A unique blend of natural compounds opposes age-related changes in gene expression related to dysregulation of cellular detoxification and antioxidant protection

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INTRODUCTION

Aging is associated with the accumulation of cellular toxins and damage. Declines in cellular detoxification mechanisms and impairments in antioxidant protection are consistently observed in aging models and likely contribute to age-associated accumulation of cellular damage. The master regulator, Nuclear factor erythroid 2-related factor 2 (Nrf2), is a transcription factor that regulates the basal and inducible expression of a large battery of genes encoding for cytoprotective factors including those that defend against electrophilic stressors and oxidative insults.

The Nrf2/electrophile response element (EpRE) Antioxidant Protection and Phase II Detoxification pathways are impaired with aging due to age-related changes in gene expression (2). A key example is the reduction in glutathione (GSH) levels in all tissues with age due primarily to declines in glutamate-cysteine ligase and glutathione synthase expression (1). Opposing these changes in gene expression may delay or attenuate the aging process.

Most anti-aging intervention strategies to date, have tested single ingredients and have focused solely on an individual gene, in an isolated tissue. A more comprehensive strategy is to examine changes in the expression patterns of multiple genes or pathways in specific tissues and then to identify a blend of phytochemicals that opposes those age-related changes. It seems prudent to examine changes that occur during middle age rather than waiting until old age when an intervention may not be as impactful. Accordingly, the purpose of this study was to test a blend of natural compounds in middle-aged mice, compared to young mice, for the ability to oppose age-related changes in the expression of Nrf2-regulated genes involved in the detoxification of xenobiotics and xenobiotic metabolites and in the synthesis and regulation of intrinsic antioxidants and antioxidant enzymes.

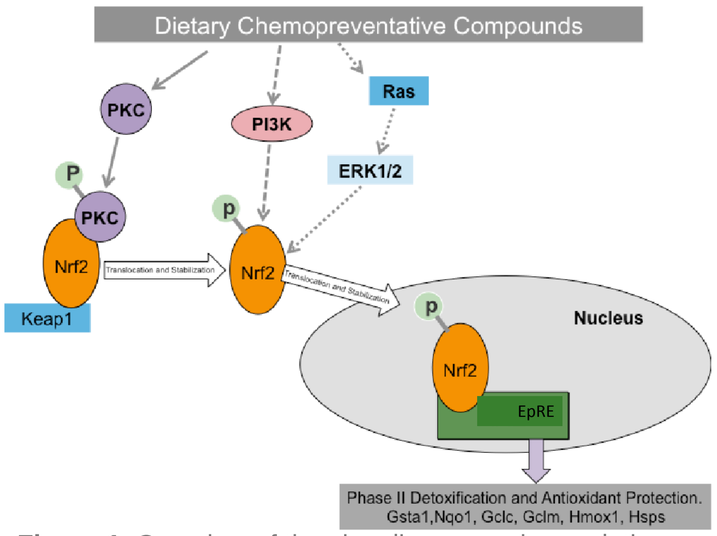

Figure 1. Overview of the signaling cascade regulating Phase II Detoxification and Antioxidant Protection; adapted from Chen and Kong (3).

METHODS

Three groups of CBA/J mice; n = 8/group.

1. Young controls (YC); age 2 mo.; AIN 93^{M} diet.
2. Middle-age controls (MAC); age 16 mo.; AIN 93^{M} diet.
3. Middle-age supplemented (MAS); age 16 mo.; AIN 93^{M} diet fortified with a **phytonutrient blend**.
   - Feeding for 3 months.

METHODS

The **phytonutrient blend** was identified based on previous *in vivo* screenings of individual ingredients that positively influenced key cytoprotective pathways and included the following components:

- *Cordyceps sinensis*
- Blood (red) orange extract
- Pomegranate whole fruit extract
- *Panax ginseng* extract
- Broccoli seed extract
- Grape seed extract

Data Analysis:
Full gene expression profiling was performed using Affymetrix Mouse Genome arrays in liver and gastrocnemius skeletal muscle tissues. Gene expression profiles and patterns were compared in order to identify changes in gene expression with age (MAC vs. YC) and in response to supplementation (MAS vs. MAC).

RESULTS

Gene Symbol	Gene Title	Liver YC vs. MAC			Liver MAC vs. MAS			Gastrocnemius YC vs. MAC			Gastrocnemius MAC vs. MAS		
			FC	p-value		FC	p-value		FC	p-value		FC	p-value
Nfe2l2	nuclear factor, erythroid derived 2, like 2	↓	-1.78	0.00	↑	1.33	0.00	NC	1.01	0.87	NC	1.11	0.14
Gclc	glutamate-cysteine ligase, catalytic subunit	NC	-1.19	0.10	NC	1.03	0.79	NC	1.14	0.09	NC	-1.03	0.65
Gclm	glutamate-cysteine ligase, modifier subunit	↓	-1.62	0.00	↓	-1.27	0.00	NC	-1.01	0.84	NC	1.04	0.68
Gsr	glutathione reductase	↓	-1.27	0.00	↑	1.62	0.00	NC	-1.08	0.21	NC	-1.09	0.21
Gss	glutathione synthetase	↑	1.17	0.01	NC	1.13	0.05	NC	1.03	0.53	NC	-1.01	0.82
Gsta3	glutathione S-transferase, alpha 3	↓	-1.09	0.03	↓	-1.10	0.02	↑	1.38	0.00	NC	-1.10	0.24
Gsta4	glutathione S-transferase, alpha 4	↑	1.50	0.00	↓	-1.25	0.01	↑	1.24	0.04	NC	1.22	0.05
Gstm1	glutathione S-transferase, mu 1	↑	2.02	0.00	↓	-1.07	0.03	NC	1.10	0.07	NC	-1.01	0.91
Gstm2	glutathione S-transferase, mu 2	↑	1.86	0.00	NC	1.02	0.82	NC	1.04	0.46	NC	1.03	0.48
Gstm3	glutathione S-transferase, mu 3	↑	2.24	0.00	NC	-1.05	0.73	NC	1.08	0.09	↓	-1.18	0.03
Gsto1	glutathione S-transferase omega 1	↑	1.28	0.02	↓	-1.53	0.00	NC	1.39	0.08	NC	-1.27	0.21
Gstt1	glutathione S-transferase, theta 1	NC	-1.02	0.74	↑	1.35	0.00	NC	-1.01	0.94	NC	-1.01	0.93
Gstt2	glutathione S-transferase, theta 2	↑	1.21	0.00	NC	-1.02	0.75	NC	1.08	0.39	NC	1.03	0.69
Glrx2	glutaredoxin 2 (thioltransferase)	↑	1.19	0.00	↓	-1.09	0.01	NC	1.06	0.09	↑	1.24	0.00
Gpx1	glutathione peroxidase 1 (cytosolic)	↑	1.75	0.00	NC	-1.08	0.05	↑	1.39	0.00	↓	-1.27	0.00
Gpx4	*glutathione peroxidase 4 (liver and testis)	↑	1.53	0.00	↓	-1.19	0.00	NC	-1.02	0.72	NC	-1.01	0.90
Gpx7	glutathione peroxidase 7	↑	1.12	0.04	NC	-1.03	0.63	↑	1.16	0.03	↓	-1.11	0.04
Gpx8	glutathione peroxidase 8 (putative)	↓	-1.18	0.00	↑	1.19	0.02	NC	-1.03	0.73	NC	-1.12	0.15
Atox1	ATX1 (antioxidant protein 1) homolog 1 (yeast)	↑	1.90	0.00	↓	-1.32	0.00	↑	1.42	0.00	↓	-1.14	0.01
Cat	catalase	↓	-1.12	0.00	↑	1.07	0.00	NC	-1.11	0.23	NC	-1.09	0.20
Sod1	superoxide dismutase 1, soluble	↑	1.15	0.00	NC	1.00	0.90	↓	-1.27	0.00	↑	1.22	0.00
Sod2	superoxide dismutase 2, mitochondrial	↓	-1.34	0.00	NC	-1.06	0.08	NC	1.03	0.54	NC	1.08	0.07
Sod3	superoxide dismutase 3, extracellular	↑	1.57	0.00	↑	1.20	0.00	↑	1.18	0.01	NC	-1.12	0.11
Dld	dihydrolipoamide dehydrogenase	↓	-1.56	0.00	NC	-1.05	0.38	NC	-1.16	0.12	NC	-1.06	0.53
Enox2	ecto-NOX disulfide-thiol exchanger 2	↓	-1.17	0.00	↑	1.13	0.03	↑	1.24	0.01	NC	1.10	0.23
Ephx2	epoxide hydrolase 2, cytoplasmic	↓	-1.24	0.00	↓	-1.15	0.02	NC	1.21	0.15	NC	-1.14	0.22
Esd	esterase D/formylglutathione hydrolase	↓	-1.15	0.01	NC	-1.07	0.39	↑	1.12	0.04	↑	1.18	0.02
Hmox1	heme oxygenase (decycling) 1	↑	2.89	0.02	NC	-1.45	0.23	↑	1.39	0.00	↓	-1.32	0.00
Hyou1	hypoxia up-regulated 1	↑	1.26	0.04	NC	1.17	0.08	NC	1.08	0.14	NC	-1.05	0.35
Lias	lipoic acid synthetase	↓	-1.58	0.00	NC	1.08	0.14	↑	1.34	0.00	NC	-1.01	0.85
Mgst1	microsomal glutathione S-transferase 1	↓	-1.26	0.00	↓	-1.12	0.00	↑	1.83	0.00	NC	1.01	0.90
Mgst3	microsomal glutathione S-transferase 3	↑	1.30	0.00	NC	-1.00	0.93	↑	1.28	0.00	↓	-1.25	0.02
Mt1	metallothionein 1	↑	3.44	0.01	↓	-3.47	0.01	↑	4.20	0.01	↓	-2.22	0.04
Mt2	metallothionein 2	↑	2.15	0.01	↓	-2.51	0.01	↑	5.22	0.01	NC	-1.95	0.12
Nqo1	NAD(P)H dehydrogenase, quinone 1	↑	1.29	0.05	NC	1.00	0.99	NC	-1.04	0.71	NC	1.18	0.15
Nqo2	NAD(P)H dehydrogenase, quinone 2	↓	-1.26	0.00	NC	-1.04	0.40	NC	-1.09	0.23	↑	1.20	0.02
Oxr1	oxidation resistance 1	↓	-1.34	0.00	NC	1.00	0.96	NC	1.02	0.80	NC	1.07	0.27
Oxsr1	oxidative-stress responsive 1	↓	-1.22	0.00	NC	1.05	0.22	NC	-1.10	0.25	NC	1.09	0.22
Perp	PERP, TP53 apoptosis effector	↓	-1.28	0.00	↑	1.20	0.00	↑	1.44	0.04	↓	-1.44	0.04
Prdx1	peroxiredoxin 1	↓	-1.19	0.01	↓	-1.31	0.00	↑	1.26	0.00	↑	1.18	0.00
Prdx2	peroxiredoxin 2	↑	1.65	0.00	↓	-1.19	0.00	↓	-1.18	0.02	↑	1.28	0.01
Prdx3	peroxiredoxin 3	↓	-1.45	0.00	↓	-1.19	0.00	NC	1.05	0.44	NC	1.11	0.06
Prdx6	peroxiredoxin 6	↑	1.08	0.04	↑	1.11	0.01	↑	1.19	0.01	NC	1.01	0.84
Sult1b1	sulfotransferase family 1B, member 1	↓	-2.14	0.00	NC	1.05	0.62	↓	-1.45	0.00	↑	1.25	0.02
Sult1c2	sulfotransferase family, cytosolic, 1C, member 2	↓	-2.23	0.00	↑	1.32	0.03	NC	-1.27	0.11	NC	1.09	0.52
Sult1e1	sulfotransferase family 1E, member 1	↑	50.22	0.02	↓	-40.10	0.02	NC	-1.06	0.67	↑	1.53	0.04
Sult2b1	sulfotransferase family, cytosolic, 2B, member 1	↑	1.11	0.04	↓	-1.13	0.03	↑	1.23	0.01	NC	-1.11	0.14
Sult3a1	sulfotransferase family 3A, member 1	↑	69.70	0.05	↓	-39.92	0.04	NC	-1.13	0.18	↑	1.38	0.01
Sult4a1	sulfotransferase family 4A, member 1	↑	1.13	0.01	↓	-1.25	0.00	NC	1.03	0.64	NC	-1.06	0.49
Tmx1	thioredoxin-related transmembrane protein 1	↓	-1.95	0.00	NC	1.04	0.16	NC	1.04	0.62	NC	1.02	0.79
Tmx2	thioredoxin-related transmembrane protein 2	↓	-1.22	0.01	↑	1.21	0.01	NC	1.00	0.99	↑	1.31	0.00
Tmx3	thioredoxin-related transmembrane protein 3	↓	-1.70	0.00	NC	-1.03	0.57	NC	-1.04	0.44	↑	1.36	0.00
Txn1	thioredoxin 1	↓	-1.05	0.05	↑	1.07	0.01	NC	1.01	0.64	NC	-1.06	0.20
Txn2	thioredoxin 2	↑	1.67	0.00	↑	1.09	0.01	NC	-1.06	0.32	NC	-1.10	0.11
Txndc11	thioredoxin domain containing 11	↑	1.27	0.02	NC	1.03	0.70	↑	1.13	0.04	NC	-1.03	0.46
Txndc12	thioredoxin domain containing 12	↓	-1.26	0.00	↑	1.26	0.00	NC	-1.02	0.81	NC	-1.07	0.50
Txndc15	thioredoxin domain containing 15	↓	-1.34	0.00	NC	-1.05	0.18	NC	1.05	0.31	↓	-1.09	0.04
Txndc17	thioredoxin domain containing 17	↑	1.29	0.00	NC	1.02	0.34	↑	1.45	0.00	↓	-1.29	0.00
Txndc9	thioredoxin domain containing 9	↓	-1.81	0.00	NC	-1.07	0.21	NC	-1.00	0.98	NC	1.05	0.50
Txnl1	thioredoxin-like 1	↓	-1.20	0.03	↑	1.18	0.01	↑	1.16	0.03	NC	1.03	0.59
Txnl4a	thioredoxin-like 4A	↑	1.21	0.00	NC	-1.07	0.05	NC	1.06	0.15	NC	-1.01	0.73
Txnl4b	thioredoxin-like 4B	↓	-1.31	0.00	↑	1.16	0.04	↓	-1.15	0.03	↑	1.29	0.01
Txnrd1	thioredoxin reductase 1	↓	-1.10	0.01	NC	1.04	0.19	NC	-1.06	0.51	NC	1.09	0.41
Txnrd2	thioredoxin reductase 2	↓	1.24	0.00	↑	1.16	0.01	NC	1.04	0.42	NC	-1.06	0.37

Table 1. Nrf2-Related Phase II Detoxification Genes. The various classes of Nrf2 related Phase II detoxification enzymes were differentially modulated in response to age and to the supplement in liver and in skeletal muscle.

RESULTS

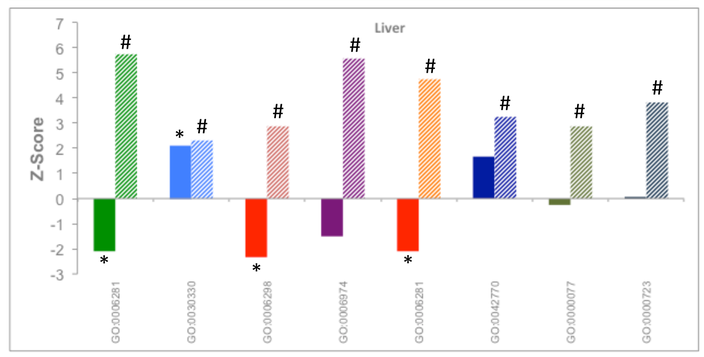

Figure 2. DNA Integrity. Changes in cytoprotective pathways responsible for maintenance of DNA integrity that were influenced by age (solid bars) and/or supplementation (hatched bars) in the liver. None of these pathways were changed in the skeletal muscle (data not shown).

- **GO:0006281** DNA repair
- **GO:0030330** DNA damage response, signal transduction by p53 class mediator
- **GO:0006298** DNA mismatch repair
- **GO:0006974** Response to DNA damage stimulus
- **GO:0042770** DNA damage response, signal transduction
- **GO:0000077** DNA damage checkpoint
- **GO:0000723** Telomere maintenance

*p<0.05 MAC vs. YC; #p<0.05 MAS vs. MAC

SUMMARY & CONCLUSIONS

1. By middle age, expression of Nrf2 was downregulated in liver, but not in skeletal muscle, suggesting that either Nrf2 is not downregulated in skeletal muscle with age or that middle-age is too early to detect changes in Nrf2.
2. The phytonutrient blend effectively opposed the downregulation of Nrf2 in liver observed in middle-age controls.
3. In addition to restoring the expression of the master regulator Nrf2, the supplement opposed age-related changes in the expression of several Nrf2-regulated genes in liver and muscle, suggesting that it may combat some negative effects of aging.
4. Although many individual genes related to cellular detoxification were changed with age, none of the GO Pathways related to detoxification had changed significantly by middle-age indicating that these pathways decline at a later age.
5. Conversely, some pathways related to DNA integrity were changed by middle-age.
6. The supplement opposed most age related changes related to DNA repair. In addition, the supplement upregulated some DNA repair pathways that had not changed by middle age suggesting that by intervening at a younger age, the supplement may have stimulated protective mechanisms *before* they had the chance to decline.
7. These effects, elicited by a mid-life nutritional intervention, will likely have positive implications for healthy human aging or 'youthspan' and warrant further investigation.

References:

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