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Effects of Equol on Gene Expression in Female Cynomolgus Monkey Iliac Arteries

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Abstract

Background and Aims—To examine effects of equol, the soy phytoestrogen metabolite, on gene expression in the monkey iliac artery.

Methods and Results—A high fat/high cholesterol diet was fed to eight ovariectomized cynomolgus monkeys for 6.5 years. After biopsy of the left iliac artery, the animals were randomized to two treatment groups for 8 months; the treatment groups were equol (23.7 mg/100 g diet, n=4) and vehicle (n=4). The right iliac artery was removed at necropsy. Gene expression in the iliac arteries in response to equol was determined by DNA microarray. Comparison of atherosclerotic lesions and plasma lipids at pre- versus post-equol treatment time points and in vehicle versus equol treatment groups did not identify any significant differences. Despite the lack of effect of equol on these parameters, 59 genes were down-regulated and 279 were up-regulated in response to equol. Comparison of these data to previous work identified 10 genes regulated in opposite directions by equol compared to presence of atherosclerosis plaque (*Menopause* 2011;18:1087–1095) and 55 genes differentially expressed in the same direction in response to both equol and estradiol (Eyster et al., *Menopause* 2013; in press).

Conclusions—Similar responses of genes to both equol and estradiol may reflect the extent to which equol serves as a natural selective estrogen receptor modulator in the arteries. Opposite responses of 10 genes to equol versus the presence of atherosclerosis implicates those genes in the potential protective effects of equol in arteries.

Keywords

equol; soy; gene expression; DNA microarray; atherosclerosis; artery

Introduction

Equol is a metabolite of the soy isoflavone, daidzein, produced by the action of gastrointestinal bacteria [1]. Equol is a ligand for estrogen receptors (ER) with greater

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potency at ER than daidzein [1]. Many claims have been made regarding the cardiovascular health benefits of soy isoflavones, and by extension, equol [2]. Studies in laboratory animals support the atheroprotective effects of dietary soy components [3, 4]. However, scientific evidence in human studies in support of these claims has been variable [2, 5]. One contributor to the variability of soy isoflavones in humans is that as many as 30–50% of individuals are not significant producers of equol from daidzein because they lack the appropriate intestinal flora [6]. This heterogeneity leads to substantial variability in the circulating concentrations of equol and daidzein and difficulties in analysis and interpretation of results.

The effects of estrogen therapy on cardiovascular diseases are controversial. When the blood vessels are healthy, estrogen appears to protect them from the development of atherosclerotic plaque [7]. However, if atherosclerosis is well-established, estrogen appears to not be beneficial and may actually increase the risk of clinical events [8, 9]. The exact mechanisms by which estrogen influences cardiovascular disease are not entirely clear, thus the evidence that daidzein, genistein, and equol can influence estrogen receptor activity does not clarify their mechanism of action in the cardiovascular system. However, these soy/soy-derived compounds have greater affinity for ERβ than for ERα [10]. Thus, these compounds may act as natural selective estrogen receptor modulators (SERMs) [11].

Similarities in the cardiovascular system of human and cynomolgus monkeys make this animal model an ideal system for the study of the effects of equol [12]. Cynomolgus monkeys develop atherosclerosis when placed on a North American-type atherogenic diet [3, 12], and the effects of estrogen and soy isoflavones on the cardiovascular system and the development of atherosclerosis have been studied extensively in cynomolgus monkeys [3, 12].

The current study was undertaken to examine global gene expression patterns in the iliac arteries of cynomolgus monkeys in response to treatment with a synthetic racemic mixture of S- and R-equol in order to identify potential equol specific effects in the absence of other isoflavone components and to avoid problems associated with variable equol conversion rates. The extent of atherosclerosis in the iliac arteries has been shown to be directly correlated with that of the coronary arteries in macaques [3]; therefore, iliac arteries were used as proxies for the coronary artery in this study. Moreover, the use of the iliac arteries permitted a longitudinal assessment of pre-versus post-equol treatment.

Methods

Animals and Study Design

Eight adult female cynomolgus macaques *(Macaca fascicularis)*, age 20 years or older, were used for this study. The animals were surgically menopausal with ovariectomy 4–6 years prior to the study. They had been housed in stable social groups of 3–4 animals each and had consumed various semi-purified high fat/high cholesterol diets for 6.5 years upon entering this study. The diet used for this study was formulated to mimic a typical North American diet and contained 0.20 mg cholesterol/Calorie of diet, 29.4% fat, and 19.8% protein from animal sources (casein/lactalbumin). The monkeys received approximately 120 kcal/kg body weight of the diet once daily for 8 months.

The left common iliac artery was biopsied to obtain pretreatment arterial tissue as described [4, 13]. Equol was added to the diet as a supplement at 23.7 mg equol/100 grams of diet (n=4); control animals received the same diet without equol (n=4). The equol supplement contained a 96.0% pure racemic mixture of S- and R-equol enantiomers in a 1:1 ratio and was provided by Solae, a division of Dupont (St. Louis, MO, USA). The dose of equol was

designed to mimic the amount of isoflavones consumed by women in several clinical trials [2] and then scaled to account for metabolic differences between women and monkeys. The dietary supplement was the only source of equol in the diet; the diet contained no soy isoflavones. After 8 months on the North American diet with equol or control treatment, the monkeys were euthanized and the post-treatment common iliac artery was collected and processed as described [14]. Briefly, the iliac artery was opened longitudinally, laid flat, and divided into 3 equal segments. One segment was fixed in paraformaldehyde, embedded in paraffin, sectioned, and stained with Verhoeff-van Gieson's stain for histologic assessment of atherosclerotic plaque intimal area. As previously described [3, 14, 15], digital images were captured from each arterial section and morphometric measurements were made to assess the extent (defined as cross-sectional area in mm^2) of iliac artery plaque [3, 15]. A second segment of iliac artery was placed in RNAlater (Sigma R-0901) and stored at −70° until us ed for extraction of total RNA. The third section was frozen and archived. At the initiation of the study (baseline) and after 3, 6, and 8 months of treatment, blood samples were obtained for the measurement of lipids and lipoproteins as described [4, 14].

All animal procedures were carried out at Wake Forest University whose facilities and laboratory animal program are fully accredited by the Association for the Assessment and Accreditation of Laboratory Animal Care. All procedures using animals conformed to State and Federal laws and were conducted in compliance with standards of the U.S. Department of Health and Human Services, and guidelines established by the Wake Forest University Animal Care and Use Committee (ACUC).

Analysis of gene expression

Total RNA was isolated from segments of iliac artery using a method designed to maximize RNA extraction from small tissue samples [13, 16]. Arterial segments ranging in size from 2.94–13.64 mg were minced in 600 μl TRI reagent (Molecular Research Center, Cincinnati, OH) and homogenized in a 2 ml tube with a 7 mm probe on a Polytron homogenizer (Kinematica, Luzern, CH). The probe was then rinsed in 400 μl of fresh TRI reagent to recover residual sample from the probe. The 2 aliquots of TRI reagent were pooled. Bromochloropropane (200 μl) and 3 M sodium acetate (60 μl) were added and the samples were centrifuged (5 min at 8,000g) to effect phase separation. The aqueous layer contraining RNA was then purified on a silica gel membrane spin column (RNeasy, Qiagen, Valencia, CA) per company instructions [16]. Gene expression signatures were analyzed using CodeLink Whole Human Genome Bioarrays (Applied Microarrays, Tempe, AZ) to compare pretreatment versus posttreatment gene expression for both equol and vehicle treated groups as described [13]. Differential expression of SET domain, bifurcated 2 (SETDB2) was identified by DNA microarray and confirmed by two-step real time reverse transcription-polymerase chain reaction (RT-PCR) as described [13]. Primers and probe were obtained from Applied Biosystems (Life Technologies; Hs00230475_m1). Data from real time PCR reactions were analyzed by qBase software as described [13].

Statistical analysis

Data are expressed as the mean ± standard error of the mean (SE), the experimental number (n) was 4 per group, and the p value was set at 0.05. Statistical analysis of DNA microarray data utilized GeneSpring GX 7.0 software (Agilent, Santa Clara, CA). Multiple testing correction used the Benjamini and Hochberg False Discovery Rate set at 0.05. Real time RT-PCR data were analyzed by paired t-test. Sizes of atherosclerotic lesions were compared by ANOVA. Differences in plasma lipid variables between equol and vehicle treated monkeys at baseline, 3, 6, and 8 months were analyzed by repeated measures ANOVA (JMP V-8, SAS, Cary, NC).

Results

Data were analyzed for statistically different expression in pretreatment versus post-equol treatment monkey iliac arteries. Gene expression data for equol treatment were corrected for vehicle control data. Transcribed sequences with no known function, genes for which the fold expression values in response to equol were less than 2.0-fold different from pretreatment values, and genes for which the average relative expression values were near the limits of detection of the assay were excluded from further analysis. Application of these criteria identified differential expression of 338 genes in the monkey iliac arteries in response to equol (Table 1). Of those genes, 59 were down-regulated by equol and 279 were up-regulated. The presence of atherosclerosis causes substantial changes in gene expression [13]. 76 of the genes that responded to equol were regulated similarly by atherosclerosis (Table 1), whereas 10 showed expression in the opposite direction to that of atherosclerosis (Table 2). Fifty-five genes showed differential expression in the same direction (increase or decrease) in response to both equol and estradiol [14] (Table 1).

The size of atherosclerotic lesions in the iliac vessels of monkeys prior to initiation of treatment with equol averaged 0.51±0.19 mm^2 with a range of 0.113–1.003 mm^2. After 8 months of treatment with equol, the average plaque size was 0.748±0.22 mm^2 with a range of 0.171–1.188 mm^2. In control animals, the size of atherosclerotic plaque lesions averaged 0.36±0.21 mm^2 with a range of 0–0.873 mm^2 prior to initiation of vehicle treatment, and 0.47±0.27 mm^2 with a range of 0.025–1.217 mm^2 after the 8 month treatment period. Comparison of pre- versus post-treatment and vehicle versus equol treatment did not identify any significant differences among lesion sizes. Plasma lipids were assessed at baseline and at 3, 6, and 8 months of the study. No significant differences in plasma lipids in response to equol treatment were identified (Table 3).

SET domain, bifurcated 2 (SETDB2) was identified by DNA microarray as a down-regulated gene in response to equol (Figure 1A). Real time RT-PCR confirmed down-regulation of SETDB2 by equol (Figure 1B).

The data set for these DNA microarrays has been deposited in the NCBI Gene Expression Omnibus (GEO; www.ncbi.nlm.nih.gov/geo) and can be accessed through GEO Series accession numbers GSE37186 and GSE26326 (GSM646184, GSM646185, GSM6946190-5).

Discussion

Of the genes in the iliac arteries that responded to equol in this study, 16% also responded to estradiol [14] but 84% did not. As equol has been shown to activate the ERβ receptor [1], these data support the concept that equol acts as a natural SERM [11]. In contrast to previous findings with soy protein and isoflavones in the cynomolgus monkey [4, 12], equol did not alter plasma lipids or lipoproteins. However, blood lipids and lipoproteins do not appear to be the sole source of positive effects of estrogens in the vasculature [15]. Thus, the gene expression effects of equol make it a potential SERM candidate for modulation of vascular biology. Moreover, soy products have also been reported to exhibit antihypertensive effects [17]. Blood pressure was not measured in the current study, but antihypertensive activity remains a potential protective mechanism of equol in the cardiovascular system. Further research to define the extent of estrogen agonist activity of equol in the arteries is warranted.

Arteries from control and equol-treated animals contained established atherosclerotic plaque prior to initiation of equol treatment. It is likely that the profound gene expression profile differences due to the presence of atherosclerosis [13, 18, 19] at least partially masked the

effects of equol on gene expression. Indeed, 86 genes appeared on the differential gene expression lists for both presence of atherosclerosis [13] and response to equol. Of those 86 genes, 76 showed the same directional changes in expression. The pattern of expression in individual arteries suggests that those changes may have been due to the presence of atherosclerotic plaque and further suggest that treatment with equol was unable to reverse the effects of atherosclerosis on expression of those genes. In contrast, the expression of ten genes was regulated in opposite directions in response to equol versus atherosclerosis. These genes may represent points of potentially atheroprotective effects of equol in the arteries. One of these genes, SETDB2, was down-regulated in response to equol but up-regulated in the presence of atherosclerosis [13]. SETDB2 (aliases CLLD8, KMT1F) is a histone 3 lysine 9 (H3K9) methyltransferase with a methyl-CpG-binding domain (MBD). H3K9 methylation is associated with chromatin condensation and transcriptional inactivation. SETDB2 may have a role in chromatin condensation/decondensation as described by Falandry and coworkers [20], or it may have a more directed role in the regulation of specific genes as described by Xu and coworkers [21], or both. In the iliac arteries, the presence of atherosclerosis increased the expression of SETDB2 [13], whereas treatment with equol resulted in a decrease in SETDB2 expression. Therefore, we can speculate that the presence of atherosclerosis would lead to chromatin condensation and repression of the expression of specific genes, whereas treatment with equol would allow chromatin decondensation and up-regulation of specific genes. This is a novel finding as the potential for equol to counter the effects of atherosclerosis on arterial gene expression at the level of histone/chromatin regulation has not previously been reported. However, the effects of regulation of the expression of this gene on arterial function remain to be determined.

Equol activates other members of the steroid receptor superfamily of nuclear receptors in addition to the estrogen receptor. For example, equol can activate peroxisome proliferator activated receptor gamma (PPARγ) [22]. A protective role for PPARγ has been identified in atherosclerosis [23]. We identified up-regulation of 7 genes associated with peroxisome synthesis and function in the monkey iliac arteries in response to equol (CAT, ECH1, HPCL2, ISOC1, PECR, PEX3, and PEX11B) which suggests that equol effectively activated PPARs in the iliac arteries. Another nuclear receptor, the pregnane X receptor (PXR, alias NR1I2) can also be activated by equol [24]. Recent evidence links PXR activation to energy metabolism [25]. Whether PPAR or PXR plays a role in the regulation of gene expression in the iliac arteries in response to equol requires further investigation.

Summary and Conclusions

The differential expression of genes in the arteries in response to equol may reflect agonism at estrogen receptors, PPARγ, or PXR. The 10 genes for which equol treatment was able to overcome the effects of atherosclerosis on expression represent potential targets for protective effects of equol in the arteries.

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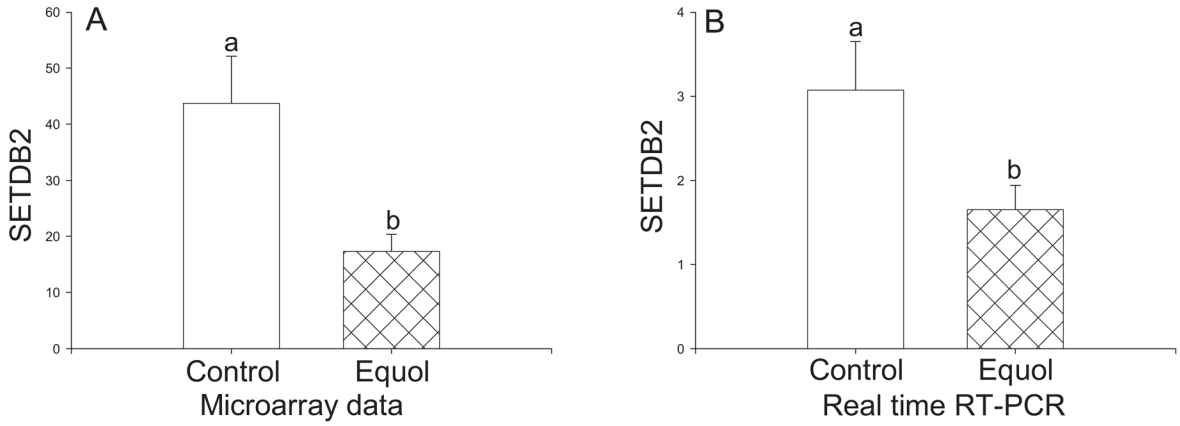

Figure 1.

Differential expression of SETDB2 in the iliac arteries of cynomolgus monkeys treated with equol for 8 months. Panel A: Data from DNA microarray analysis, n=4/group. Panel B: Data from real time RT-PCR analysis. Data are expressed as the mean ± SEM. Statistical analysis utilized Student's t-test. Bars with different letter superscripts denote that the data for those groups are significantly different from each other, $p<0.05$.

Table 1

Differentially expressed genes in the iliac arteries of ovariectomized cynomolgus macaques treated with equol for 8 months. Gene expression values are shown for pretreatment baseline data (PreTrt; n=4) and for post equol treatment (PostTrt; n=4). Fold expression data (Fold) compare posttreatment to pretreatment values for each gene (PostTrt/PreTrt). The GenBank accession number (GenBank ACCN#) and p value for statistical significance (p val) are also shown.

Genbank ACCN #	Gene Name	PreTrt	PostTrt	Fold	p val
	Lipid/Fatty acid biosynthesis/metabolism				
NM_012260	2-hydroxyphytanoyl-CoA lyase (HPCL2)	1.11	3.06	2.8	0.027
NM_020676	abhydrolase domain containing 6 (ABHD6)	0.89	2.04	2.3	0.010
NM_030925	calcium binding protein 39-like (CAB39L)	6.04	2.83	0.5	0.006
NM_001752	catalase (CAT)	0.40	1.21	3.0	0.017
NM_000896	cytochrome P450, family 4, subfamily F, polypeptide 3 (CYP4F3)	4.56	9.39	2.1	0.003
NM_003676	degenerative spermatocyte homolog, lipid desaturase (DEGS)	2.96	6.41	2.2	0.030
NM_016245	dehydrogenase/reductase (SDR family) member 8 (DHRS8)	32.11	15.13	0.5	0.000
NM_001398	enoyl Coenzyme A hydratase 1, peroxisomal (ECH1)	0.82	1.77	2.2	0.024
NM_002395	malic enzyme 1, NADP(+)-dependent, cytosolic (ME1)	0.35	1.25	3.6	0.027
NM_015922	NAD(P) dependent steroid dehydrogenase-like (NSDHL)	0.71	2.42	3.4	0.021
NM_003846	peroxisomal biogenesis factor 11B (PEX11B)	0.95	2.79	2.9	0.025
NM_003630	peroxisomal biogenesis factor 3 (PEX3)	2.13	4.40	2.1	0.033
NM_018441	peroxisomal trans-2-enoyl-CoA reductase (PECR)[b]	1.80	6.11	3.4	0.048
NM_153613	lysophosphatidylcholine acyltransferase 4	0.52	1.10	2.1	0.043
NM_016048	isochorismatase domain containing 1 (ISOC1)	0.58	1.66	2.9	0.013
	Glucose metabolism				
NM_000188	hexokinase 1 (HK1)[b]	3.48	8.61	2.5	0.036
NM_002300	lactate dehydrogenase B (LDHB)	25.88	54.82	2.1	0.031
NM_021965	phosphoglucomutase 5 (PGM5)	0.40	1.14	2.9	0.042
NM_005609	phosphorylase, glycogen; muscle (PYGM)	1.86	7.48	4.0	0.028
	ATP synthesis				
NM_000143	fumarate hydratase (FH)	6.04	16.44	2.7	0.028
NM_002080	glutamic-oxaloacetic transaminase 2, mitochondrial (GOT2)	3.16	9.66	3.1	0.031
NM_005896	isocitrate dehydrogenase 1 (NADP+), soluble (IDH1)	1.77	3.95	2.2	0.039
NM_002168	isocitrate dehydrogenase 2 (NADP+), mitochondrial (IDH2)	0.42	1.28	3.0	0.001

Genbank ACCN #	Gene Name	PreTrt	PostTrt	Fold	p val
NM_005000	NADH dehydrogenase (ubiquinone) 1 alpha subcomplex, 5, 13kDa (NDUFA5) [b]	27.84	13.27	0.5	0.011
NM_003002	succinate dehydrogenase complex, subunit D (SDHD)	17.66	35.44	2.0	0.011
	Protein metabolism				
NM_183050	branched chain keto acid dehydrogenase E1, beta polypeptide (BCKDHB)	1.74	3.67	2.1	0.017
NM_152740	3-hydroxyisobutyrate dehydrogenase (HIBADH)[b]	2.11	5.98	2.8	0.021
	General Enzymes/Metabolism				
NM_138340	abhydrolase domain containing 3 (ABHD3)[b]	0.68	1.86	2.7	0.047
NM_018641	carbohydrate (chondroitin 4) sulfotransferase 12 (CHST12)[a]	3.39	7.48	2.2	0.028
NM_020682	Cyt19 protein (CYT19)	1.69	3.65	2.2	0.007
NM_032857	lactamase, beta (LACTB)[b]	57.55	29.57	0.5	0.050
NM_022493	nuclear prelamin A recognition factor-like (NARFL)	0.48	1.48	3.1	0.003
NM_033452	tripartite motif-containing 47 (TRIM47)[a,b]	2.07	5.79	2.8	0.020
NM_017590	ubiquitous tetratricopeptide containing protein RoXaN (RoXaN)	3.15	6.75	2.1	0.001
	Signal transduction				
NM_002922	regulator of G-protein signalling 1 (RGS1)	17.34	3.04	0.2	0.006
NM_003390	WEE1 homolog (WEE1)[a]	4.67	1.76	0.4	0.005
NM_007314	v-abl Abelson murine leukemia viral oncogene homolog 2 (ABL2)	2.31	0.88	0.4	0.011
NM_002928	regulator of G-protein signalling 16 (RGS16)	77.98	32.00	0.4	0.007
AK124904	similar to Rho/Rac guanine nucleotide exchange factor[a]	4.11	1.72	0.4	0.002
NM_152422	protein tyrosine phosphatase domain containing 1 (PTPDC1)	1.00	0.43	0.4	0.032
NM_006622	polo-like kinase 2 (Drosophila) (PLK2)	11.41	5.36	0.5	0.002
NM_006224	phosphotidylinositol transfer protein (PITPN)	1.49	0.73	0.5	0.019
NM_015093	mitogen-activated protein kinase kinase kinase 7 interacting protein 2 (MAP3K7IP2)[a]	17.52	8.63	0.5	0.031
NM_014836	Rho-related BTB domain containing 1 (RHOBTB1)	50.87	25.28	0.5	0.004
NM_022456	RAB3A interacting protein (rabin3) (RAB3IP)	1.93	0.97	0.5	0.025
NM_003706	phospholipase A2, group IVC (PLA2G4C)	15.29	7.69	0.5	0.000
NM_014225	protein phosphatase 2 (formerly 2A), regulatory subunit A (PR 65), alpha (PPP2R1A)[a]	14.80	28.40	1.9	0.001
NM_198938	prostaglandin E synthase 2 (PTGES2)	0.57	1.13	2.0	0.028
NM_031417	MAP/microtubule affinity-regulating kinase 4 (MARK4)	0.49	0.98	2.0	0.000
NM_016656	Ras-related GTP binding B (RRAGB)	1.17	2.35	2.0	0.040

Genbank ACCN #	Gene Name	PreTrt	PostTrt	Fold	p val
NM_004162	RAB5A, member RAS oncogene family (RAB5A)	1.68	3.38	2.0	0.009
NM_015915	spastic paraplegia 3A (autosomal dominant) (SPG3A)	2.09	4.22	2.0	0.013
NM_006290	tumor necrosis factor, alpha-induced protein 3 (TNFAIP3)	1.61	3.28	2.0	0.025
NM_016573	Gem-interacting protein (GMIP)	0.53	1.08	2.0	0.045
NM_007182	Ras association (RalGDS/AF-6) domain family 1 (RASSF1)[b]	17.18	35.09	2.0	0.034
U70667	Fas-ligand associated factor 1	16.80	34.35	2.0	0.045
NM_001343	disabled homolog 2, mitogen-responsive phosphoprotein (DAB2)	2.56	5.26	2.1	0.027
NM_206835	TNF receptor-associated factor 7 (TRAF7)[b]	5.40	11.10	2.1	0.028
NM_014602	phosphoinositide-3-kinase, regulatory subunit 4, p150 (PIK3R4)	1.75	3.63	2.1	0.029
NM_052902	serine/threonine kinase 11 interacting protein (STK11IP)	2.08	4.38	2.1	0.020
NM_005219	diaphanous homolog 1 (DIAPH1)	5.41	11.53	2.1	0.017
NM_015937	phosphatidylinositol glycan, class T (PIGT)	6.61	14.23	2.2	0.008
NM_020983	adenylate cyclase 6 (ADCY6)	3.31	7.18	2.2	0.008
NM_012478	WW domain binding protein 2 (WBP2)[a,b]	7.10	15.45	2.2	0.029
NM_003022	SH3 domain binding glutamic acid-rich protein like (SH3BGRL)	32.43	71.01	2.2	0.038
NM_005486	target of myb1-like 1 (TOM1L1)	0.71	1.56	2.2	0.026
NM_000115	endothelin receptor type B (EDNRB)	1.18	2.61	2.2	0.010
N22508	similar to tumor necrosis factor receptor 2[a]	3.29	7.43	2.3	0.006
NM_013314	B-cell linker (BLNK)	5.13	11.59	2.3	0.000
NM_002480	protein phosphatase 1, regulatory (inhibitor) subunit 12A (PPP1R12A)	13.27	30.19	2.3	0.046
NM_013994	discoidin domain receptor family, member 1 (DDR1)	0.76	1.73	2.3	0.001
NM_145245	similar to ecotropic viral integration site 5; Neuroblastoma stage 4S gene[a]	3.15	7.19	2.3	0.038
NM_000678	adrenergic, alpha-1D-, receptor (ADRA1D)	0.46	1.05	2.3	0.042
NM_015609	putative MAPK activating protein PM20, PM21	1.20	2.74	2.3	0.000
NM_002227	Janus kinase 1 (a protein tyrosine kinase) (JAK1)	1.56	3.57	2.3	0.018
NM_016151	thousand and one amino acid protein kinase (TAO1)[a]	6.10	14.17	2.3	0.000
NM_002336	low density lipoprotein receptor-related protein 6 (LRP6)[a]	12.20	28.48	2.3	0.013
NM_014376	cytoplasmic FMR1 interacting protein 2 (CYFIP2)	0.91	2.15	2.4	0.013
NM_001242	tumor necrosis factor receptor superfamily, member 7 (TNFRSF7)[b]	0.82	1.96	2.4	0.032
NM_182759	TAFA3 protein (TAFA3)[a]	3.48	8.58	2.5	0.006

Nutr Metab Cardiovasc Dis. Author manuscript; available in PMC 2015 April 01.

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NM_198964	parathyroid hormone-like hormone (PTHLH)[a]	11.91	29.65	2.5	0.001
NM_021044	desert hedgehog homolog (DHH)	0.66	1.67	2.5	0.011
NM_000801	FK506 binding protein 1A, 12kDa (FKBP1A)	4.60	11.75	2.6	0.043
NM_007369	G protein-coupled receptor 161 (GPR161)[a]	5.37	13.79	2.6	0.002
NM_198452	Similar to calcium/calmodulin-dependent protein kinase 1, beta	1.49	3.85	2.6	0.005
NM_004383	c-src tyrosine kinase (CSK)	1.90	4.97	2.6	0.021
NM_007178	serine/threonine kinase receptor associated protein (STRAP)[b]	2.76	7.26	2.6	0.036
NM_018010	estrogen-related receptor beta like 1 (ESRRBL1)[b]	0.87	2.30	2.6	0.037
NM_024832	Ras and Rab interactor 3 (RIN3)[a]	1.05	2.80	2.7	0.033
NM_020421	aarF domain containing kinase 1 (ADCK1)	0.48	1.28	2.7	0.040
NM_181784	sprouty-related, EVH1 domain containing 2 (SPRED2)	1.06	2.84	2.7	0.039
NM_003405	tyrosine 3-monooxygenase/tryptophan 5-monooxygenase activation protein, eta (YWHAH)[b]	5.74	15.56	2.7	0.027
NM_014220	transmembrane 4 superfamily member 1 (TM4SF1)	2.01	5.61	2.8	0.050
NM_012302	latrophilin 2 (LPHN2)	1.72	4.85	2.8	0.039
NM_016639	tumor necrosis factor receptor superfamily, member 12A (TNFRSF12A)[b]	10.41	29.44	2.8	0.027
NM_002836	protein tyrosine phosphatase, receptor type, A (PTPRA)[b]	2.83	8.12	2.9	0.017
NM_016478	nuclear interacting partner of anaplastic lymphoma kinase (ALK) (NIPA)	0.58	1.73	3.0	0.018
NM_015662	selective LIM binding factor, rat homolog (SLB)	0.59	1.77	3.0	0.007
NM_004838	homer homolog 3 (HOMER3)[a]	0.50	1.60	3.2	0.048
NM_016586	MAP3K12 binding inhibitory protein 1 (MBIP)	1.84	5.95	3.2	0.005
NM_017790	regulator of G-protein signalling 3 (RGS3)[a,b]	1.66	5.59	3.4	0.015
NM_005493	RAN binding protein 9 (RANBP9)	0.32	1.09	3.4	0.023
NM_003239	transforming growth factor, beta 3 (TGFB3)	0.30	1.08	3.6	0.005
NM_005737	ADP-ribosylation factor-like 7 (ARL7)[a]	0.30	1.15	3.8	0.028
NM_006271	S100 calcium binding protein A1 (S100A1)	1.18	4.74	4.0	0.012
NM_006374	serine/threonine kinase 25 (STK25)[b]	2.28	9.30	4.1	0.048
NM_002755	mitogen-activated protein kinase kinase 1 (MAP2K1)[b]	1.84	7.53	4.1	0.016
NM_005539	inositol polyphosphate-5-phosphatase, 40kDa (INPP5A)	3.77	15.80	4.2	0.016
NM_030798	Williams-Beuren syndrome chromosome region 16 (WBSCR16)[a]	0.23	1.00	4.3	0.014

Nutr Metab Cardiovasc Dis. Author manuscript; available in PMC 2015 April 01.

Genbank ACCN #	Gene Name	PreTrt	PostTrt	Fold	p val
NM_212492	G protein pathway suppressor 1 (GPS1)	1.29	8.00	6.2	0.017
	Transcriptional regulation				
NM_199072	I-mfa domain-containing protein (HIC)[a]	11.74	2.58	0.2	0.001
NM_001806	CCAAT/enhancer binding protein (C/EBP), gamma (CEBPG)	13.79	4.21	0.3	0.024
NM_014112	[1]trichorhinophalangeal syndrome I (TRPS1)[a]	29.14	12.57	0.4	0.001
NM_005437	nuclear receptor coactivator 4 (NCOA4)	16.28	7.85	0.5	0.014
BC033086	transcription factor 19 (SC1)	8.41	4.06	0.5	0.018
NM_005069	single-minded homolog 2 (SIM2)[b]	19.17	37.60	2.0	0.038
NM_018433	jumonji domain containing 1A (JMJD1A)	133.85	262.61	2.0	0.042
NM_003419	zinc finger protein 345 (ZNF345)	0.94	1.86	2.0	0.047
NM_003743	nuclear receptor coactivator 1 (NCOA1)	1.21	2.44	2.0	0.008
NM_173539	zinc finger protein 596 (ZNF596)	0.78	1.64	2.1	0.014
NM_017761	proline-rich nuclear receptor coactivator 2 (PNRC2)	5.05	10.84	2.1	0.007
NM_001517	general transcription factor IIH, polypeptide 4, 52kDa (GTF2H4)	2.47	5.40	2.2	0.004
NM_002017	Friend leukemia virus integration 1 (FLI1)	0.96	2.28	2.4	0.019
M94046	zinc finger protein (MAZ) mRNA[a]	10.92	26.50	2.4	0.006
NM_001206	basic transcription element binding protein 1 (BTEB1)[a]	0.79	1.95	2.5	0.019
NM_021994	zinc finger protein (C2H2 type) 277 (ZNF277)[b]	1.12	2.82	2.5	0.025
NM_013260	transcriptional regulator protein (HCNGP)	1.04	2.70	2.6	0.005
NM_014423	ALL1 fused gene from 5q31 (AF5Q31)[b]	2.41	6.33	2.6	0.032
NM_016535	zinc finger protein 581 (ZNF581)[a,b]	2.35	6.44	2.7	0.009
NM_001164	amyloid beta (A4) precursor protein-binding, family B, member 1 (Fe65) (APBB1)	0.92	2.61	2.8	0.011
NM_003457	zinc finger protein 207 (ZNF207)	5.51	15.79	2.9	0.008
NM_002398	Meis1, myeloid ecotropic viral integration site 1 homolog (MEIS1)	1.29	3.70	2.9	0.031
NM_030767	AT-hook transcription factor (AKNA)	0.98	3.07	3.1	0.009
NM_003575	zinc finger protein 282 (ZNF282)	0.45	1.45	3.2	0.017
NM_004118	forkhead-like 18 (Drosophila) (FKHL18)[a]	3.74	12.10	3.2	0.041
NM_014067	LRP16 protein (LRP16)	0.33	1.10	3.3	0.001
NM_003418	zinc finger protein 9 (a cellular retroviral nucleic acid binding protein) (ZNF9)	7.95	28.17	3.5	0.029
NM_005610	retinoblastoma binding protein 4 (RBBP4)	2.98	11.36	3.8	0.004

Nutr Metab Cardiovasc Dis. Author manuscript; available in PMC 2015 April 01.

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NM_020338	retinoic acid induced 17 (RAI17), mRNA	0.66	2.53	3.8	0.019
NM_019102	homeo box A5 (HOXA5)	0.73	2.80	3.8	0.031
NM_021078	GCN5 general control of amino-acid synthesis 5-like 2 (yeast) (GCN5L2)[b]	1.31	5.13	3.9	0.014
NM_014583	LIM and cysteine-rich domains 1 (LMCD1)	0.83	3.37	4.1	0.039
U38904	zinc finger protein C2H2-25 mRNA	0.35	1.44	4.1	0.032
NM_002167	inhibitor of DNA binding 3, dominant negative helix-loop-helix protein (ID3)[a]	2.17	10.63	4.9	0.024
	Nucleic acid synthesis and regulation				
NM_004396	DEAD (Asp-Glu-Ala-Asp) box polypeptide 5 (DDX5)[a]	3.57	1.34	0.4	0.031
NM_006284	TAF10 RNA polymerase II, TATA box binding protein (TBP)-associated factor, 30kDa (TAF10)	1.95	0.74	0.4	0.001
NM_031915	SET domain, bifurcated 2 (SETDB2)[b]	43.73	17.33	0.4	0.019
NM_015450	protection of telomeres 1 (POT1)[a]	12.54	5.47	0.4	0.013
NM_139281	WD repeat domain 36 (WDR36)	4.28	1.94	0.5	0.002
NM_145715	tigger transposable element derived 2 (TIGD2)[b]	8.72	4.07	0.5	0.017
NM_019070	DEAD (Asp-Glu-Ala-Asp) box polypeptide 49 (DDX49)	13.89	6.92	0.5	0.001
NM_000553	Werner syndrome (WRN)	3.59	1.79	0.5	0.000
NM_006469	influenza virus NS1A binding protein (IVNS1ABP)	18.57	9.56	0.5	0.008
NM_005777	RNA binding motif protein 6 (RBM6)	2.98	5.89	2.0	0.038
NM_024096	XTP3-transactivated protein A (XTP3TPA)[b]	3.64	7.20	2.0	0.033
NM_015117	zinc finger CCCH type domain containing 3 (ZC3HDC3)[a]	1.67	3.34	2.0	0.000
NM_018119	polymerase (RNA) III (DNA directed) polypeptide E (80kD) (POLR3E)[b]	0.75	1.52	2.0	0.011
NM_012232	polymerase I and transcript release factor (PTRF)[a]	4.03	8.34	2.1	0.006
NM_020385	XPMC2 prevents mitotic catastrophe 2 homolog (XPMC2H)	0.79	1.64	2.1	0.002
NM_014706	squamous cell carcinoma antigen recognised by T cells 3 (SART3)	1.40	2.91	2.1	0.001
NM_001499	GLE1 RNA export mediator-like (yeast) (GLE1L)[b]	0.80	1.68	2.1	0.025
NM_003883	histone deacetylase 3 (HDAC3)[b]	3.57	7.51	2.1	0.036
NM_016732	RNA binding protein (hnRNP-associated with lethal yellow) (RALY)	4.27	9.02	2.1	0.014
NM_005915	MCM6 minichromosome maintenance deficient 6 (MCM6)	1.47	3.18	2.2	0.025
NM_024844	pericentrin 1 (PCNT1)	2.45	5.48	2.2	0.008
NM_004504	HIV-1 Rev binding protein (HRB)	1.08	2.42	2.2	0.044

Genbank ACCN #	Gene Name	PreTrt	PostTrt	Fold	p val
NM_014596	zinc ribbon domain containing, 1 (ZNRD1)[b]	1.55	3.56	2.3	0.000
NM_022874	survival of motor neuron 1, telomeric (SMN1)	8.55	20.03	2.3	0.041
NM_006828	activating signal cointegrator 1 complex 3 (ASCC3) (helicase HELIC1)[b]	0.77	1.82	2.4	0.024
NM_032361	THO complex 3 (THOC3)	1.97	4.66	2.4	0.016
NM_006999	polymerase (DNA directed) sigma (POLS)[b]	0.90	2.29	2.5	0.034
NM_006924	splicing factor, arginine/serine-rich 1 (splicing factor 2) (SFRS1)	2.89	7.92	2.7	0.013
NM_031266	heterogeneous nuclear ribonucleoprotein A/B (HNRPAB)[b]	2.86	8.36	2.9	0.018
L29065	DNA-binding protein A gene	2.90	8.97	3.1	0.040
NM_004593	splicing factor, arginine/serine-rich 10 (transformer 2 homolog) (SFRS10)[b]	2.71	8.61	3.2	0.018
NM_006391	importin 7 (IPO7)[b]	1.36	4.51	3.3	0.007
NM_031492	hypothetical protein similar to RNA-binding protein lark (MGC10871)	1.17	3.92	3.4	0.029
NM_004247	U5 snRNP-specific protein, 116 kD (U5-116KD)	0.56	1.93	3.4	0.001
NM_002695	polymerase (RNA) II (DNA directed) polypeptide E, 25kDa (POLR2E)[a]	0.39	1.36	3.5	0.017
NM_014740	DEAD (Asp-Glu-Ala-Asp) box polypeptide 48 (DDX48)	4.97	19.13	3.8	0.022
NM_002694	polymerase (RNA) II (DNA directed) polypeptide C, 33kDa (POLR2C)	1.81	6.99	3.9	0.007
NM_201224	DEAD (Asp-Glu-Ala-Asp) box polypeptide 47 (DDX47)[b]	0.86	3.91	4.5	0.016
NM_006026	H1 histone family, member X (H1FX)[b]	1.23	7.78	6.3	0.026
NM_004964	histone deacetylase 1 (HDAC1)	0.28	1.84	6.6	0.005
NM_001126	adenylosuccinate synthase (ADSS)[b]	1.13	2.81	2.5	0.010
NM_003875	guanine monophosphate synthetase (GMPS)	1.63	3.47	2.1	0.010
NM_004075	cryptochrome 1 (photolyase-like) (CRY1)	0.77	1.70	2.2	0.021
NM_175886	phosphoribosyl pyrophosphate synthetase 1-like 1 (PRPS1L1)	1.04	2.74	2.6	0.018
	Defense/Immune system				
NM_002982	chemokine (C-C motif) ligand 2 (CCL2)[b]	5.21	1.29	0.2	0.022
NM_024711	immune associated nucleotide 2 (hIAN2)[b]	37.14	17.51	0.5	0.032
NM_000585	interleukin 15 (IL15)	2.99	1.42	0.5	0.001
NM_000538	regulatory factor X-associated protein (RFXAP)	1.29	2.53	2.0	0.026
NM_006688	complement component 1, q subcomponent-like 1 (C1QL1)	3.14	6.34	2.0	0.009
NM_009588	lymphotoxin beta (TNF superfamily, member 3) (LTB)[a]	1.57	3.24	2.1	0.010

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NM_018844	B-cell receptor-associated protein 29 (BCAP29)[b]	3.13	7.35	2.3	0.040
NM_197974	butyrophilin, subfamily 3, member A3 (BTN3A3)[b]	2.36	5.55	2.4	0.044
NM_021160	HLA-B associated transcript 5 (BAT5)[b]	3.14	9.37	3.0	0.012
NM_006858	interleukin 1 receptor-like 1 ligand (IL1RL1LG)	0.43	1.35	3.1	0.009
NM_030789	histocompatibility (minor) 13 (HM13)[b]	1.95	6.72	3.4	0.007
NM_005335	hematopoietic cell-specific Lyn substrate 1 (HCLS1)	0.33	1.24	3.8	0.015
	Proteases and their regulators				
NM_030660	Machado-Joseph disease (ataxin 3) (MJD)	2.77	1.03	0.4	0.003
NM_032802	putative intramembrane cleaving protease (SPPL2A)	7.61	3.57	0.5	0.003
NM_006036	putative prolyl oligopeptidase	3.35	1.66	0.5	0.003
NM_016022	likely ortholog of C elegans anterior pharynx defective 1A (APH-1A)	0.94	1.85	2.0	0.010
NM_006215	serine (or cysteine) proteinase inhibitor, A4 (antitrypsin) (SERPINA4)	1.31	2.77	2.1	0.004
NM_013379	dipeptidylpeptidase 7 (DPP7)[a,b]	9.54	22.95	2.4	0.047
NM_139159	dipeptidylpeptidase 9 (DPP9)[a]	1.21	3.09	2.6	0.005
NM_004279	peptidase (mitochondrial processing) beta (PMPCB)	8.87	23.35	2.6	0.039
NM_005468	N-acetylated alpha-linked acidic dipeptidase-like 1 (NAALADL1)	0.96	2.83	2.9	0.003
NM_007173	protease, serine, 23 (SPUVE)	2.45	7.87	3.2	0.048
NM_016134	[2]plasma glutamate carboxypeptidase (PGCP)[b]	0.86	3.63	4.2	0.015
	Cell cycle/cell fate				
AF220656	apoptosis-associated nuclear protein PHLDA1 (PHLDA1)	2.67	1.02	0.4	0.005
NM_006716	activator of S phase kinase (ASK)[a]	7.94	3.51	0.4	0.013
NM_022476	fused toes homolog (mouse) (FTS)	2.38	5.25	2.2	0.024
NM_005190	cyclin C (CCNC)	0.61	1.42	2.3	0.049
NM_024094	defective in sister chromatid cohesion homolog 1 (MGC5528)	33.98	82.09	2.4	0.019
NM_020812	dedicator of cytokinesis 6 (DOCK6)	1.71	4.24	2.5	0.042
NM_194271	ring finger protein 34 (RNF34)	1.52	3.81	2.5	0.004
NM_017747	ankyrin repeat and KH domain containing 1 (ANKHD1)	1.50	3.79	2.5	0.027
NM_032038	spinster-like (SPINL)[b]	2.60	6.81	2.6	0.030
NM_006283	transforming, acidic coiled-coil containing protein 1 (TACC1)	1.81	4.91	2.7	0.011
NM_002455	metaxin 1 (MTX1)[b]	2.06	5.87	2.8	0.017

Nutr Metab Cardiovasc Dis. Author manuscript; available in PMC 2015 April 01.

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NM_004642	CDK2-associated protein 1 (CDK2AP1)[b]	3.62	11.18	3.1	0.016
NM_016238	anaphase promoting complex subunit 7 (ANAPC7)	1.92	6.13	3.2	0.011
NM_004765	B-cell CLL/lymphoma 7C (BCL7C)[a]	2.69	8.96	3.3	0.001
NM_004632	death associated protein 3 (DAP3)[b]	1.58	5.58	3.5	0.018
NM_005426	tumor protein p53 binding protein, 2 (TP53BP2)	1.59	6.08	3.8	0.004
NM_001760	cyclin D3 (CCND3)[b]	1.76	8.95	5.1	0.014
	Adhesion				
NM_016174	cerebral endothelial cell adhesion molecule 1 (CEECAM1)[a]	7.68	15.94	2.1	0.009
NM_005245	FAT tumor suppressor homolog 1 (FAT)	0.96	2.07	2.2	0.002
NM_003872	neuropilin 2 (NRP2)	1.96	4.25	2.2	0.009
NM_006670	trophoblast glycoprotein (TPBG)	1.59	3.46	2.2	0.007
NM_021181	SLAM family member 7 (SLAMF7)[a]	3.46	8.27	2.4	0.015
NM_032457	BH-protocadherin (brain-heart) (PCDH7)[b]	20.88	53.94	2.6	0.042
NM_004148	ninjurin 1 (NINJ1)[b]	2.26	7.14	3.2	0.026
NM_001423	epithelial membrane protein 1 (EMP1)	0.44	1.75	4.0	0.021
NM_005529	heparan sulfate proteoglycan 2 (perlecan) (HSPG2)[a]	1.18	8.27	7.0	0.007
	Extracellular matrix				
NM_032048	elastin microfibril interfacer 2 (EMILIN2)[b]	11.80	5.58	0.5	0.012
NM_000088	collagen, type I, alpha 1 (COL1A1)[a]	72.58	138.33	1.9	0.032
NM_002023	fibromodulin (FMOD)	1.48	3.14	2.1	0.006
NM_000095	cartilage oligomeric matrix protein (COMP)[b]	6.21	13.88	2.2	0.021
NM_130444	collagen, type XVIII, alpha 1 (COL18A1)	1.37	3.16	2.3	0.005
NM_022664	extracellular matrix protein 1 (ECM1)[a]	0.34	1.21	3.6	0.040
NM_199235	collectin sub-family member 11 (COLEC11)[a]	1.13	4.07	3.6	0.007
	Chaperonins				
NM_024610	HSPB (heat shock 27kDa) associated protein 1 (HSPBAP1)	18.15	8.28	0.5	0.019
NM_006431	chaperonin containing TCP1, subunit 2 (beta) (CCT2)[a]	52.85	24.46	0.5	0.001
NM_012111	AHA1, activator of heat shock 90kDa protein ATPase homolog 1 (AHSA1)[b]	4.21	8.56	2.0	0.041
NM_006221	protein (peptidyl-prolyl cis/trans isomerase) NIMA-interacting 1 (PIN1)	2.66	5.89	2.2	0.048

Nutr Metab Cardiovasc Dis. Author manuscript; available in PMC 2015 April 01.

Genbank ACCN #	Gene Name	PreTrt	PostTrt	Fold	p val
NM_006112	peptidylprolyl isomerase E isoform 1; cyclophilin 33[b]	3.51	12.41	3.5	0.034
NM_144617	heat shock protein, alpha-crystallin-related, B6 (HSPB6)[a]	0.19	1.11	5.8	0.016
	Oxidation-reduction				
NM_000096	ceruloplasmin (ferroxidase) (CP)[b]	2.70	9.56	3.5	0.010
NM_014080	dual oxidase 2 (DUOX2)[a]	6.30	18.92	3.0	0.002
NM_015913	endoplasmic reticulum thioredoxin superfamily member, 18 kDa (TLP19)[b]	1.17	3.31	2.8	0.037
NM_016275	selenoprotein T (SELT)[b]	2.40	8.77	3.7	0.024
NM_000178	glutathione synthetase (GSS)	0.87	2.11	2.4	0.005
	Cytoskeleton				
NM_001376	dynein, cytoplasmic, heavy polypeptide 1 (DNCH1)	45.02	22.20	0.5	0.003
AA912262	similar to thymosin beta-4	21.92	10.96	0.5	0.007
NM_014900	COBL-like 1 (COBLL1)	4.83	9.91	2.1	0.034
NM_032608	myosin XVIIIB (MYO18B)	2.06	4.37	2.1	0.026
NM_015033	formin binding protein 1 (FNBP1)[a]	19.47	41.60	2.1	0.019
NM_001978	erythrocyte membrane protein band 49 (dematin) (EPB49)[b]	4.48	9.64	2.2	0.023
NM_014000	vinculin (VCL)	8.07	23.87	3.0	0.011
NM_021738	supervillin (SVIL)	1.75	5.30	3.0	0.010
NM_053024	profilin 2 (PFN2)	1.15	3.58	3.1	0.006
NM_000366	tropomyosin 1 (alpha) (TPM1)	4.22	15.86	3.8	0.017
NM_006400	dynactin 2 (p50) (DCTN2)[b]	1.69	7.74	4.6	0.029
	Organelle function				
NM_001011	ribosomal protein S7 (RPS7)[a]	76.57	29.38	0.4	0.030
NM_014445	stress-associated endoplasmic reticulum protein 1 (SERP1)	21.88	8.61	0.4	0.003
AK057656	similar to mitochondrial processing peptidase beta subunit[a]	14.41	6.46	0.4	0.016
NM_001028	ribosomal protein S25 (RPS25)	15.99	7.64	0.5	0.018
NM_013237	px19-like protein (PX19)	26.54	13.02	0.5	0.001
NM_014713	lysosomal-associated protein transmembrane 4 alpha (LAPTM4A)	64.63	133.42	2.1	0.027
NM_006855	KDEL (Lys-Asp-Glu-Leu) endoplasmic reticulum protein retention receptor 3 (KDELR3)	2.67	5.94	2.2	0.027
NM_002902	reticulocalbin 2, EF-hand calcium binding domain (RCN2)	4.68	10.61	2.3	0.044

Genbank ACCN #	Gene Name	PreTrt	PostTrt	Fold	p val
NM_006351	translocase of inner mitochondrial membrane 44 homolog (TIMM44)	1.29	2.95	2.3	0.004
NM_002901	reticulocalbin 1, EF-hand calcium binding domain (RCN1)	3.31	7.64	2.3	0.030
NM_032478	mitochondrial ribosomal protein L38 (MRPL38)[a]	4.24	11.15	2.6	0.002
NM_172251	mitochondrial ribosomal protein L54 (MRPL54)	0.32	1.01	3.2	0.002
	Protein processing				
NM_016480	poly(A) binding protein interacting protein 2 (PAIP2)	50.05	98.55	2.0	0.015
NM_052870	sorting nexin associated golgi protein 1 (SNAG1)[b]	0.92	1.82	2.0	0.021
NM_182551	lysocardiolipin acyltransferase 1	0.85	1.69	2.0	0.011
NM_007230	mannosidase, alpha, class 1B, member 1 (MAN1B1)[b]	0.89	2.44	2.7	0.034
NM_002676	phosphomannomutase 1 (PMM1)[b]	0.88	2.44	2.8	0.004
NM_000434	sialidase 1 (lysosomal sialidase) (NEU1)[b]	0.79	3.77	4.8	0.009
NM_003032	sialyltransferase 1 (beta-galactoside alpha-2,6-sialyltransferase) (SIAT1)	1.92	3.87	2.0	0.011
NM_005216	dolichyl-diphosphooligosaccharide-protein glycosyltransferase (DDOST)[b]	6.91	20.35	2.9	0.031
NM_003863	dolichyl-phosphate mannosyltransferase polypeptide 2, regulatory subunit (DPM2)[b]	2.74	11.14	4.1	0.008
NM_005500	SUMO-1 activating enzyme subunit 1 (SAE1)	0.84	2.30	2.7	0.009
NM_012214	mannosyl (alpha-1,3-)-glycoprotein beta-1,4-N-acetylglucosaminyltransferase, isoenzyme A (MGAT4A)	6.14	2.92	0.5	0.003
NM_020156	core 1 UDP-galactose:N-acetylgalactosamine-alpha-R beta 1,3-galactosyltransferase (C1GALT1)	8.35	4.22	0.5	0.022
NM_002047	glycyl-tRNA synthetase (GARS)	13.70	6.61	0.5	0.000
NM_001751	cysteinyl-tRNA synthetase (CARS)	0.27	1.00	3.7	0.002
NM_004184	tryptophanyl-tRNA synthetase (WARS)	1.15	2.45	2.1	0.003
	Vesicle transport				
NM_018261	SEC3-like 1 (SEC3L1)	6.17	2.72	0.4	0.000
NM_015386	component of oligomeric golgi complex 4 (COG4)	1.75	3.55	2.0	0.044
NM_007277	SEC6-like 1 (S cerevisiae) (SEC6L1)[b]	2.80	6.87	2.5	0.045
NM_138567	synaptotagmin VIII (SYT8)[a]	0.44	1.17	2.7	0.048
NM_198398	serologically defined breast cancer antigen 84 (SDBCAG84)[b]	7.49	24.60	3.3	0.040
NM_004781	vesicle-associated membrane protein 3 (cellubrevin) (VAMP3)	2.65	10.01	3.8	0.045
	Ubiquitin system and related				
NM_016406	Ufm1-conjugating enzyme 1 (Ufc1)	3.20	1.38	0.4	0.000

Genbank ACCN #	Gene Name	PreTrt	PostTrt	Fold	p val
NM_006313	ubiquitin specific protease 15 (USP15)	4.99	2.41	0.5	0.001
NM_014412	Siah-interacting protein (SIP)	17.39	8.70	0.5	0.011
NM_006156	neural precursor cell expressed, developmentally down-regulated 8 (NEDD8)	13.02	6.66	0.5	0.016
NM_006913	ring finger protein 5 (RNF5)[b]	7.32	14.39	2.0	0.006
NM_018438	F-box protein 6 (FBXO6)[b]	2.76	5.76	2.1	0.033
NM_003939	beta-transducin repeat containing (BTRC)	0.92	1.93	2.1	0.049
NM_020892	deltex homolog 2 (DTX2)	0.75	1.62	2.2	0.025
NM_012308	F-box and leucine-rich repeat protein 11 (FBXL11)[a]	8.93	21.07	2.4	0.016
NM_022039	split hand/foot malformation (ectrodactyly) type 3 (SHFM3)[b]	2.88	7.38	2.6	0.040
NM_016129	COP9 constitutive photomorphogenic homolog subunit 4 (COPS4)	4.44	13.16	3.0	0.035
NM_006837	COP9 constitutive photomorphogenic homolog subunit 5 (COPS5)[b]	1.89	7.74	4.1	0.022
NM_173647	ring finger protein 149 (RNF149)[b]	154.37	68.53	0.4	0.021
	Channels/transporters				
NM_001679	ATPase, Na+/K+ transporting, beta 3 polypeptide (ATP1B3)	3.00	1.25	0.4	0.039
NM_003562	solute carrier family 25 (oxoglutarate carrier), member 11 (SLC25A11)	2.12	4.19	2.0	0.004
NM_015945	solute carrier family 35, member C2 (SLC35C2)	1.60	3.18	2.0	0.009
NM_014437	solute carrier family 39 (zinc transporter), member 1 (SLC39A1)	1.53	3.10	2.0	0.037
NM_001038	sodium channel, nonvoltage-gated 1 alpha (SCNN1A)	0.59	1.21	2.1	0.030
NM_022003	FXYD domain containing ion transport regulator 6 (FXYD6)	0.91	1.90	2.1	0.029
NM_004732	potassium voltage-gated channel, shaker-related subfamily, beta member 3 (KCNAB3)	4.04	8.72	2.2	0.041
NM_001695	ATPase, H+ transporting, lysosomal 42kDa, V1 subunit C, isoform 1 (ATP6V1C1)[b]	2.56	5.59	2.2	0.029
NM_001293	chloride channel, nucleotide-sensitive, 1A (CLNS1A)	11.66	25.98	2.2	0.034
NM_133496	solute carrier family 30 (zinc transporter), member 7 (SLC30A7)[b]	0.99	2.28	2.3	0.040
NM_005689	ATP-binding cassette, sub-family B (MDR/TAP), member 6 (ABCB6)	0.56	1.29	2.3	0.015
NM_018344	solute carrier family 29 (nucleoside transporters), member 3 (SLC29A3)	4.20	9.70	2.3	0.001
NM_015638	transient receptor potential cation channel, subfamily C, member 4 associated protein (TRPC4AP)[b]	0.87	2.15	2.5	0.035
NM_004955	solute carrier family 29 (nucleoside transporters), member 1 (SLC29A1)	2.27	6.16	2.7	0.023
NM_000387	solute carrier family 25, member 20 (SLC25A20)	0.53	1.49	2.8	0.020
NM_017458	major vault protein (MVP)[a]	1.07	3.18	3.0	0.045

Genbank ACCN #	Gene Name	PreTrt	PostTrt	Fold	p val
NM_199037	sodium channel, voltage-gated, type I, beta (SCN1B)[a]	2.17	6.53	3.0	0.008
NM_005765	ATPase, H+ transporting, lysosomal accessory protein 2 (ATP6AP2)	0.39	1.27	3.3	0.022
NM_004317	arsA arsenite transporter, ATP-binding, homolog 1 (ASNA1)[a]	0.31	1.01	3.3	0.013
NM_016016	solute carrier family 25 member 39 (SLC25A39)[b]	1.37	4.90	3.6	0.014
	Unknown function				
NM_018103	leucine rich repeat containing 5 (LRRC5)[b]	40.70	15.24	0.4	0.026
AK124720	similar to paraneoplastic antigen MA1 (PNMA1)	4.37	8.60	2.0	0.004
NM_001294	cleft lip and palate associated transmembrane protein 1 (CLPTM1)	2.89	5.76	2.0	0.004
NM_014254	transmembrane protein 5 (TMEM5)	4.66	10.19	2.2	0.042
NM_014575	schwannomin interacting protein 1 (SCHIP1)	2.12	5.11	2.4	0.046
NM_138373	myeloid-associated differentiation marker (MYADM)	0.35	1.02	2.9	0.015
NM_152285	arrestin domain containing 1 (ARRDC1)[a,b]	0.62	1.81	2.9	0.020

[a] Differential expression with both Equol and Estradiol [14].

[b] Differential expression with both Equol and Atherosclerosis [13].

Table 2

Genes in the iliac arteries of ovariectomized cynomolgus macaques that were regulated in opposite directions in response to equol treatment compared to the presence of atherosclerosis. Ten genes were up-regulated by equol but down-regulated in the presence of atherosclerosis or vice versa. Data are expressed as fold expression; posttreatment versus pretreatment values for equol (Fold Equol; PostTrt/PreTrt) and presence versus absence of atherosclerosis [13] (Fold Athero; presence/absence). The GenBank accession number (GenBank ACCN#) and gene ontology for each gene are also shown.

Genbank ACCN #	Gene Name	Fold Equol	Fold Athero	Gene Ontology
NM_005069	single-minded homolog 2 (SIM2)	2.0	0.4	Transcriptional regulation
NM_032457	BH-protocadherin (brain-heart) (PCDH7)	2.6	0.3	Adhesion
NM_001978	erythrocyte membrane protein band 49 (dematin) (EPB49)	2.2	0.4	Cytoskeleton
NM_024711	immune associated nucleotide 2 (hIAN2)	0.5	2.0	Defense/Immune system
NM_032048	elastin microfibril interfacer 2 (EMILIN2)	0.5	2.4	Extracellular matrix
NM_173647	ring finger protein 149 (RNF149)	0.4	2.3	Ubiquitin system and related
NM_032857	lactamase, beta (LACTB)	0.5	3.1	Catabolic/Metabolism
NM_031915	SET domain, bifurcated 2 (SETDB2)	0.4	2.3	Nucleic acid regulation
NM_145715	tigger transposable element derived 2 (TIGD2)	0.5	2.3	Nucleic acid regulation
NM_018103	leucine rich repeat containing 5 (LRRC5)	0.4	2.4	Unknown function

Table 3

Plasma lipids and body weight (BW, kg) in equol treated (n=4) ovariectomized cynomolgus macaques at baseline and at 3, 6, and 8 months (m) of treatment. Abbreviations: total plasma cholesterol (TPC, mmol/L), triglycerides (TG, mmol/L), high density lipoprotein cholesterol (HDLC, mmol/L), low density lipoprotein plus very low density lipoprotein cholesterol (LDL+VLDL, mmol/L).

	BW (kg)	TPC	TG	HDLC	LDL+VLDL	TPC/HDLC
Baseline	2.85±0.18	428.75±21.5	32.25±1.7	37±8.7	391.75±15.3	13.29±2.5
3 m	3.04±0.21	340.5±28.6	35.25±0.5	38.3±7.7	302.25±21.1	9.59±1.3
6 m	3.02±0.19	337.5±34.2	29.5±1.2	33±9.4	304.50±38.4	13.30±4.5
8 m	3.01±0.17	372.25±25.9	34.75±2.6	23±6.6	349.25±28.5	23.04±9.2