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**Progress report on SPS 211259**

**Project title: Impact of walnut ellagitannins on immune and inflammatory cell function**

**Sponsor: UConn Health**

**RNA preparation and Next-generation sequencing**

PBMC preps from eleven volunteers taken before and after a 3-week walnut intervention period (56 g/day) were used to prepare RNA for RNA-seq analysis at the UConn Center for Genome Innovation (CGI). Sufficient RNA was available to perform RNA-seq in duplicate for all but three samples, which were processed as a single (Table 1). Some of the replicates showed RBC contamination, so all hemoglobin transcripts were removed. RNA-seq data for volunteer 1011 showed that one replicate was an extreme outlier from all other samples, so this outlier sample was excluded from further analysis.

**Bioinformatic analysis**

Gene expression data were processed to obtain transcript-level counts, which were then aggregated to gene-level counts. Duplicate samples showed good agreement. Data were processed to obtain transcripts per kilobase million (TPM). Using raw counts, DESeq2 was used to identify differentially expressed genes following the walnut intervention.Principal component analysis did not resolve the data into before and after walnut intervention groups (not shown). The table at the end shows gene expression changes with a p<0.001 (excluding genes with low expression and a log2-fold change less than 0.75).

**Analysis of gene expression changes associated with walnut consumption**

DEseq2 analysis of the RNA-seq data was performed to identify gene expression changes induced by walnut consumption. Values generated by DEseq2 include the Log2-fold change, the p-value and the padjusted-value (corrected for multiple tests). Top gene expression changes based on statistical significance are shown in Table 2 (novel transcripts and low-expressors were removed). Two genes showed expression changes with a padjusted < 0.05: FOSB and NAMPT. Both genes were increased in subjects following walnut consumption, and both are typically associated with enhanced cellular proliferation. FOSB is an immediate early gene activated by growth factors in numerous cell types and NAMPT is a multifunctional protein that maintains cellular energy levels in proliferating cells and, when secreted, has growth factor activity of its own. The next most significantly changed gene following walnut consumption was RASGEF1B, which was increased post walnut consumption with a padjusted < 0.1. Although less statistically significant than FOSB and NAMPT, it is notable that RASGEF1B encodes a Ras guanine nucleotide exchange factor (GEF), a key pro-growth signaling protein expressed in many different cell types. Overall, these gene expression changes point to a stronger proliferative capacity of PBMCs following walnut intervention.



A final gene of note activated by walnut consumption is the AHR gene. Although not statistically prominent, this gene may be biologically relevant given that the AHR protein encodes the aryl hydrocarbon receptor transcription factor that can bind walnut-derived urolithins. AHR links chemical stimuli with transcriptional responses. It is not clear why its expression is higher after walnut consumption. One possibility is that the cells increase its expression in response to AHR binding to one or more urolithin metabolites.

**Subject-specific gene expression changes**

To assess the gene expression changes following walnut consumption on an individual subject basis, before and after line graphs were generated for FOSB, NAMPT, RASGEF1B, and AHR (Fig 1). For most subjects, expression of these 4 genes was increased following walnut consumption. However, data also shows subject variability; in some cases, no response or a decrease in expression is observed. To understand how the variability in the gene activation relates in the different subjects, a table was generated showing the fold-activation of each gene in each subject (Table 3; gene activation shown in red, gene repression shown in blue). This table shows a general coregulation of this gene set, suggesting a coordinated gene expression response. It also shows that some subjects respond more strongly than others, which may be related to metabolic variables.

**Figure 1:** Lines graphs showing mRNA expression changes in individual subjects before and after 3-week walnut consumption.

A group of graphs with different colored lines

Description automatically generated

**Summary**

Walnut consumption was associated with an increased expression of a set of genes involved in promoting cell activation and proliferation. The data also pointed to range of individual responses, with most individuals activating these genes.

It is not clear which cell types in the PBMC pool display the gene expression changes identified, or the impact these genes on immune responses. Based on the function of the genes identified, stronger activation responses might be expected. It is also unclear which walnut components are responsible for these changes.

**Gene expression changes sorted by p-adjusted**

Genes with a base mean > 50; log2 change > 0.75; p-value < 0.001

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Gene name** | **Description** | **baseMean** | **log2Change** | **padj** | **pvalue** |
| FOSB | FosB proto-oncogene, AP-1 transcription factor subunit [Source:HGNC Symbol;Acc:HGNC:3797] | 4727.95 | 2.305 | 0.0162 | 3.94E-07 |
| NAMPT | nicotinamide phosphoribosyltransferase [Source:HGNC Symbol;Acc:HGNC:30092] | 6496.32 | 0.891 | 0.045 | 2.19E-06 |
| RASGEF1B | RasGEF domain family member 1B [Source:HGNC Symbol;Acc:HGNC:24881] | 1259.83 | 1.005 | 0.0855 | 8.31E-06 |
| EMP1 | epithelial membrane protein 1 [Source:HGNC Symbol;Acc:HGNC:3333] | 395.74 | 1.198 | 0.1283 | 2.81E-05 |
| USP3-AS1 | USP3 antisense RNA 1 [Source:HGNC Symbol;Acc:HGNC:44140] | 61.56 | 0.757 | 0.1722 | 4.18E-05 |
| A2M | alpha-2-macroglobulin [Source:HGNC Symbol;Acc:HGNC:7] | 51.92 | 0.825 | 0.2508 | 9.50E-05 |
| AHR | aryl hydrocarbon receptor [Source:HGNC Symbol;Acc:HGNC:348] | 2830.22 | 0.807 | 0.2508 | 1.03E-04 |
| RASD1 | ras related dexamethasone induced 1 [Source:HGNC Symbol;Acc:HGNC:15828] | 103.11 | 0.796 | 0.2508 | 1.04E-04 |
| NAMPTP1 | nicotinamide phosphoribosyltransferase pseudogene 1 [Source:HGNC Symbol;Acc:HGNC:17633] | 507.7 | 0.757 | 0.4079 | 3.77E-04 |
| TP53INP2 | tumor protein p53 inducible nuclear protein 2 [Source:HGNC Symbol;Acc:HGNC:16104] | 384.66 | 0.701 | 0.4079 | 3.36E-04 |
| UBE2FP1 | UBE2F pseudogene 1 [Source:HGNC Symbol;Acc:HGNC:44535] | 60.26 | 0.918 | 0.4103 | 4.72E-04 |
| TRIB1 | tribbles pseudokinase 1 [Source:HGNC Symbol;Acc:HGNC:16891] | 2623.32 | 0.721 | 0.4148 | 5.79E-04 |
| AREG | amphiregulin [Source:HGNC Symbol;Acc:HGNC:651] | 613.01 | 1.496 | 0.4289 | 7.21E-04 |
| SGK1 | serum/glucocorticoid regulated kinase 1 [Source:HGNC Symbol;Acc:HGNC:10810] | 3535.06 | 0.713 | 0.4567 | 8.36E-04 |
| PER1 | period circadian regulator 1 [Source:HGNC Symbol;Acc:HGNC:8845] | 6251.42 | 0.745 | 0.4917 | 1.18E-03 |
| SOCS1 | suppressor of cytokine signaling 1 [Source:HGNC Symbol;Acc:HGNC:19383] | 432.65 | 0.716 | 0.5185 | 1.39E-03 |
| ADM | adrenomedullin [Source:HGNC Symbol;Acc:HGNC:259] | 322.01 | 0.785 | 0.5647 | 1.66E-03 |
| IFNG | interferon gamma [Source:HGNC Symbol;Acc:HGNC:5438] | 77.45 | 0.787 | 0.5758 | 1.83E-03 |
| ID1 | inhibitor of DNA binding 1 [Source:HGNC Symbol;Acc:HGNC:5360] | 282.39 | 1.059 | 0.6194 | 2.39E-03 |
| TAMALIN | trafficking regulator and scaffold protein tamalin [Source:HGNC Symbol;Acc:HGNC:18707] | 895.02 | 0.755 | 0.6608 | 3.10E-03 |
| CLIC1 | chloride intracellular channel 1 [Source:HGNC Symbol;Acc:HGNC:2062] | 86.96 | -1.174 | 0.7278 | 4.14E-03 |
| IGLV3-21 | immunoglobulin lambda variable 3-21 [Source:HGNC Symbol;Acc:HGNC:5905] | 358.15 | -1.212 | 0.7501 | 4.71E-03 |
| LRG1 | leucine rich alpha-2-glycoprotein 1 [Source:HGNC Symbol;Acc:HGNC:29480] | 179.25 | 1.092 | 0.7699 | 5.18E-03 |
| NR4A2 | nuclear receptor subfamily 4 group A member 2 [Source:HGNC Symbol;Acc:HGNC:7981] | 2220.02 | 1.121 | 0.7732 | 5.47E-03 |
| TNFRSF17 | TNF receptor superfamily member 17 [Source:HGNC Symbol;Acc:HGNC:11913] | 93.22 | -1.248 | 0.8521 | 7.34E-03 |
| MAFF | MAF bZIP transcription factor F [Source:HGNC Symbol;Acc:HGNC:6780] | 1061.09 | 0.898 | 0.868 | 7.74E-03 |
| IGLV3-19 | immunoglobulin lambda variable 3-19 [Source:HGNC Symbol;Acc:HGNC:5903] | 158.88 | -0.935 | 0.868 | 7.95E-03 |
| CD83 | CD83 molecule [Source:HGNC Symbol;Acc:HGNC:1703] | 1514.56 | 0.734 | 0.8704 | 8.77E-03 |
| PDK4 | pyruvate dehydrogenase kinase 4 [Source:HGNC Symbol;Acc:HGNC:8812] | 2075.87 | 0.703 | 0.8704 | 8.27E-03 |
| IGHM | immunoglobulin heavy constant mu [Source:HGNC Symbol;Acc:HGNC:5541] | 1267.61 | -1.031 | 0.8704 | 8.33E-03 |
| BHLHA15 | basic helix-loop-helix family member a15 [Source:HGNC Symbol;Acc:HGNC:22265] | 68.24 | -1.094 | 0.8704 | 8.46E-03 |
| TNFSF9 | TNF superfamily member 9 [Source:HGNC Symbol;Acc:HGNC:11939] | 87.5 | 0.763 | 0.8905 | 1.01E-02 |
| TNFRSF12A | TNF receptor superfamily member 12A [Source:HGNC Symbol;Acc:HGNC:18152] | 182.47 | 0.915 | 0.9965 | 3.97E-02 |
| DUSP4 | dual specificity phosphatase 4 [Source:HGNC Symbol;Acc:HGNC:3070] | 536.33 | 0.881 | 0.9965 | 1.67E-02 |
| EREG | epiregulin [Source:HGNC Symbol;Acc:HGNC:3443] | 236.09 | 0.763 | 0.9965 | 4.64E-02 |
| RGS1 | regulator of G protein signaling 1 [Source:HGNC Symbol;Acc:HGNC:9991] | 1735.42 | 0.73 | 0.9965 | 1.87E-02 |
| OSBP2 | oxysterol binding protein 2 [Source:HGNC Symbol;Acc:HGNC:8504] | 348.19 | -0.518 | 0.9965 | 4.27E-02 |
| PF4 | platelet factor 4 [Source:HGNC Symbol;Acc:HGNC:8861] | 149.55 | -0.599 | 0.9965 | 3.62E-02 |
| MYRF | myelin regulatory factor [Source:HGNC Symbol;Acc:HGNC:1181] | 169.81 | -0.678 | 0.9965 | 1.79E-02 |
| IGFBP2 | insulin like growth factor binding protein 2 [Source:HGNC Symbol;Acc:HGNC:5471] | 65.19 | -0.75 | 0.9965 | 4.71E-02 |
| JCHAIN | joining chain of multimeric IgA and IgM [Source:HGNC Symbol;Acc:HGNC:5713] | 4071.65 | -0.88 | 0.9965 | 2.55E-02 |
| EGR1 | early growth response 1 [Source:HGNC Symbol;Acc:HGNC:3238] | 4411.15 | -0.955 | 0.9965 | 2.66E-02 |
| MAGI2-AS3 | MAGI2 antisense RNA 3 [Source:HGNC Symbol;Acc:HGNC:40862] | 119.95 | -1.035 | 0.9965 | 3.17E-02 |