioc/html/GeneOverlap.%20html) [59](#_ENREF_59).

**Pathway analysis**

Gene expression data obtained from ArrayExpress was analysed using the IPA (Ingenuity Pathway Analysis) Core Analysis function (QIAGEN) under default parameters. Significance testing was performed using the following methods: 1) Ratio of the number of differentially expressed genes from the uploaded dataset that map to an IPA pathway divided by the total number of molecules that exist in the pathway. 2) Fischer’s exact test, used to calculate the probability that the association between the genes in the uploaded dataset and the canonical pathway is explained by chance alone. P-values of <0.05 were considered significant. 3) Benjamini-Hochberg procedure, used to calculate the false discovery rate and correct for multiple-testing [9](#_ENREF_9). Adjusted P-values of <0.05 were considered significant. 4) Fold change cut-off of >0.5, <-0.5 log(differential expression, DE) applied to all datasets. 5) z-score analysis, used as a statistical measure of the match between expected relationship direction and observed gene expression of the uploaded dataset. Positive and negative z-scores indicate upregulated and downregulated pathways, respectively. In line with IPA cut-off values, z-scores of >2.0 or < -2.0 were considered significant.

Statistical overrepresentation testing of gene lists was performed using the publicly available PANTHER (Protein ANalysis THrough Evolutionary Relationships) Classification System [46](#_ENREF_46), <http://www.pantherdb.org/>. Over-represented pathways were determined using the binomial distribution test [16](#_ENREF_16). P-values of <0.05 were considered significant.

**Results**

**Gene expression changes associated with dietary omega-3 fatty acids** EPA/DHA**, flavonoids and resveratrol**

The total number of significant gene expression changes associated with dietary intervention with the functional foods addressed in this study were 3,403 for EPA/DHA (mean: 1,213; range: 542 - 1,827), 2,703 for flavonoids (mean: 1,480; range: 915 – 2,045) and 3,464 for resveratrol (mean: 925; range: 555 – 1,424). Gene lists representing all genes significantly regulated in response to these treatment groups are included in *Appendix 2*. Significant overlap was observed between the gene sets for EPA/DHA and flavonoids (p = 4.67 x 10-25) and flavonoids and resveratrol (p = 0.015), but not for EPA/DHA and resveratrol (p = 0.624). A total of 141 genes were common across the three treatment groups (*Appendix 2*, Table A12). Statistical overrepresentation testing of these overlapping gene sets was performed using the PANTHER Classification System, which compares user defined gene lists to a reference gene list within the PANTHER database and determines whether a particular class of genes is over- or under-represented with respect to a particular biological process or pathway [44](#_ENREF_44). Significantly over-represented pathways are represented in Figure 1.

**Determining regulatory pathways affected by dietary omega-3 fatty acids** EPA/DHA**, flavonoids and resveratrol**

For pathway analysis, differential gene expression data for all significantly regulated genes under each of the treatment conditions was parsed into the IPA platform and enrichment analysis performed using the Core Analysis function. This provides an overview of biological relationships, mechanisms, functions and pathways relating to the uploaded dataset based on log fold changes in gene expression. Gene lists were filtered to include genes with a log fold change of more than 0.5. To correct for multiple-testing, datasets were adjusted using the Benjamini-Hochberg procedure [9](#_ENREF_9). The top canonical pathways associated with dietary intervention with EPA/DHA, flavonoids and resveratrol are shown in Figure 2 and *Appendix 3* (Tables A13-A15). Intervention with EPA/DHA enriched for genes implicated in the anti-proliferative role of TOB (Transducer Of ERBB2) in T cell signaling, as well as PPAR (peroxisome proliferator receptor activator) signaling and autoimmune disorders; flavonoids activated PI3K signaling in B lymphocytes, iCOS-iCOSL signaling in T helper cells and cancer pathways; and resveratrol associated with dendritic cell maturation and phagosome formation. Comparative enrichment analysis of significantly regulated canonical pathways indicated overlap between the different treatment groups, as listed in Table 2. PPARα/RXRα (retinoid X receptor) activation was common to all treatment interventions. A regulatory upstream connection and network predicted for PPAR signaling in response to omega-3 (EPA/DHA) treatment intervention is illustrated in Figure 3, based on IPA Casual Network Analysis [37](#_ENREF_37). EPA/DHA and flavonoids shared 7/15 common canonical pathways, EPA/DHA and resveratrol shared 8/15 pathways, and flavonoids and resveratrol shared 2/15 pathways. There was an enrichment of pathways relating to immune response, disease signalling, cardiovascular function and signal transduction across all treatment conditions (Table 2).

**Dietary omega-3 fatty acids** EPA/DHA**, flavonoids and resveratrol target disease pathways**

To investigate the role of EPA/DHA, flavonoids and resveratrol in the prevention and/or treatment of disease, IPA Core Analysis was used to identify the top disease pathways and biological functions associated with significantly regulated gene sets for these treatment conditions. This identified processes relevant to tumorigenesis, inflammation and autoimmune disorders, Figure 4A. The net effect of gene expression changes was addressed using z-scores which predict an increase (positive z-score) or decrease (negative z-score) in pathway activation. Based on this analysis, EPA/DHA was suggested to have anti-tumorigenic properties (increased apoptosis of tumor cell lines; decreased aggregation of blood cells) , flavonoids had both anti-tumorigenic (decreased growth of melanoma) and pro-tumorigenic properties (decreased apoptosis and necrosis of prostate cancer cell lines; increased cell viability of cervical cancer cell lines), and resveratrol was strongly enriched for pro-tumorigenic processes (decreased apoptosis of breast cancer cell lines; increased migration, invasion and proliferation of tumor cell lines), Figure 4B.

**Omega-3 fatty acids** EPA/DHA **modulate genes implicated in breast cancer through GWAS**

To further address how EPA/DHA, flavonoids and resveratrol may converge onto major disease pathways, gene expression changes associated with these functional foods were compared against GWAS hits for different biological functions and diseases. Hypergeometric distribution testing using the GeneOverlap package in R was used to determine significant overlap between the GWAS datasets and genes associated with dietary interventions with EPA/DHA, flavonoids and resveratrol. As illustrated in Figure 5A, significant overlap was observed between the two lists representing genes regulated in response to EPA/DHA and those implicated in breast cancer risk from GWAS. A total of 32 genes were identified as being common between the two gene lists. Pathway analysis of this gene set identified enrichment of genes implicated in metabolic processes (Figure 5B) and developmental pathways (Figure 5C). No other significant associations were found between gene lists for the functional foods and diseases/biological functions tested, Figure 5A.

**Discussion**

There is a growing interest in the therapeutic potential of omega-3 fatty acids and polyphenol-rich foods in the prevention and treatment of chronic disease [61](#_ENREF_61); [62](#_ENREF_62); [41](#_ENREF_41); [58](#_ENREF_58); [22](#_ENREF_22); [51](#_ENREF_51); [60](#_ENREF_60); [55](#_ENREF_55); [7](#_ENREF_7); [40](#_ENREF_40); [24](#_ENREF_24); [63](#_ENREF_63); [26](#_ENREF_26). The protective role of these functional foods has in part been attributed to their antioxidant and anti-apoptotic effects [68](#_ENREF_68); [13](#_ENREF_13); [49](#_ENREF_49); [66](#_ENREF_66); [30](#_ENREF_30). However, the exact biological mechanisms underpinning the functional aspects of these bioactive compounds are not fully understood. In this communication, pathway analysis was performed to identify the major biological processes associated with genes targeted by dietary intervention with omega-3 fatty acids EPA/DHA, flavonoids and resveratrol. Better understanding of the potential synergistic effects of these functional foods in modulating complex biological and pathological processes has important therapeutic implications for the prevention and management of disease.

Enrichment analysis of overlapping gene sets significantly regulated in response to dietary interventions with EPA/DHA, flavonoids and resveratrol identified apoptosis, interleukin signaling and angiogenesis as common pathways across all treatment groups (Figure 1). In addition, EPA/DHA and flavonoids enriched for pathways relevant to cellular proliferation and migration, B and T cell activation, neuroendocrine signaling and cardiovascular function; flavonoids and resveratrol enriched for signal transduction, immune response and neuroendocrine pathways; and EPA/DHA and resveratrol enriched for pathways associated with neurodegeneration (Figures 1 and 2). PPARα/RXRα activation was identified as a significantly regulated pathway common to all three treatment interventions (Figure 2). PPAR signaling was also found to be significantly associated with EPA/DHA (*Appendix 3*, Table A13). Omega-3 fatty acids, flavonoids and resveratrol are known ligands of PPARγ [23](#_ENREF_23); [53](#_ENREF_53); [12](#_ENREF_12), which plays a key role in the regulation of apoptosis, cellular proliferation, glucose and lipid metabolism, inflammatory responses, oxidative stress, endothelial function and cancer [43](#_ENREF_43). We would therefore expect to see strong regulation of PPAR-specific target genes in our analysis. However, casual analysis for potential upstream regulators in IPA [37](#_ENREF_37) suggested only marginal involvement of PPARG and PPARA in the regulation of genes differentially expressed in the treatment intervention groups studied, Figure 3A. This may reflect the long-term and systemic nature of the studies included in our analysis; even if PPARs played a significant role in triggering gene expression changes at the start of treatment intervention, expression of direct PPAR targets are likely stabilised via multiple feedback mechanisms. Nonetheless, more long-term differential gene expression profiles triggered by these early induced changes are clearly represented in our functional network analysis of primary PPAR targets (Figure 3B). On the other hand, continuous activation of receptors can eventually cause an adaptive cellular response and shutting down of the expression of the receptors and their downstream pathways. It would therefore be interesting to follow this line of investigation into interrogation of organismal and tissue responses to short- and long-term exposures to these bioactive compounds.

Analysis of disease pathways associated with the functional foods analysed in this study indicated anti-tumorigenic properties for EPA/DHA, Figure 4B, which is supported by the literature [50](#_ENREF_50); [17](#_ENREF_17); [47](#_ENREF_47). In contrast, flavonoids were linked to both anti- and pro-tumorigenic processes, whereas resveratrol was strongly enriched for pro-tumorigenic pathways. Resveratrol is well documented for its modulation of the cell cycle in models of cancer [2](#_ENREF_2); [34](#_ENREF_34); [8](#_ENREF_8); [5](#_ENREF_5); [42](#_ENREF_42); [54](#_ENREF_54); [70](#_ENREF_70). These conflicting results may reflect tissue-specific gene expression profiles (adipose verses skeletal muscle) or phenotypic variations across the study cohorts (overweight/obese male verses postmenopausal female) and warrant further investigation.

Our diet is influenced by many factors including age, sex, education, ethnicity and socioeconomic pressures. Psychological determinants of diet such as mood and stress levels are also very influential [1](#_ENREF_1); [65](#_ENREF_65); [45](#_ENREF_45); [29](#_ENREF_29). To address overlap between nutrition and key biological processes and common diseases potentially modified by dietary interventions, GWAS datasets for ageing, cognition, breast cancer, CVD, diabesity, neurodegeneration and psychiatric disorders were compared against gene expression changes associated with EPA/DHA, flavonoids and resveratrol. Significant overlap was observed between the EPA/DHA and breast cancer gene sets (Figure 5), emphasizing the anti-tumorigenic properties of this functional food.

There are several limitations of this study, most notably the comparison of small cohort trials with varied treatment interventions and methodologies, including, and not limited to, cohort size, subject variables, length of intervention, type and dosage of supplement tested, tissue type used for gene expression analysis, and type of microarray technology used for gene expression profiling. Correction for the effect of cofounding variables such as age, sex, health status, genetic variation etc. on gene expression changes across the different study cohorts included in our analysis could not be accounted for in this study. A further limitation of this study is the absence of a matched control group; not every study included in this analysis had gene expression data for no-treatment intervention. Nonetheless, this study identifies several potentially important genes and regulatory pathways targeted through human dietary interventions with omega-3 fatty acids EPA/DHA, flavonoids and resveratrol. This is exemplified through significant overlap of the EPA/DHA gene set, identified from pathway analysis as having an anti-tumorigenic profile, with GWAS hits for breast cancer.

In summary, gene expression changes associated with dietary omega-3 fatty acids EPA/DHA, flavonoids and resveratrol converged onto important biological and disease pathways, including immune response and cell cycle regulation, suggesting a potentially important role for these bioactive compounds in the prevention and treatment of dietary-related disease. The findings of this study warrant further investigation into the synergistic effect of these functional foods in the management of non-communicable diseases.

**Financial Support**

This research received no specific grant from any funding agency, commercial or not-for-profit sectors.

**Conflict of Interest**

None

**Authorship**

AW/PQ/JPS/JPQ were involved in the study design. AW/OV were involved in the generation and interpretation of data.

**Figure 1. Enrichment analysis of overlapping gene sets associated with dietary interventions with omega-3 fatty acids EPA/DHA, flavonoids and resveratrol.** Gene expression data was downloaded from ArrayExpress (<https://www.ebi.ac.uk/arrayexpress/>). Studies included in the analysis are detailed in Table 1. Significant gene expression changes are represented as a Venn diagram showing treatment-specific and overlapping profiles across the three dietary interventions. For gene lists included in this analysis, see *Appendix 2* (Tables A9-A12). Overlapping gene sets (omega-3 and flavonoids, 494 genes; omega-3 and resveratrol, 420 genes; flavonoids and resveratrol, 349 genes; all treatments, 141 genes) were subjected to statistical overrepresentation testing using PANTHER pathway analysis software. Significantly over-represented pathways are listed for each of the overlapping gene sets. Bold font indicates common pathways across the different gene sets. †Withstood Bonferroni correction for multiple-testing. Abbreviations: *CCKR, cholecystokinin receptor; CRF, Cortocotropin releasing factor; JAK/STAT, Janus kinase/Signal transducer and activator of transcription; mAChR, Muscarinic acetylcholine receptor; PDGF, Platelet-derived growth factor; PKB, protein kinase B; TGF, Transforming growth factor; TRHR, Thyrotropin-releasing hormone receptor; 5-HT2, 5-hydroxytryptamine/serotonin*.

**Figure 2. Top 20 canonical pathways significantly regulated following dietary intervention with omega-3 (EPA/DHA), flavonoids and resveratrol.** Functional enrichment analysis of differentially regulated gene sets was performed using IPA Core Analysis. Fold change cut-off of >0.5 and <-0.5 log(DE) was applied. Statistical significance was determined using the Benjamini-Hochberg procedure for multiple-testing correction. Intensity of a block colour corresponds to -log(P-value). All significant pathways for each treatment group are listed in *Appendix 3*; common significant pathways across the different treatment groups are listed in Table 2.

**Figure 3. PPARG signaling network predicted for differentially expressed genes in response to omega-3 (EPA/DHA) treatment intervention. A,** PPARG as an upstream regulatory connection generated using IPA upstream analysis. Red, green and grey blocks respectively show functions differentially up-regulated, down-regulated and not significantly changed in response to EPA/DHA treatment intervention. Significant activators are represented by red lines connected to red boxes; significant inhibitors by blue lines connected to green boxes. Intensity of a block colour corresponds to –log(fold change). **B,** PPARG regulatory network was generated using the IPA network algorithm. Input gene IDs were used as seed functions and their associations retrieved from IPA’s knowledge base. The maximum number of nodes allowed in one network was set at 35. Solid arrows represent known connections (experimentally validated functional interactions); dashed lines represent suggested connections (based on detected associations between proteins). The shapes of blocks correspond to classes of general molecular functions. Red blocks represent differentially over-expressed genes; green blocks represent under-expressed genes, grey blocks represent genes that have not changed based on a fold change cut-off of >0.5 and <-0.5 log(DE). Intensity of a block colour corresponds to log(DE).

**Figure 4. Top disease pathways and biological functions enriched for genes significantly regulated following dietary intervention with omega-3 (EPA/DHA), flavonoids and resveratrol.** Functional enrichment analysis of differentially regulated gene sets was performed using IPA Core Analysis. Fold change cut-off of >0.5 and <-0.5 log(DE) was applied. **A,** Statistical significance was determined using the Benjamini-Hochberg procedure for multiple-testing correction. Intensity of a block colour corresponds to -log(P-value). **B,** Net effects of gene expression changes on pathway activation or repression were determined using activation z-scores (threshold, <-2.0; >2.0). Intensity of a block colour corresponds to downregulated (blue) and upregulated (orange) pathways.

**Figure 5. Significance testing of overlap between genes regulated in response to functional foods and GWAS hits for common diseases/biological functions. A**,Gene lists representing all genes significantly regulated in response to dietary interventions with omega-3 fatty acids EPA/DHA, flavonoids and resveratrol (see Table 1 for study details) were compared against gene lists identified from genome wide association studies (GWAS, see *Appendix 1* for study details) for ageing, breast cancer, cognition, cardiovascular disease (CVD), diabesity, neurodegeneration and psychiatric disorders. Numbers in brackets represent the number of genes within each gene list. GeneOverlap and GeneOverlapMatrix functions available in R were used to calculate and visualise significant overlap between the gene lists tested. Fischer’s exact test was used to calculate p-values which are stated within each panel of the grid. Colour key represents odds ratio values. \*Significant overlap (p<0.05). **B-C**, Statistical overrepresentation testing was performed using the binomial statistics tool available through PANTHER to identify biological processes (**B**) and pathway classifications (**C**) for common genes associated with breast cancer and dietary intervention with omega-3 (EPA/DHA). Uploaded genes were compared to a reference list containing all human genes within the PANTHER database in order to statistically determine over- or under- representation of PANTHER classification categories using the binomial distribution test [16](#_ENREF_16). Associated genes are listed on the right of the bar charts. Abbreviations: *EGF, Epidermal growth factor; FGF, fibroblast growth factor; Flav., flavonoids; HIF, hypoxia-inducible factor; IGF, insulin-like growth factor; mGluR, metabotropic glutamate receptor, Nb, nucleobase; PDGF, platelet-derived growth factor; PKB, protein kinase B; Resv., resveratrol*.

**References:**

1. Adam TC & Epel ES (2007) Stress, eating and the reward system. *Physiol Behav* **91**, 449-458.

2. Ahmad N, Adhami VM, Afaq F *et al.* (2001) Resveratrol Causes WAF-1/p21-mediated G1-phase Arrest of Cell Cycle and Induction of Apoptosis in Human Epidermoid Carcinoma A431 Cells. *Clinical Cancer Research* **7**, 1466-1473.

3. Alles B, Samieri C, Feart C *et al.* (2012) Dietary patterns: a novel approach to examine the link between nutrition and cognitive function in older individuals. *Nutr Res Rev* **25**, 207-222.

4. Anjos T, Altmae S, Emmett P *et al.* (2013) Nutrition and neurodevelopment in children: focus on NUTRIMENTHE project. *Eur J Nutr* **52**, 1825-1842.

5. Bai Y, Mao QQ, Qin J *et al.* (2010) Resveratrol induces apoptosis and cell cycle arrest of human T24 bladder cancer cells in vitro and inhibits tumor growth in vivo. *Cancer Sci* **101**, 488-493.

6. Barker ED, Kirkham N, Ng J *et al.* (2013) Prenatal maternal depression symptoms and nutrition, and child cognitive function. *Br J Psychiatry* **203**, 417-421.

7. Batra P & Sharma AK (2013) Anti-cancer potential of flavonoids: recent trends and future perspectives. *3 Biotech* **3**, 439-459.

8. Benitez DA, Pozo-Guisado E, Alvarez-Barrientos A *et al.* (2007) Mechanisms involved in resveratrol-induced apoptosis and cell cycle arrest in prostate cancer-derived cell lines. *J Androl* **28**, 282-293.

9. Benjamini Y & Hochberg Y (1995) Controlling the False Discovery Rate: A Practical and Powerful Approach to Multiple Testing. *Journal of the Royal Statistical Society. Series B (Methodological)* **57**, 289-300.

10. Boomgaarden I, Egert S, Rimbach G *et al.* (2010) Quercetin supplementation and its effect on human monocyte gene expression profiles in vivo. *Br J Nutr* **104**, 336-345.

11. Bouwens M, van de Rest O, Dellschaft N *et al.* (2009) Fish-oil supplementation induces antiinflammatory gene expression profiles in human blood mononuclear cells. *Am J Clin Nutr* **90**, 415-424.

12. Calleri E, Pochetti G, Dossou KSS *et al.* (2014) Resveratrol and its metabolites bind to PPARs. *Chembiochem* **15**, 1154-1160.

13. Cameron AR, Anton S, Melville L *et al.* (2008) Black tea polyphenols mimic insulin/insulin-like growth factor-1 signalling to the longevity factor FOXO1a. *Aging Cell* **7**, 69-77.

14. Cannella C, Savina C & Donini LM (2009) Nutrition, longevity and behavior. *Arch Gerontol Geriatr* **49 Suppl 1**, 19-27.

15. Chapple IL, Milward MR, Ling-Mountford N *et al.* (2012) Adjunctive daily supplementation with encapsulated fruit, vegetable and berry juice powder concentrates and clinical periodontal outcomes: a double-blind RCT. *J Clin Periodontol* **39**, 62-72.

16. Cho RJ & Campbell MJ (2000) Transcription, genomes, function. *Trends Genet* **16**, 409-415.

17. Cockbain AJ, Toogood GJ & Hull MA (2012) Omega-3 polyunsaturated fatty acids for the treatment and prevention of colorectal cancer. *Gut* **61**, 135-149.

18. Cui X, Jin Y, Singh UP *et al.* (2012) Suppression of DNA damage in human peripheral blood lymphocytes by a juice concentrate: a randomized, double-blind, placebo-controlled trial. *Mol Nutr Food Res* **56**, 666-670.

19. Davis S & Meltzer PS (2007) GEOquery: a bridge between the Gene Expression Omnibus (GEO) and BioConductor. *Bioinformatics* **23**, 1846-1847.

20. Dawson K, Zhao L, Adkins Y *et al.* (2012) Modulation of blood cell gene expression by DHA supplementation in hypertriglyceridemic men. *J Nutr Biochem* **23**, 616-621.

21. De Spirt S, Sies H, Tronnier H *et al.* (2012) An encapsulated fruit and vegetable juice concentrate increases skin microcirculation in healthy women. *Skin Pharmacol Physiol* **25**, 2-8.

22. Dragan S, Nicola T, Ilina R *et al.* (2007) Role of multi-component functional foods in the complex treatment of patients with advanced breast cancer. *Rev Med Chir Soc Med Nat Iasi* **111**, 877-884.

23. Edwards IJ & O'Flaherty JT (2008) Omega-3 Fatty Acids and PPARgamma in Cancer. *PPAR Res* **2008**, 358052.

24. Edwards RL & Kroon PA (2014) Inhibition of VEGF Signaling by Polyphenols in Relation to Atherosclerosis and Cardiovascular Disease. 281-325.

25. Esfahani A, Wong JM, Truan J *et al.* (2011) Health effects of mixed fruit and vegetable concentrates: a systematic review of the clinical interventions. *J Am Coll Nutr* **30**, 285-294.

26. Fabian CJ, Kimler BF & Hursting SD (2015) Omega-3 fatty acids for breast cancer prevention and survivorship. *Breast Cancer Res* **17**, 62.

27. Feart C, Samieri C, Alles B *et al.* (2013) Potential benefits of adherence to the Mediterranean diet on cognitive health. *Proc Nutr Soc* **72**, 140-152.

28. Feart C, Samieri C & Barberger-Gateau P (2015) Mediterranean diet and cognitive health: an update of available knowledge. *Curr Opin Clin Nutr Metab Care* **18**, 51-62.

29. Ganasegeran K, Al-Dubai SA, Qureshi AM *et al.* (2012) Social and psychological factors affecting eating habits among university students in a Malaysian medical school: a cross-sectional study. *Nutr J* **11**, 48.

30. Hajianfar H, Paknahad Z & Bahonar A (2013) The effect of omega-3 supplements on antioxidant capacity in patients with type 2 diabetes. *Int J Prev Med* **4**, S234-238.

31. Jacka FN, Ystrom E, Brantsaeter AL *et al.* (2013) Maternal and early postnatal nutrition and mental health of offspring by age 5 years: a prospective cohort study. *J Am Acad Child Adolesc Psychiatry* **52**, 1038-1047.

32. Jacobs DR, Jr., Gross MD & Tapsell LC (2009) Food synergy: an operational concept for understanding nutrition. *Am J Clin Nutr* **89**, 1543S-1548S.

33. Jacobs DR & Steffen LM (2003) Nutrients, foods, and dietary patterns as exposures in research: a framework for food synergy. *Am J Clin Nutr* **78**, 508S–513S.

34. Joe AK, Liu H, Suzui M *et al.* (2002) Resveratrol Induces Growth Inhibition, S-phase Arrest, Apoptosis, and Changes in Biomarker Expression in Several Human Cancer Cell Lines. *Clinical Cancer Research* **8**, 893-903.

35. Kolehmainen M, Mykkanen O, Kirjavainen PV *et al.* (2012) Bilberries reduce low-grade inflammation in individuals with features of metabolic syndrome. *Mol Nutr Food Res* **56**, 1501-1510.

36. Konings E, Timmers S, Boekschoten MV *et al.* (2014) The effects of 30 days resveratrol supplementation on adipose tissue morphology and gene expression patterns in obese men. *Int J Obes (Lond)* **38**, 470-473.

37. Kramer A, Green J, Pollard J, Jr. *et al.* (2014) Causal analysis approaches in Ingenuity Pathway Analysis. *Bioinformatics* **30**, 523-530.

38. Lamprecht M, Obermayer G, Steinbauer K *et al.* (2013) Supplementation with a juice powder concentrate and exercise decrease oxidation and inflammation, and improve the microcirculation in obese women: randomised controlled trial data. *Br J Nutr* **110**, 1685-1695.

39. Lamprecht M, Oettl K, Schwaberger G *et al.* (2007) Several indicators of oxidative stress, immunity, and illness improved in trained men consuming an encapsulated juice powder concentrate for 28 weeks. *J Nutr* **137**, 2737-2741.

40. Laviano A, Rianda S, Molfino A *et al.* (2013) Omega-3 fatty acids in cancer. *Curr Opin Clin Nutr Metab Care* **16**, 156-161.

41. Manach C, Mazur A & Scalbert A (2005) Polyphenols and prevention of cardiovascular diseases. *Curr Opin Lipidol* **16**, 77-84.

42. Mao QQ, Bai Y, Lin YW *et al.* (2010) Resveratrol confers resistance against taxol via induction of cell cycle arrest in human cancer cell lines. *Mol Nutr Food Res* **54**, 1574-1584.

43. Marion-Letellier R, Savoye G & Ghosh S (2016) Fatty acids, eicosanoids and PPAR gamma. *Eur J Pharmacol* **785**, 44-49.

44. Mi H, Muruganujan A, Casagrande JT *et al.* (2013) Large-scale gene function analysis with the PANTHER classification system. *Nat Protoc* **8**, 1551-1566.

45. Mikolajczyk RT, El Ansari W & Maxwell AE (2009) Food consumption frequency and perceived stress and depressive symptoms among students in three European countries. *Nutr J* **8**, 31.

46. Myi H, Muruganujan A, Dong S *et al.* (2009) An Introduction to the PANTHER Pathway Resource. *NCI Nature Pathway Interaction Database*.

47. Notarnicola M, Tutino V, Tafaro A *et al.* (2013) Antitumorigenic effect of dietary natural compounds via lipid metabolism modulation in Apc(Min/+) mice. *Anticancer Res* **33**, 3739-3744.

48. Pallauf K, Giller K, Huebbe P *et al.* (2013) Nutrition and healthy ageing: calorie restriction or polyphenol-rich "MediterrAsian" diet? *Oxid Med Cell Longev* **2013**, 707421.

49. Pandey KB & Rizvi SI (2009) Plant polyphenols as dietary antioxidants in human health and disease. *Oxid Med Cell Longev* **2**, 270-278.

50. Petrik MBH, McEntee MF, Chiu C-H *et al.* (1999) Omega-3 Fatty Acids Reduce Intestinal Tumors In Mice With a Defect in the APC Gene. *Journal of the American Dietetic Association* **99**, A11.

51. Petro TM (2011) Regulatory role of resveratrol on Th17 in autoimmune disease. *Int Immunopharmacol* **11**, 310-318.

52. Prado EL & Dewey KG (2014) Nutrition and brain development in early life. *Nutr Rev* **72**, 267-284.

53. Quang TH, Ngan NT, Minh CV *et al.* (2013) Anti-inflammatory and PPAR transactivational properties of flavonoids from the roots of Sophora flavescens. *Phytother Res* **27**, 1300-1307.

54. Quoc Trung L, Espinoza JL, Takami A *et al.* (2013) Resveratrol induces cell cycle arrest and apoptosis in malignant NK cells via JAK2/STAT3 pathway inhibition. *PLoS One* **8**, e55183.

55. Ramos-Romero S, Perez-Cano FJ, Perez-Berezo T *et al.* (2012) Effect of a cocoa flavonoid-enriched diet on experimental autoimmune arthritis. *Br J Nutr* **107**, 523-532.

56. Ritchie ME, Phipson B, Wu D *et al.* (2015) limma powers differential expression analyses for RNA-sequencing and microarray studies. *Nucleic Acids Res* **43**, e47.

57. Roll S, Nocon M & Willich SN (2011) Reduction of common cold symptoms by encapsulated juice powder concentrate of fruits and vegetables: a randomised, double-blind, placebo-controlled trial. *Br J Nutr* **105**, 118-122.

58. Sagar SM, Yance D & Wong RK (2006) Natural health products that inhibit angiogenesis: a potential source for investigational new agents to treat cancer—Part 1. *Current Oncology* **13**, 14-26.

59. Shen L (2015) GeneOverlap: An R package to test and visualize gene overlaps. *R Package.* [*http://shenlab-sinai.github.io/shenlab-sinai/*](http://shenlab-sinai.github.io/shenlab-sinai/).

60. Shukla Y & Singh R (2011) Resveratrol and cellular mechanisms of cancer prevention. *Ann N Y Acad Sci* **1215**, 1-8.

61. Simopoulos AP (2002) Omega-3 fatty acids in inflammation and autoimmune diseases. *J Am Coll Nutr* **21**, 495-505.

62. Stoclet JC, Chataigneau T, Ndiaye M *et al.* (2004) Vascular protection by dietary polyphenols. *Eur J Pharmacol* **500**, 299-313.

63. Tanaka T (2014) Flavonoids for allergic diseases: present evidence and future perspective. *Curr Pharm Des* **20**, 879-885.

64. Timmers S, Konings E, Bilet L *et al.* (2011) Calorie restriction-like effects of 30 days of resveratrol supplementation on energy metabolism and metabolic profile in obese humans. *Cell Metab* **14**, 612-622.

65. Torres SJ & Nowson CA (2007) Relationship between stress, eating behavior, and obesity. *Nutrition* **23**, 887-894.

66. Tulio AZ, Jr., Chang C, Edirisinghe I *et al.* (2012) Berry fruits modulated endothelial cell migration and angiogenesis via phosphoinositide-3 kinase/protein kinase B pathway in vitro in endothelial cells. *J Agric Food Chem* **60**, 5803-5812.

67. Vauzour D, Rodriguez-Mateos A, Corona G *et al.* (2010) Polyphenols and human health: prevention of disease and mechanisms of action. *Nutrients* **2**, 1106-1131.

68. Wang S, DeGroff VL & Clinton SK (2003) Tomato and soy polyphenols reduce insulin-like growth factor-I-stimulated rat prostate cancer cell proliferation and apoptotic resistance in vitro via inhibition of intracellular signaling pathways involving tyrosine kinase. *J Nutr* **133**, 2367-2376.

69. Welter D, MacArthur J, Morales J *et al.* (2014) The NHGRI GWAS Catalog, a curated resource of SNP-trait associations. *Nucleic Acids Res* **42**, D1001-1006.

70. Yuan L, Zhang Y, Xia J *et al.* (2015) Resveratrol induces cell cycle arrest via a p53-independent pathway in A549 cells. *Mol Med Rep* **11**, 2459-2464.

**Table 1.** Microarray study descriptions included in the pathway analysis

| Treatment | ArrayExpress ID | Study description a | Study cohort | Supplementation description | Array ID | Citation |
| --- | --- | --- | --- | --- | --- | --- |
| Omega-3 | E-GEOD-48368 | Gene expression profile of PBMCs after intake of fish oil for seven weeks | n=17 healthy subjects | Fish oil capsules providing a daily intake of 1.6 g EPA/DHA (0.7 g EPA; 0.9 g DHA) | Illumina HumanHT-12 V4.0 expression beadchip array | - |
| E-GEOD-12375 | Gene expression profile of PBMCs after intake of fish oil for six months | n=23 healthy elderly subjects (15 males, 8 females); age 69.9 (67–76) years; BMI 26.5 (21.7–33.6) kg/m2; triglycerides 1.0 (0.4–2.0) mmol/L | Fish oil capsules providing a daily intake of 1.8g EPA/DHA (1,093 ± 17 mg EPA; 847 ± 23 mg DHA) | NuGO human Hs1a520180 array | [11](#_ENREF_11) |
| E-GEOD-20114 | Gene expression profile of PBMCs after DHA supplementation for 90 days | n=4 hypertriglyceridemic but otherwise healthy men; age 39-66 years; BMI 22-35 kg/m2; triglycerides 1.7–4.5 mmol/L | DHA oil capsules providing a daily intake of 3 g DHA and 0 g EPA | Affymetrix GeneChip Human Genome U133 Plus 2.0 array | [20](#_ENREF_20) |
| Flavonoids | E-GEOD-13899 | Gene expression profile of monocytes after quercetin supplementation for 2 weeks | n=4 healthy subjects (2 males, 2 females); age 24 (SD 2·8) years; BMI 21.1 (SD 1.9); systolic/ diastolic BP 122/73 (SD 5/5) mmHg; triglycerides 1.2 (SD 0.4) mmol/L | Capsules providing a daily intake of 150 mg quercetin (naturally occurring polyphenol of the flavonoid subclass *flavonols*) | Affymetrix GeneChip Human Genome U133 Plus 2.0 array | [10](#_ENREF_10) |
| E-GEOD-34145 | Gene expression profile of PBMCs following dietary intake of anthocyanins for 8 weeks | n=3 overweight female subjects; 53 (SD 6) years; BMI 31.4 (SD 4.7) kg/m2; systolic/ diastolic BP 147/93 (SD 18/9) mmHg; triglycerides 2.2 (SD 0.7) mmol/L | Daily consumption of bilberry puree and dried bilberries equivalent of 400 g fresh bilberries (contain the highest levels of polyphenols, particularly anthocyanins of the flavonoid subclass *flavanols*, amongst the commonly consumed berries). Other sources of carbohydrates in the subjects’ diet were substituted with the provided bilberry products. | Illumina Human-6 v2 Expression BeadChip | [35](#_ENREF_35) |
| Resveratrol | E-GEOD-42432 | Gene expression profile of adipose tissue following 30 days resveratrol supplementation | n=9 obese but otherwise healthy male subjects; age 40-65 years; 28-36 kg/m2 | Double-blind randomised crossover study; daily intake of placebo and 150 mg/day resveratrol [99% resVida] with a 4-week washout period in between | Affymetrix Human Gene 1.1 ST Array [HuGene-1\_1-st] | [36](#_ENREF_36) |
| E-GEOD-41168 | Gene expression profile of adipose tissue following 12 weeks resveratrol supplementation | n=13 healthy, postmenopausal female subjects | Daily supplementation with 75 mg/day resveratrol | Affymetrix GeneChip Human Genome U133 Plus 2.0 Array | - |
| E-GEOD-41168 | Gene expression profile of skeletal muscle (vastus lateralis) following 12 weeks resveratrol supplementation | n=9 healthy, postmenopausal female subjects | Daily supplementation with 75 mg/day resveratrol | Affymetrix GeneChip Human Genome U133 Plus 2.0 Array | - |
| E-GEOD-32357 | Gene expression profile of skeletal muscle (vastus lateralis) following 30 days resveratrol supplementation | n=10 obese but otherwise healthy male subjects 52.5 (SEM 2.1) years; BMI 31.5 (SEM 0.8) kg/m2; systolic/ diastolic BP 131/82 (SEM 3.1/2.5) mmHg; triglycerides 1.9 (SEM 0.2) mmol/L | Double-blind randomised crossover study; daily intake of placebo and 150 mg/day resveratrol [99% resVida] with a 4-week washout period in between | Affymetrix Human Gene 1.1 ST Array [HuGene-1\_1-st] | [64](#_ENREF_64) |

Abbreviations: *BMI, body mass index; BP, blood pressure; EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid; PBMCs, peripheral blood mononuclear cells*. a Gene expression changes were analysed in the same individuals using baseline measurements verses treatment intervention unless stated otherwise. Available study cohort subject demographics are presented as mean (range, SD or SEM).

**Table 2.** Common canonical pathways significantly regulated following dietary intervention with omega-3 (EPA/DHA), flavonoids and resveratrol

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Functional Group | Canonical Pathway | Omega-3 (EPA/DHA) | Flavonoids | Resveratrol |
| Immune response pathways | PPARα/RXRα Activation | **2.20 x 10-2** | **1.99 x 10-2** ↓ | **1.97 x 10-2** |
| IL-10 Signaling | **9.53 x 10-4** | 4.48 x 10-1 | **8.69 x 10-3** |
| Glucocorticoid Receptor Signaling | **9.51 x 10-3** | **2.07 x 10-3** | 5.22 x 10-1 |
| Natural Killer Cell Signaling | 5.33 x 10-1 | **6.51 x 10-3** | **3.73 x 10-2** |
| Leukocyte Extravasation Signaling | **4.17 x 10-2** | 4.43 x 10-1 | **8.70 x 10-3** |
| Regulation of IL-2 Expression in Activated and Anergic T Lymphocytes | **1.37 x 10-2** | **1.60 x 10-2** | - |
| IL-6 Signaling | **4.75 x 10-2** | 3.01 x 10-1 | **4.38 x 10-2** |
| Signal transduction | Protein Kinase A Signaling | **3.36 x 10-2** | **3.26 x 10-4** | 4.11 x 10-1 |
| NF-κB Signaling | **5.47 x 10-3** | **3.26 x 10-3** | 2.97 x 10-1 |
| HIPPO signaling | **1.72 x 10-2** | 2.53 x 10-1 | **1.58 x 10-2** |
| Sumoylation Pathway | **2.29 x 10-2** | **7.85 x 10-3** | - |
| Disease pathways | Systemic Lupus Erythematosus Signaling | **4.89 x 10-2** | **2.66 x 10-3** | 3.83 x 10-1 |
| Hepatic Fibrosis / Hepatic Stellate Cell Activation | **2.79 x 10-2** | - | **2.51 x 10-2** |
| Cardiovascular system | Intrinsic Prothrombin Activation Pathway | **2.76 x 10-2** | - | **2.60 x 10-2** |
| Metabolism | Creatine-phosphate Biosynthesis | **3.12 x 10-2** | - | **3.02 x 10-2** |

Functional enrichment analysis of gene sets differentially regulated by dietary intervention with omega-3 fatty acids EPA/DHA, flavonoids or resveratrol was performed using IPA software. Fold change cut-off of >0.5 and <-0.5 log(differential expression, DE) was applied. Statistical significance was determined using the Benjamini-Hochberg procedure. Bold font indicates pathways that withstood multiple-testing correction (adjusted p<0.05). Net effects of gene expression changes on pathway activation or repression were determined using activation z-scores (threshold, <-2.0; >2.0). ↓ Downregulated pathways; ↑ upregulated pathways.