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Soy food supplementation, dietary fat reduction and peripheral blood gene expression in postmenopausal women -A randomized, controlled trial

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Abstract

We evaluated the potential of gene expression profiling in peripheral blood mononuclear cells (PBMCs) collected at baseline and at the completion of a 8-week controlled dietary intervention. Healthy postmenopausal women were randomized to a very-low-fat diet (VLFD; 11% of energy as fat) (n=21), a Step 1 diet (25% energy as fat) supplemented with soy food (SFD; 50 mg isoflavones/d) (n=20), or a control Step 1 diet (CD; 27% energy as fat) with no soy food (n=18). All diets were prepared at the General Clinical Research Center of the University of Southern California. We did not observe any gene that showed variable response across the three dietary interventions. However, there were notable changes in gene expression associated with the intervention in the VLFD and SFD groups. Our findings suggest that the expression of nicotinamide phosphoribosyltransferase (NAMPT) and genes related to Fc gamma R-mediated phagocytosis and cytokine interactions may be significantly altered in association with dietary fat reduction and soy supplementation. Gene expression changes in NAMPT were somewhat dampened with adjustment for weight but changes related to Fc gamma R-mediate phagocytosis and cytokine interactions remained largely unchanged. In summary, PBMCs can reveal novel gene expression changes in association with controlled dietary intervention.

Keywords

soy supplementation; dietary fat reduction; peripheral blood gene expression

1. Introduction

There is now compelling epidemiologic evidence from case-control and prospective cohort studies that regular soy food consumers experience a lower risk of breast cancer development and recurrence [1, 2]. However, the molecular/biochemical basis by which soy food intake may protect against breast cancer remains poorly understood. It has been speculated that the effect of soy on breast cancer could be mediated through a hormonal mechanism but results from meta-analyses of clinical studies showed no significant association between soy intervention and circulating estrogen levels [3] or mammographic density [4]. The role of dietary fat and breast cancer risk remains controversial with most of the prospective cohort studies showing no significant relation between risk and fat intake

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[5]. However, significant positive associations in relation to total fat intake [6] and saturated fat [7] were reported in recent cohort studies. The Women's Health Initiative Dietary Modification Trial also reported a marginally statistically significant 9% reduction in breast cancer incidence among women in the low fat dietary pattern group compared with women in the control group [8].

In the past decade the application of DNA microarray technology has opened up new opportunities to study the effects of food components and nutrients in the control of cellular processes [9]. The growing acceptance to conduct gene expression studies using peripheral blood mononuclear cells (PBMCs) as a surrogate for changes in tissues is particularly attractive because blood samples are often the only biological samples that can be readily collected in studies conducted in healthy volunteers [10]. We took advantage of a previously reported controlled randomized 2-month dietary intervention study in healthy women [11] to examine the effects of modification in the intake of dietary fat/carbohydrates, soy supplementation versus a control diet on gene expression in PBMC. Subjects in this study consumed a very-low-fat diet (VLFD; 11% of energy as fat, 68% energy as carbohydrate), a Step 1 diet (25% of energy as fat, 56% of energy as carbohydrate) supplemented with soy food (SFD; 50 mg isoflavones per day), or a control Step I diet (CD; 27% of energy as fat, 54% of energy as carbohydrate) with no soy food. All the foods for these isocaloric meals for the intervention study were prepared in the Bionutrition Kitchen of the General Clinical Research Center at the University of Southern California [11]. Weight loss was not an intended objective of our intervention study and the average weight loss was about 2 kg in each of the three arms (see below). Although we found no significant differences in serum sex hormones, lipid, growth factors and other biomarkers at the completion of the intervention between women in SFD and VLFD groups and those in the CD group, there were serological changes within each of the diet groups associated with intervention. These changes include: 1) significant reduction (range: 6% to 11%) in total cholesterol in all 3 dietary groups and significant reduction in LDL cholesterol (12%) in the VLFD group; 2) significant reduction (range: 33% to 47%) in leptin concentrations in all 3 groups; and 3) reductions (range: 13% to 33%) in insulin concentrations in all 3 groups which were statistically significant in the CD and SFD arms [11]. Using RNA samples that were isolated from the fasting blood specimens that were collected at baseline and at the completion of the intervention, we conducted a microarray study to evaluate gene expression changes in association with the 3 diets. We report here results we obtained on analysis of 58 women (17 CD, 20 SFD, 21 VLFD) in which gene expression profiles at baseline and at the completion of the 8 weeks of intervention were compared within each treatment arm.

2. Methods

2.1 Study subjects

Details of the study subjects have been reported previously [11]. Briefly, this study included healthy postmenopausal (> 1 year since the last menstrual period) who were not current users of menopausal hormone therapy (i.e., stopped use > 6 months before entering study). Participants were not on any special diets (e.g., low-fat, high-fiber, high-protein) and did not have a history of cancer, diabetes, cardiovascular diseases or other chronic diseases. A total of 59 subjects were included in the trial and were randomly assigned to one of the three intervention groups: 20 in SFD group, 21 in VLFD group, and 18 in CD group. Participants were blinded to the diet regimen they were assigned. All procedures for recruitment were approved by the USC institutional review board, and all subjects signed a written informed consent.

2.2 Dietary interventions

During the 8-week study period, subjects received daily meals that included breakfast, lunch, dinner and a morning and an evening snack that were prepared in the Bionutrition Department's Research Kitchen at the General Clinical Research Center at the Los Angeles County USC Medical Center weekly [11]. Details of the food preparation, storage and delivery have been reported previously [11]. In brief, the SFD intervention included 50 mg isoflavones/d (approximately 15 g soy protein); the VLFD intervention was designed to contain 12% of energy from fat and we used legumes as a primary source of protein along with fish, lean chicken and low-fat dairy products; the CD intervention followed the American Dietetic Association guidelines for a healthy balanced diet – about 50% of energy from carbohydrate, 30% from fat and 20% from protein [12]. Since weight change was not a designed purpose in the protocol, participants were weighed every 2 weeks and their diets were adjusted to higher or lower caloric diets if they had lost or gained > 2kg from the initial baseline weight. Although there were no differences in weight change between the 3 diet groups, there was a small but significant reduction in body weight in all 3 dietary arms: -1.9 kg in the CD group, -2.5 kg in the SFD group and -2.8 kg in the VLFD group.

2.3 Data collection

A baseline questionnaire including demographic characteristics, menstrual and reproductive and menopausal factors was administered to participants. Body weight, blood pressure measurements and blood samples were obtained at baseline and every 2 weeks during follow-up. Blood specimens were collected between 6am and 11am after subjects had fasted for 12 hours. On the day of blood drawn, subjects also collected an overnight urine sample into a plastic bottle which contained 1 g ascorbic acid. Urine samples were separated into 100mL aliquots and stored at -20 degree. Participants completed a daily log of foods consumed and the logs were used as one of the measures for compliance. Urinary isoflavone concentrations were measured; levels remained very low for women in the CD and VLFD diet but increased more than 10-fold for women in the SFD showing good compliance [11]. As for subjects in VLFD and CD groups, high density lipoprotein (HDL) and triglyceride concentrations were used as an indirect marker for compliance [11].

2.4 RNA isolation and microarray profiling

Total RNA was extracted from peripheral blood samples collected from each subject at baseline and at the end of the 8 weeks of intervention using commercial reagents including Trizol (Invitrogen) followed by further purification with Qiagen RNeasy kit (Qiagen, Valencia, CA). We quantified RNA yield by spectrophotometric analysis using the convention that 1 OD at 260 nm equals 40 micrograms RNA per ml. The absorbance was checked at 260 and 280 nm for determination of sample concentration and purity. All the samples had ratios of A260/A280 between 1.9 and 2.1 with a value of 2.0 for pure RNA. Biotin-labeled cRNA was prepared from total RNA and hybridized to Affymetrix Human Genome U95Av2 arrays (Affymetrix, Santa Clara, CA, USA) which interrogated 12,625 genes. Probe synthesis, hybridization and initial expression analysis were performed according to protocol as recommended by the manufacturer. The gene expression studies were conducted using core facilities at the Childrens' Hospital of Los Angeles and Keck School of Medicine at USC [13]. Briefly the RNA was used to generate cDNA by reverse transcriptase, then cRNA using RNA polymerase II. In this step, biotinylated, fluorescently labeled nucleotides were incorporated into the cRNA. This labeled mixture was then fragmented and hybridized with the GeneChip. Amplification of the signal is achieved using fluorescently labeled avidin. Arrays were read in a confocal laser scanner and the data analyzed using the R programming language and packages from the Bioconductor web site (<http://www.bioconductor.org>).

We successfully obtained both pre- and post- treatment gene expression measurements on 58 participants (20 in SFD group, 21 in VLFD group, and 17 in CD group) but only obtained baseline gene expression measurements on 1 subject in the CD group. We did not include this subject in the analysis. These microarrays were processed at 3 different runs (approximately 4 months apart) during a period of 11 months between 2001 and 2002. Pre- and post- treatment gene expression measurements for the same subject were done in the same batch.

2.5 Statistical Analysis

Gene expression microarray data preprocessing, including background correction, quantile normalization and summarization, were performed by Robust Multiarray Analysis (RMA) algorithm [14]. The microarray data were preprocessed separately by date of run, so that the primary dependent variable, difference between baseline and endpoint, should not be subject to effects of analysis batch. No evidence of a batch effect was confirmed using a principle component analysis (PCA) to visualize whether the primary dependent variable varied by batch.

Before testing for treatment effects, we removed Affymetrix internal control probes from the data set, and applied a non-specific filter to remove low-varying probes. The probes were ranked based on the interquartile range (IQR) of the difference in expression levels between baseline and week 8. The top 50% were selected for further analysis ($n=6279$ probes). We used analysis of variance to compare the mean gene expression changes across the three diet groups for each probe. Univariate gene tests were conducted using a false-discovery rate (FDR) to control for multiple comparisons. To incorporate biological knowledge into the analysis, an adapted gene set enrichment analysis (GSEA) approach was used to identify a subset of genes differentially induced by the dietary interventions [15]. When several probes were mapped to a single gene, we picked up the most variable probe, as measured by IQR. Via Entrez Gene ID, probes were first mapped to Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways [16], one of the most widely used pathway databases, and 197 pathways were identified. However, only pathways with at least 10 genes were kept, leaving a total of 147 pathways and 2048 probes. Changes of gene expression from baseline to week 8 (log fold change) was regressed on dietary intervention groups, and an F-test was performed to test the overall association between dietary interventions and gene expression change for each of the 2048 probes. For each of the 147 pathways, an enrichment score was generated by summing over the scaled deviation of the observed minus expected F-statistic

for the probes in the pathway: $ES_{SCORE} = \sqrt{N_g} \sum_{i=1}^{N_g} (F_i - E(F)) / S(F)$, where N_g is the number of genes in the pathway, F_i the observed F statistic for the i^{th} probe in the pathway, $E(F)$ the expected value of F and $S(F)$ its standard deviation under the null. We computed p-values by assuming that the ES score approximated a standard normal variate. False-discovery rate (FDR)-adjusted p-values were computed using the Benjamini & Hochberg method [17].

We then used repeated measures analysis of variance (ANOVA) to assess the level of gene expression changes within each of the three dietary groups. First, the gene set enrichment analysis within each diet group was used. The same approach used for between group analyses was applied here. Briefly, the top 50% most variable probes were selected based on IQR of the difference in expression levels between baseline and week 8 within each intervention group respectively, and the most variable probe was chosen if several probes mapped to a single gene. Requiring at least 10 genes per pathway, we identified 148, 151 and 146 pathways in SFD, VLFD and CD groups, respectively. The enrichment scores were then generated using the standard enrichment score for t-tests proposed by Irizzary et al.

[15]. 2-sided parametric p-values were obtained and further adjusted using Benjamini & Hochberg method (17).

Finally, we added a fold change cut-off criteria in addition to the repeated measures ANOVA to assess the level of gene expression changes within each of the three dietary groups. To make it possible to compare fold change between groups, we used the 6279 probes which were selected based on variation across the whole samples. Probes were identified as up-regulated or down-regulated if: (1) average unadjusted fold change was greater than 1.25 (up-regulated) or less than 0.80 fold (down-regulated); and (2) paired t-test p-value < 0.05 (see Tables 1-4, columns 3 and 4). We also show fold-changes that adjusted for body mass index (mg/kg^2) as a time-dependent covariate (see Tables 1-4, columns 5 and 6). We further identified if any pathways existed in those up- and down-regulated genes in each dietary group using KEGG database.

3. Results

Overall, the effects of SFD and VLFD intervention groups on gene expression changes from baseline to week 8 were similar (Figure 1 a and b). Both groups showed more pronounced down-regulation but very limited up-regulation in gene expression levels. The CD group, on the other hand, indicated a relatively balanced change in both up- and down-regulation sides with slightly more probes on the up-regulation side showing stronger statistical significance (Figure 1 c). Despite suggested differences observed in figure 1, when we performed ANOVA analyses by controlling for FDR, there were no statistically significant differences between the diet groups. With the relatively modest sample size in this study, we conducted further analyses to examine changes within a diet group. At the same time, GSEA did not identify any pathways with an excess representation of genes showing variation in response (all $p > 0.05$).

However, the gene set enrichment analysis within each dietary group suggested there were groups of genes significantly induced by the dietary interventions, and the effects were much more pronounced in SFD and VLFD groups than in CD group. While 51 and 86 pathways were identified in SFD and VLFD groups, only 3 pathways were found in CD group (Supplementary Table 1, 2 and 3). Three pathways – “Chemokine signaling pathway”, “Fc gamma R-mediated phagocytosis” and “Natural killer cell mediated cytotoxicity” were the top 3 hits in both SFD and VLFD groups. “Metabolic pathway” was the most affected group of genes in the CD group. We then picked up significantly changed probes if the average unadjusted fold change was greater than 1.25-fold (up-regulated) or less than 0.80 fold (down-regulated) and the paired t-test p-value < 0.05. In the VLFD arm 160 probes (representing 134 unique genes) showed significant down-expression (<0.80 fold) (Table 1, Columns 3 and 4) compared to 77 probes (representing 67 unique genes) in the SFD arm (Table 2, Columns 3 and 4) and 5 probes (representing 5 unique genes) in the CD arm (Table 3, Columns 3 and 4). Very few genes showed significantly over-expression (>1.25-fold) after dietary treatment (none in the SFD, 1 probe in the VLFD and 7 probes in the CD arms, Table 4, Columns 3 and 4). In the SFD, 16 of the 77 probes showed at least 30% lower gene expression levels after 2 months of soy supplementation. The top 5 most down-regulated genes in terms of fold change were DEFA1 (0.48 fold, $P=0.008$), FCGR3B (0.52 fold, $P=0.006$), NAMPT (0.55 fold, $P=0.026$), FCGR2A (0.61 fold, $P=0.019$), PTGS2 (0.63 fold, $P=0.017$). In the VLFD arm, 31 of the 160 probes showed at least 30% lower gene expression levels after intervention. The top 5 most down-regulated genes were FCGR3B (0.49 fold, $P=0.01$), NAMPT (0.54, $P=0.027$), FCGR2A (0.56 fold, $P=0.005$), BCL2A1 (0.57, $P=0.004$), and ANXAS (0.58 fold, $P=0.039$). Out of the 77 down-regulated probes in SFD group, 50 are also among the 160 down-expressed probes in VLFD group. In fact, 3

genes (FCGR3B, NAMPT, FCGR2A) showed largest fold changes in both the VLFD group and the soy group without adjustment for BMI.

We also show the fold-change results with adjustment for BMI as a time-dependent covariate. In the VLFD group, there were very slight changes in the fold-changes of the top five genes (FCGR3B, NAMPT, FCGR2A, BCL2A1, ANXA3) although there were some weakening of the p values (Table 1, columns 5 and 6). In the SFD arm, there were also some changes in the fold-changes of the top five genes (DEFA1, FCGR3B, NAMPT, FCGR2A, PTGS2); 2 the 5 top genes (DEFA1, FCGR3B) remained statistically significant (Table 2, columns 5 and 6) and the 3 genes were borderline statistically significant.

Using the 10 genes per pathway criteria, the 160 probes down-regulated in VLFD group were found in 3 pathways: “Pathways in cancer” (N=13 genes), “Chemokine signaling pathway” (N=13 genes) “Natural killer cell mediated cytotoxicity pathway” (N=11 genes). Two pathways were found in the SFD arm when using at least 5 genes/pathway criteria (insulin signaling pathway and pathways in cancer; each has 5 genes) but no particular pathway was identified in the 77 under-expressed probes in SFD group when using the 10 genes/pathway criterion.

4. Discussion

We evaluated the potential of gene expression changes in peripheral blood samples in a 8-week dietary intervention of 58 healthy postmenopausal women who were randomized to a very-low-fat diet (VLFD; 11% of energy as fat), a Step 1 diet supplemented with soy food (SFD; 50 mg isoflavones per day), or a control Step 1 diet (CD, 27% of energy as fat, no isoflavones per day). Weight maintenance was largely achieved (~3% of weight loss in the three groups) in our study. We did not observe any significant differences in gene expression changes between the three groups after correction for multiple testing. Two other studies have compared gene expression profiles of diets with very different macronutrient composition [18, 19]. In a 10 week dietary intervention study which randomized participants to a diet of 20-25% fat energy or a diet of 40-45% fat energy, gene expression responses in adipose tissue to the two diets did not differ [18]. In contrast, significant differences in gene expression profiles were reported in a study that compared PBMC gene expression responses to a high-carbohydrate (47% of energy from carbohydrate, 19% of energy from protein) or a high-protein (14% of energy from carbohydrate, 58% of energy from protein) before and 2 hours after consumption of these breakfasts [19]. However, postprandial gene expression changes may not apply to longer term dietary intervention studies (see below). Results from our study and those of Dahlman et al. [18] suggest that changes in a wide range of macronutrients (at least in terms of dietary fat from 11% to 45% fat energy) may have little effect on gene expression.

Despite the lack of differences in responses between diets, we found statistically significant changes in gene expression between baseline and after 8 weeks of intervention within diets. The highest number of gene changes was in the VLFD group (135 genes), intermediate in the SFD group (67 genes), and lowest in the CD group (12 genes). The few changes in the control arm are consistent with findings in previous studies [20, 21]. No significant gene expression change in prostate epithelium was found among men randomized to a standard American diet (35% of energy as fat) whereas significant changes were observed among those randomized to a very low fat diet (<20% of energy as fat) for 6 weeks [20]. Similarly, no genes were found to be differentially expressed between baseline and repeat breast and adipose tissue biopsies among women who remained on a standard diet (34% of energy as fat) whereas many significant changes were found among those randomized to a 1-month calorie-restricted diet (~60% of usual calorie)[21].

The relatively modest changes we found in the VLFD and SFD groups are also not surprising. The changes we observed were modest both in terms of the percent of genes (~2.5% of the top 6279 probes) implicated and the magnitude of change (>1.25 fold or <0.80 fold). The relative stability of gene expression in our participants may be related, in part, that they were healthy and that weight loss was modest (~3% of body weight) during the study. Healthy subjects have a remarkable capacity to maintain homeostasis, both through direct metabolic regulation and compensation of an altered diet [22]. Studies that have reported larger magnitude of gene expression changes and/or larger percent of affected genes differed from our study in several ways. These studies have investigated postprandial dietary effects 2-6 hours after an intervention [19, 23, 24] or they were longer-term studies in which substantial weight loss (i.e., 5-10% of baseline body weight) occurred because of the intervention [18, 21, 25-27]. In fasting studies, most pathways were already changed after 24 hours [28] suggesting that for intervention studies of 2-3 months duration, it may be helpful to have multiple collections of biological specimens to examine the more immediate gene expression changes as well as the sustained changes after a fixed period of intervention.

There was overlap in the genes that were altered in the SFD and VLFD; 50 of the 77 genes significantly altered in the SFD were also changed in the VLFD. Nicotinamide phosphoribosyl-transferase (NAMPT) was one of the genes that showed the largest fold change in expression in the SFD and VLFD group (0.54-fold in the VLFD and 0.55-fold in SFD); the change was dampened after adjustment for BMI (0.55-fold in the VLFD and 0.63-fold in SFD). NAMPT is produced predominantly in peripheral blood and the levels are 4-5 times higher than those in liver, adipose or other tissues [29]. NAMPT was first known as pre-B cell colony enhancing factor (PBEF) when it was identified by a screen of a human peripheral blood lymphocyte cDNA library and was reported as a cytokine capable of stimulating the maturation of B cell precursors [30]. NAMPT was subsequently rediscovered as an adipocytokine and was renamed visfatin and was reported to possess insulin-mimetic properties [31] although this remains highly controversial [32]. Revello et al. showed that NAMPT catalyzes the biosynthesis of nicotinamide adenine dinucleotide (NAD), an essential coenzyme in cellular redox reactions [33, 34]. In short, NAMPT is now known as NAMPT/visfatin/PBEF and has numerous roles including regulating NAD availability, controlling cellular metabolism, inflammatory response, and possibly insulin secretion [35, 36]. NAMPT overexpression has been found in colorectal, breast and other cancers and in studies of clinical outcome, increased NAMPT expression has been associated with poorer outcome [37].

Serum levels of NAMPT are clearly modifiable and have been associated with weight and insulin concentrations in some cross-sectional studies [36]. Friebe et al [29] reported significant reductions in serum NAMPT (~20%) 6 months following bariatric surgery in fourteen persons. A similar magnitude of NAMPT reduction was reported in normal weight persons (n=15) who engaged in a 6 month vigorous exercise program. However, NAMPT expression levels were not measured in these participants [29]. The significance of our observation of reduced gene expression of NAMPT in the SFD and VLFD group is not clear but further investigation seems warranted given that NAMPT has been suggested as a key to mammalian health and longevity [38] and a molecular link between metabolism, inflammation and cancer [39].

Women in the SFD and VLFD group also showed significantly reduced expression of FCGR3B and FCGR2A. Human neutrophils interact with immune complexes through these two distinct FC receptors which are constitutively expressed and bind the Fc domain of IgG to elicit responses including phagocytosis and oxidative burst [40-42]. Both FCGR3B and FCGR2A are important in promoting immune-complex mediated inflammation [43, 44].

Genetic variation of FCGR3B has been implicated in rheumatoid arthritis and other autoimmune diseases [45]. Interestingly, FCGR2A interacts with high affinity to C-reactive protein (CRP) [46, 47]. While CRP is an accepted risk factor for coronary artery disease, the functional status of FCGR2A may be important since CRP mediates its biological effects via binding and internalization through the FCGR2A receptors [48, 49]. It should be noted that several of the genes that showed significant changes before and after intervention in the VLFD group are involved in Fc gamma R-mediated phagocytosis (PAK1, RAC2, RAF1, LYN, PTPRC, GSN) or cytokine receptor interaction (IL8, IL13RA1, TNFSF10, CXCR1, CXCR2, FAS, TGFBR2, CSF3F, IFNGR1) related to chemotaxis and neutrophil recruitment. Some of these genes (FAS, IFNGR1, CXCR1, PAK1, LYN, PTPRC) also showed significant changes before and after intervention in the SFD group. Interesting there are strong correlations (0.6 to 0.9) in the expression of NAMPT and many of the top genes in the inflammatory and metabolic pathways (data not shown).

The inducible prostaglandin-endoperoxide synthase (PTGS2), also known as cyclooxygenase (COX)-2 was one of the top five differentially changed genes in the SFD group (0.63-fold). Soy genistein has been shown to decrease the synthesis of prostaglandins in a range of normal and tumor cells [50-52]. The major action of soy genistein on the prostaglandin pathway appears to be the inhibition of the expression of COX-2 [53]. Although microarray gene expression studies in cell lines and animal models [54-60] have been conducted to better understand the cellular effects of soy, only one of these studies investigated gene expression changes in PMBC in women. In the study by Niculesiu et al. [61], 30 healthy postmenopausal women were randomized to soy isoflavone or placebo capsules for 84 days. In both of our studies, calmodulin (CALM1) and adducin (ADD3) were significantly down-regulated. Adducins are a family of cytoskeleton proteins that bind with high affinity to calmodulin and are substrates for protein kinases A and C [62]. Calmodulin is a calcium-binding protein that mediates many different cellular processes including inflammation, immune response, and others [63]. A difference in our results relates to the BAZ1A gene; this bromodomain protein is involved in chromatin remodeling [64] and was down-regulated in our SFD group (0.68-fold) but was up-regulated (4.3 fold) in the previous study [61]. The fact we did not find a larger number of overlapping genes in our two studies is not too surprising given that the dose of soy supplementation was more than 15 times higher in the study by Niculesiu et al [61]. In addition, an individual's ability to form the metabolite equol from daidzein, a key soy isoflavone was suggested to influence gene expression changes [61]. Most (17 of 20) of the women in the SFD were non-equol producers [11] and thus our results reflect largely those of non-equol producers.

There are, however, limitations of our study. We did not assess if there was a change in leukocyte subpopulation counts in the study. Sample limitations precluded validation of the top changes in genes detected by our microarray analysis by RT-PCR. Although we collected blood specimens at baseline and every 2 weeks during the 8 weeks of intervention, our microarray analysis was limited to a comparison of gene expression at baseline and at the end of 8 weeks and we were unable to examine gene expression levels at other times (e.g., week 2) because of the high cost of the microarray analyses. It is conceivable that our results may be more reflective of the gene expression profile reached at homeostasis in response to the dietary changes and that earlier changes associated with the intervention were not examined. Previous studies suggest that gene expression changes in relation to macronutrient changes may be relatively subtle unless there is also caloric restriction and substantial weight loss [18]. In fact, it is difficult to compare our results to those of previous studies which were designed to examine gene expression changes in relation to changes in fatty acid composition [18, 24, 65, 66], carbohydrate quality [67, 68], or caloric restriction [18, 21, 26]. Similarly, results on gene expression changes observed in postprandial studies [19, 23, 24] may not be applicable in longer term dietary intervention studies.

5. Summary

Substantial changes in macronutrient (11% of energy as fat, 65% of energy as carbohydrate) and soy supplementation (50 mg isoflavone per day) in an isocaloric intervention setting had relatively modest effects in gene expression in PBMC. Novel findings from our study suggest that expression of NAMPT and genes related to Fc gamma R-mediated phagocytosis and cytokine receptor interactions may be significantly altered in association with dietary fat reduction and soy supplementation that cannot be accounted for completely by changes in weight. Soy supplementation may additionally influence expression of PTGS2, calmodulin and adducin genes. It will be important to confirm our findings and to investigate whether soy supplementation or dietary fat reduction directly influences the expression and/or serum NAMPT concentrations or whether this represents a secondary adaptive or compensatory response to the dietary changes.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Figure 1.

Volcano plots of the 6279 probes in each dietary intervention group. Y-axis represents the $-\log_{10}(\text{p-value})$ from the paired t-test of each probe. X-axis represents the average fold change – from baseline to week 8.

Table 1
Down regulated genes in very low-fat diet (VLDF)

Affy ID	Gene symbol	Crude average fold change ¹	P-value ¹	Adjusted average fold change ²	P-value ²
31499_s_at	FCGR3B	0.493	0.010	0.514	0.026
33849_at	NAMPT	0.540	0.027	0.551	0.053
37689_s_at	FCGR2A	0.562	0.005	0.572	0.013
2002_s_at	BCL2A1	0.574	0.004	0.588	0.012
31792_at	ANXA3	0.584	0.039	0.568	0.047
34435_at	AQP9	0.585	0.041	0.597	0.075
1369_s_at	IL8	0.587	0.027	0.555	0.025
34666_at	SOD2	0.610	0.019	0.609	0.033
40082_at	ACSL1	0.627	0.042	0.628	0.065
2024_s_at	LYN	0.634	0.002	0.638	0.005
34498_at	VNN2	0.638	0.042	0.670	0.096
596_s_at	CSF3R	0.643	0.029	0.663	0.061
41300_s_at	ITM2B	0.647	0.006	0.668	0.019
34665_g_at	FCGF2B	0.648	0.013	0.670	0.032
1033_g_at	CXCR2	0.662	0.028	0.668	0.049
40519_at	PTPRC	0.665	0.010	0.670	0.023
36207_at	SEC14L1	0.668	0.033	0.675	0.058
38895_i_at	NCF4	0.672	0.017	0.696	0.039
32616_at	LYN	0.672	0.012	0.677	0.026
1402_at	LYN	0.672	0.011	0.671	0.021
34777_at	ADM	0.676	0.016	0.686	0.021
1352_at	CXCR1	0.677	0.029	0.673	0.040
34890_at	ATP6V1A	0.683	0.004	0.687	0.009
1774_at	MXD1	0.688	0.026	0.687	0.040
38894_g_at	NCF4	0.691	0.048	0.708	0.086
34951_at	GPR109B	0.694	0.017	0.716	0.025
411_i_at	IFITM2	0.695	0.012	0.690	0.018
39858_s_at	STX11	0.696	0.001	0.743	0.008
40171_at	FRAT2	0.697	0.005	0.712	0.012
38581_at	GNAQ	0.699	0.008	0.694	0.014
1868_g_at	CFLAR	0.700	0.013	0.713	0.030
32737_at	RAC2	0.705	0.001	0.728	0.004
402_s_at	ICAM3	0.706	0.016	0.716	0.034
41819_at	FYB	0.707	0.006	0.702	0.012
38138_at	S100A11	0.707	0.016	0.739	0.045

Affy ID	Gene symbol	Crude average fold change ¹	P-value ¹	Adjusted average fold change ²	P-value ²
1441_s_at	FAS	0.710	0.014	0.693	0.016
953_g_at	FKBP1A	0.713	0.002	0.702	0.003
39809_at	HBP1	0.713	0.010	0.733	0.029
36243_at	TLR1	0.715	0.031	0.748	0.081
1164_at	UBE2D1	0.715	0.014	0.731	0.035
2049_s_at	JUNB	0.715	0.018	0.750	0.042
160027_s_at	IGF2R	0.715	0.026	0.711	0.040
1440_s_at	FAS	0.716	0.012	0.701	0.014
1038_s_at	IFNGR1	0.717	0.022	0.737	0.052
38354_at	CEBPB	0.718	0.029	0.762	0.087
38403_at	LAMP2	0.719	0.041	0.718	0.066
35892_at	CR1	0.719	0.007	0.732	0.019
226_at	PRKAR1A	0.720	0.016	0.727	0.034
40520_g_at	PTPRC	0.722	0.007	0.731	0.019
181_g_at	MBOAT7	0.723	0.032	0.729	0.054
1457_at	JAK1	0.727	0.005	0.726	0.011
37651_at	RCOR1	0.727	0.023	0.745	0.053
1913_at	CCNG2	0.728	0.014	0.723	0.024
31448_s_at	LILRA2	0.728	0.012	0.754	0.037
31895_at	BACH1	0.729	0.022	0.768	0.067
36623_at	ALPL	0.729	0.005	0.725	0.006
37603_at	IL1RN	0.730	0.003	0.729	0.006
227_g_at	PRKAR1A	0.732	0.038	0.744	0.075
228_at	RALB	0.732	0.002	0.728	0.004
37328_at	PLEK	0.738	0.007	0.738	0.015
41469_at	PI3	0.738	0.026	0.736	0.038
40567_at	TUBA1B	0.740	0.011	0.756	0.023
1715_at	TNFSF10	0.740	0.036	0.748	0.062
38381_at	STX3	0.741	0.022	0.729	0.024
38411_at	SORL1	0.744	0.022	0.744	0.038
38110_at	SDCBP	0.745	0.039	0.756	0.076
33705_at	PDE4B	0.747	0.017	0.744	0.030
262_at	AMD1	0.748	0.032	0.742	0.047
826_at	DDX3X	0.749	0.004	0.740	0.008
33339_g_at	STAT1	0.749	0.010	0.758	0.024
38765_at	DICER1	0.749	0.041	0.742	0.058
37693_at	NUMB	0.750	0.027	0.757	0.052

Affy ID	Gene symbol	Crude average fold change ¹	P-value ¹	Adjusted average fold change ²	P-value ²
36472_at	NMI	0.751	0.028	0.770	0.064
38130_s_at	GK3P	0.752	0.019	0.769	0.043
41735_at	DENND3	0.753	0.037	0.781	0.090
37099_at	ALOX5AP	0.754	0.047	0.729	0.039
504_at	UBE2D3	0.757	0.003	0.763	0.008
957_at	ARRB2	0.757	0.007	0.780	0.022
305_g_at	SOS2	0.758	0.004	0.756	0.009
38123_at	CDC123	0.758	0.011	0.766	0.025
36781_at	SERPINA1	0.759	0.014	0.794	0.047
41409_at	C1orf38	0.759	0.010	0.782	0.029
1150_at	PTPRE	0.760	0.004	0.781	0.015
32612_at	GSN	0.760	0.025	0.772	0.053
1814_at	TGFBR2	0.760	0.014	0.768	0.030
40568_at	ATP6V1B2	0.760	0.037	0.778	0.077
676_g_at	IFITM1	0.761	0.019	0.767	0.035
40172_g_at	FRAT2	0.761	0.011	0.769	0.021
39708_at	STAT3	0.762	0.036	0.777	0.077
1074_at	RAB1A	0.762	0.008	0.774	0.021
37095_r_at	FPR2	0.764	0.022	0.765	0.036
623_s_at	RAB2A	0.764	0.004	0.774	0.013
37009_at	CAT	0.765	0.024	0.795	0.071
31675_s_at	PTENP1	0.765	0.043	0.788	0.094
32916_at	PTPRE	0.765	0.012	0.784	0.034
245_at	SELL	0.767	0.009	0.768	0.017
37985_at	LMNB1	0.767	0.024	0.787	0.057
1917_at	RAF1	0.767	0.005	0.768	0.010
37644_s_at	FAS	0.768	0.043	0.755	0.053
1560_g_at	PAK2	0.768	0.002	0.755	0.003
34413_at	NDEL1	0.769	0.030	0.769	0.047
794_at	PTPN6	0.770	0.006	0.795	0.020
34642_at	YWHAZ	0.771	0.008	0.771	0.015
35259_s_at	SFRS2IP	0.771	0.026	0.771	0.045
33855_at	GRB2	0.771	0.003	0.774	0.007
35966_at	QPCT	0.772	0.038	0.790	0.081
853_at	NFE2L2	0.773	0.012	0.777	0.025
41333_at	ACAP2	0.773	0.007	0.772	0.015
359_at	IL13RA1	0.773	0.009	0.772	0.017

Affy ID	Gene symbol	Crude average fold change ¹	P-value ¹	Adjusted average fold change ²	P-value ²
1984_s_at	ARHGDIB	0.774	0.003	0.777	0.008
268_at	PECAM1	0.774	0.012	0.781	0.028
37685_at	PICALM	0.775	0.040	0.783	0.069
38831_f_at	GNB2	0.775	0.021	0.805	0.064
33821_at	ELOVL5	0.776	0.031	0.780	0.053
32776_at	RALB	0.778	0.023	0.776	0.038
41594_at	JAK1	0.778	0.004	0.770	0.007
180_at	MBOAT7	0.778	0.007	0.767	0.010
37855_at	CTBS	0.778	0.003	0.788	0.009
969_s_at	USP9X	0.780	0.020	0.802	0.056
1558_g_at	PAK1	0.780	0.009	0.783	0.019
908_at	IFIT2	0.781	0.045	0.763	0.041
32978_g_at	FAM65B	0.781	0.049	0.807	0.112
38580_at	GNAQ	0.781	0.006	0.776	0.010
36684_at	AMD1	0.783	0.014	0.766	0.016
31510_s_at	H3F3B	0.784	0.005	0.795	0.015
40698_at	CLEC2B	0.784	0.044	0.788	0.073
1928_s_at	SMAD2	0.784	0.001	0.778	0.002
33146_at	MCL1	0.784	0.009	0.788	0.016
36336_s_at	SBNO2	0.784	0.027	0.835	0.099
1272_at	EIF2S3	0.784	0.031	0.802	0.073
467_at	OSTF1	0.785	0.027	0.804	0.067
36678_at	TAGLN2	0.786	0.046	0.783	0.068
34544_at	ZNF267	0.786	0.024	0.815	0.070
35811_at	RNF13	0.787	0.019	0.801	0.046
41438_at	OSBPL8	0.787	0.040	0.778	0.053
34946_at	IGSF6	0.788	0.048	0.794	0.082
1903_at		0.789	0.024	0.787	0.031
276_at	DNAJA1	0.790	0.023	0.785	0.032
32179_s_at	SNAP23	0.790	0.040	0.780	0.050
1245_i_at	PAK2	0.791	0.006	0.774	0.007
1557_at	PAK1	0.792	0.006	0.782	0.010
31431_at	FCGRT	0.792	0.004	0.819	0.015
777_at	GDI2	0.793	0.011	0.811	0.034
33372_at	RAB31	0.794	0.019	0.798	0.037
36099_at	SFRS1	0.795	0.029	0.786	0.039
1117_at	CDA	0.796	0.011	0.810	0.021

Affy ID	Gene symbol	Crude average fold change ¹	P-value ¹	Adjusted average fold change ²	P-value ²
39302_at	DSC2	0.796	0.013	0.807	0.022
877_at	CREB1	0.797	0.012	0.780	0.013
36313_at	EVI2A	0.797	0.044	0.813	0.093
670_s_at	CREB5	0.797	0.014	0.798	0.013
370_at	STK38	0.797	0.028	0.802	0.052
40019_at	EVI2B	0.798	0.037	0.818	0.089
39857_at	STX11	0.798	0.017	0.816	0.046
277_at	MCL1	0.799	0.012	0.807	0.025
39319_at	LCP2	0.799	0.041	0.831	0.107
32963_s_at	RRAGD	0.799	0.015	0.815	0.038
432_s_at	TRAC	0.799	0.008	0.794	0.015
35767_at	GABARAPL2	0.800	0.035	0.805	0.066
38129_at	GK	0.800	0.031	0.812	0.059
33924_at	DENND5A	0.800	0.029	0.814	0.061

¹Crude average fold change <0.80 and p value <0.05

²Adjusting BMI as a categorical variable (BMI<=25, >25 and <=30, >30)

Table 2
Down regulated genes in soy food diet (SFD) group

Affy ID	Gene symbol	Crude average fold change ¹	P-value ¹	Adjusted average fold change ²	P-value ²
31506_s_at	DEFA1	0.478	0.008	0.493	0.015
31499_s_at	FCGR3B	0.517	0.006	0.567	0.022
33849_at	NAMPT	0.548	0.026	0.628	0.081
37689_s_at	FCGR2A	0.608	0.019	0.674	0.066
1069_at	PTGS2	0.632	0.017	0.680	0.054
31792_at	ANXA3	0.638	0.036	0.728	0.115
32814_at	IFIT1	0.645	0.046	0.758	0.165
35472_at	KCNJ15	0.647	0.034	0.715	0.106
34666_at	SOD2	0.654	0.026	0.727	0.087
40519_at	PTPRC	0.669	0.023	0.690	0.050
37971_at	BAZ1A	0.680	0.016	0.718	0.047
38765_at	DICER1	0.683	0.017	0.742	0.062
32034_at	ZNF217	0.683	0.037	0.716	0.081
226_at	PRKAR1A	0.683	0.010	0.713	0.032
34665_g_at	FCGR2B	0.684	0.020	0.728	0.062
41300_s_at	ITM2B	0.685	0.017	0.714	0.039
2024_s_at	LYN	0.704	0.024	0.759	0.074
36243_at	TLR1	0.707	0.026	0.763	0.085
227_g_at	PRKAR1A	0.708	0.024	0.742	0.060
33705_at	PDE4B	0.713	0.018	0.758	0.054
38581_at	GNAQ	0.717	0.036	0.743	0.082
34951_at	GPR109B	0.718	0.030	0.790	0.107
34890_at	ATP6V1A	0.720	0.003	0.741	0.012
1164_at	UBE2D1	0.720	0.011	0.757	0.039
1441_s_at	FAS	0.723	0.022	0.762	0.066
1457_at	JAK1	0.723	0.032	0.717	0.047
38110_at	SDCBP	0.725	0.042	0.774	0.109
1038_s_at	IFNGR1	0.727	0.029	0.771	0.085
40520_g_at	PTPRC	0.730	0.015	0.736	0.027
38895_i_at	NCF4	0.731	0.035	0.777	0.097
36207_at	SEC14L1	0.732	0.040	0.788	0.108
31895_at	BACH1	0.733	0.036	0.785	0.100
35153_at	NBN	0.737	0.032	0.785	0.089
38129_at	GK	0.740	0.008	0.768	0.028
37644_s_at	FAS	0.740	0.027	0.771	0.073

Affy ID	Gene symbol	Crude average fold change ¹	P-value ¹	Adjusted average fold change ²	P-value ²
38130_s_at	GK3P	0.741	0.025	0.783	0.077
510_g_at	SMAD4	0.745	0.028	0.746	0.048
33462_at	P2RY14	0.747	0.044	0.803	0.128
36809_at	CLC	0.747	0.007	0.774	0.024
1352_at	CXCR1	0.747	0.029	0.821	0.104
1440_s_at	FAS	0.748	0.014	0.788	0.049
432_s_at	TRAC	0.748	0.034	0.735	0.037
38411_at	SORL1	0.752	0.034	0.775	0.070
40067_at	ELF1	0.758	0.027	0.780	0.062
33103_s_at	ADD3	0.759	0.047	0.770	0.084
34312_at	NCOA2	0.759	0.037	0.794	0.086
41469_at	PI3	0.761	0.003	0.817	0.012
39809_at	HBP1	0.762	0.034	0.798	0.069
41438_at	OSBPL8	0.763	0.049	0.798	0.111
1245_i_at	PAK2	0.764	0.009	0.794	0.030
33855_at	GRB2	0.765	0.011	0.794	0.035
504_at	UBE2D3	0.771	0.037	0.797	0.081
34642_at	YWHAZ	0.773	0.033	0.783	0.062
812_at	PPP1R2	0.773	0.025	0.775	0.041
36825_at	TRIM22	0.773	0.025	0.790	0.057
38123_at	CDC123	0.775	0.038	0.797	0.082
1031_at	SRPK1	0.775	0.015	0.802	0.046
1903_at		0.775	0.039	0.780	0.058
853_at	NFE2L2	0.777	0.012	0.816	0.033
1984_s_at	ARHGDIB	0.779	0.029	0.784	0.051
41144_g_at	CALM1	0.781	0.008	0.786	0.018
1074_at	RAB1A	0.784	0.035	0.797	0.071
40441_g_at	SERBP1	0.785	0.040	0.785	0.061
35872_at	PDE3B	0.786	0.013	0.776	0.018
879_at	MX2	0.787	0.048	0.847	0.153
1913_at	CCNG2	0.788	0.030	0.843	0.109
39376_at	HIPK1	0.788	0.034	0.806	0.065
36684_at	AMD1	0.789	0.017	0.816	0.052
39858_s_at	STX11	0.791	0.042	0.817	0.098
623_s_at	RAB2A	0.793	0.048	0.826	0.121
36215_at	PRKACB	0.794	0.033	0.786	0.045
32067_at	CREM	0.794	0.033	0.798	0.059
31510_s_at	H3F3B	0.795	0.045	0.807	0.087

Affy ID	Gene symbol	Crude average fold change ¹	P-value ¹	Adjusted average fold change ²	P-value ²
36808_at	PTPN22	0.797	0.045	0.767	0.030
36110_at	RAB5A	0.797	0.037	0.821	0.089
34930_at	GPR65	0.799	0.028	0.812	0.058
35934_at	EIF2S3	0.800	0.036	0.806	0.061

¹Crude average fold change <0.80 and p value <0.05

²Adjusting BMI as a categorical variable (BMI≤25, >25 and ≤30, >30)

Table 3
Down regulated genes in control diet (CD) group

Affy ID	Gene symbol	Crude average fold change ¹	P-value ¹	Adjusted average fold change ²	P-value ²
36809_at	CLC	0.581	0.035	0.606	0.061
36623_at	ALPL	0.740	0.039	0.755	0.064
36630_at	TSC22D3	0.786	0.015	0.784	0.020
34543_at	MXD1	0.788	0.048	0.803	0.080
1984_s_at	ARHGDIB	0.790	0.043	0.797	0.065

¹Crude average fold change <0.80 and p value <0.05

²Adjusting BMI as a categorical variable (BMI≤25, >25 and ≤30, >30)

Table 4

Up regulated genes in VLFD group and CD group

Affy ID	Gene symbol	Crude average fold change ¹	P-value ¹	Adjusted average fold change ²	P-value ²
VLFD group					
40428_i_at	PLGLB1	1.287	0.025	1.327	0.024
Control Group					
1725_s_at	UBE3A	1.256	0.011	1.223	0.024
38711_at	CLASP2	1.267	0.008	1.291	0.007
34397_at	LUC7L3	1.270	0.0003	1.272	0.001
33186_i_at		1.287	0.009	1.310	0.008
37050_r_at	TOMM34	1.289	0.027	1.291	0.037
40613_at	C6orf62	1.300	0.035	1.283	0.057
35681_r_at	ZEB2	1.306	0.027	1.281	0.047

¹Crude average fold change <0.80 and p value <0.05²Adjusting BMI as a categorical variable (BMI<=25, >25 and <=30, >30)