References

Allam, F., et al. (2013). "Grape powder supplementation prevents oxidative stress-induced anxiety-like behavior, memory impairment, and high blood pressure in rats." J Nutr **143**(6): 835-842.

We examined whether or not grape powder treatment ameliorates oxidative stress-induced anxiety-like behavior, memory impairment, and hypertension in rats. Oxidative stress in Sprague-Dawley rats was produced by using L-buthionine-(S,R)-sulfoximine (BSO). Four groups of rats were used: 1) control (C; injected with vehicle and provided with tap water), 2) grape powder-treated (GP; injected with vehicle and provided for 3 wk with 15 g/L grape powder dissolved in tap water), 3) BSO-treated [injected with BSO (300 mg/kg body weight), i.p. for 7 d and provided with tap water], and 4) BSO plus grape powder-treated (GP+BSO; injected with BSO and provided with grape powder-treated tap water). Anxiety-like behavior was significantly greater in BSO rats compared with C or GP rats ($P < 0.05$). Grape powder attenuated BSO-induced anxiety-like behavior in GP+BSO rats. BSO rats made significantly more errors in both short- and long-term memory tests compared with C or GP rats ($P < 0.05$), which was prevented in GP+BSO rats. Systolic and diastolic blood pressure was significantly greater in BSO rats compared with C or GP rats ($P < 0.05$), whereas grape powder prevented high blood pressure in GP+BSO rats. Furthermore, brain extracellular signal-regulated kinase-1/2 (ERK-1/2) was activated ($P < 0.05$), whereas levels of glyoxalase-1 (GLO-1), glutathione reductase-1 (GSR-1), calcium/calmodulin-dependent protein kinase type IV (CAMK-IV), cAMP response element-binding protein (CREB), and brain-derived neurotrophic factor (BDNF) were significantly less ($P < 0.05$) in BSO but not in GP+BSO rats compared with C or GP rats. We suggest that by regulating brain ERK-1/2, GLO-1, GSR-1, CAMK-IV, CREB, and BDNF levels, grape powder prevents oxidative stress-induced anxiety, memory impairment, and hypertension in rats.

Anastasius, N., et al. (2009). "Evidence that low-dose, long-term genistein treatment inhibits oestradiol-stimulated growth in MCF-7 cells by down-regulation of the PI3-kinase/Akt signalling pathway." J Steroid Biochem Mol Biol **116**(1-2): 50-55.

The reduced incidence of breast cancer in certain Eastern countries has been attributed to high soy diets although this evidence is simply epidemiological. One of the major constituents of soy is genistein, but paradoxically this phytoestrogen binds to oestrogen receptors and stimulates growth at concentrations that would be achieved by a high soy diet, but inhibits growth at high experimental concentrations. To determine the effects of low-dose, long-term genistein exposure we have cultured MCF-7 breast cancer cells in 10 nM genistein for 10-12 weeks and investigated whether or not this long-term genistein treatment (LTGT) altered the expression of oestrogen receptor alpha (ERalpha) and the activity of the PI3-K/Akt signalling pathway. This is known to be pivotal in the signalling of mitogens such as oestradiol (E(2)), insulin-like growth factor-1 (IGF-1) and epidermal growth factor (EGF). LTGT significantly reduced the growth promoting effects of E(2) and increased the dose-dependent growth-inhibitory effect of the PI3-K inhibitor, LY 294002, compared to untreated control MCF-7 cells. This was associated with a significant decreased protein expression of total Akt and phosphorylated Akt but not ERalpha. Rapamycin, an inhibitor of one of the down-stream targets of Akt, mammalian target of rapamycin (mTOR), also dose-dependently inhibited growth but the response to this drug was similar in LTGT and control MCF-7 cells. The protein expression of liver receptor homologue-1 (LRH1), an orphan nuclear receptor implicated in tumourigenesis was not affected by LTGT. The results show that LTGT results in a down-regulation of the PI3-K/Akt signalling pathway and may be a mechanism through which genistein could offer protection against breast cancer.

Arambasic, J., et al. (2013). "Alpha-lipoic acid upregulates antioxidant enzyme gene expression and enzymatic activity in diabetic rat kidneys through an O-GlcNAc-dependent mechanism." Eur J Nutr **52**(5): 1461-1473.

PURPOSE: The combined hyperglycemia lowering and antioxidant actions of alpha-lipoic acid (LA) contribute to its usefulness in preventing renal injury and other diabetic complications. The precise mechanisms by which LA alters diabetic oxidative renal injury are not known. We hypothesized that LA through its hypoglycemic effect lowers O-GlcNAcylation which influences the expression and activities of antioxidant enzymes which assume important roles in preventing diabetes-induced oxidative renal injury. METHODS: An experimental model of diabetes was induced in rats by the administration of 40 mg/kg streptozotocin (STZ) intraperitoneally (i.p.) for five consecutive days. LA was applied at a dose of 10 mg/kg i.p. for 4 weeks, starting from the last day of STZ administration. RESULTS: An improved glycemic status of LA-treated diabetic rats was accompanied by a significant suppression of oxidative stress and a reduction of oxidative damage of lipids, proteins and DNA. LA treatment normalized CuZn-superoxide dismutase (SOD) and catalase activities in renal tissue of diabetic rats. These changes were allied with upregulated gene expression and lower levels of O-GlcNA glycosylation. The accompanying increase in MnSOD activity was only linked with upregulated gene expression. The observed antioxidant enzyme gene regulation was accompanied by nuclear translocation of Nuclear factor-erythroid-2-related factor 2 (Nrf2), enhanced expression of heat shock proteins (HSPs) and by reduction in O-GlcNAcylation of HSP90, HSP70, and extracellular regulated kinase and p38. CONCLUSION: alpha-Lipoic acid administration activates a coordinated cytoprotective response against diabetes-induced oxidative injury in kidney tissue through an O-

GlcNAc-dependent mechanism.

Arredondo, F., et al. (2010). "After cellular internalization, quercetin causes Nrf2 nuclear translocation, increases glutathione levels, and prevents neuronal death against an oxidative insult." Free Radic Biol Med **49**(5): 738-747.

In this work we describe the protective effects of quercetin against H(2)O(2) in 24-h-pretreated neuronal cultures. We explored quercetin availability and subcellular fate through the use of HPLC-Diode Array Detection (DAD), epifluorescence, and confocal microscopy. We focused on quercetin modulation of thiol-redox systems by evaluating changes in mitochondrial thioredoxin Trx2, the levels of total glutathione (GSH), and the expression of the gamma-glutamate-cysteine ligase catalytic subunit (GCLC), the rate-limiting enzyme of GSH synthesis, by the use of Western blot, HPLC, and real-time PCR techniques, respectively. We further explored the activation of the protective NF-E2-related factor 2 (Nrf2)-dependent signaling pathway by quercetin using immunocytochemistry techniques. Our results showed rapid quercetin internalization into neurons, reaching the nucleus after its addition to the culture. Quercetin pretreatment increased total GSH levels, but did not increase Trx2. Interestingly it caused Nrf2 nuclear translocation and significantly increased GCLC gene expression. At the moment of H(2)O(2) addition, intracellular quercetin or related metabolites were undetectable in the cultures although quercetin pretreatment prevented neuronal death from the oxidant exposure. Our findings suggest alternative mechanisms of quercetin neuroprotection beyond its long-established ROS scavenging properties, involving Nrf2-dependent modulation of the GSH redox system.

Bak, M. J., et al. (2012). "Procyanidins from wild grape (Vitis amurensis) seeds regulate ARE-mediated enzyme expression via Nrf2 coupled with p38 and PI3K/Akt pathway in HepG2 cells." Int J Mol Sci **13**(1): 801-818.

Banerjee, S., et al. (2012). "Phosphorylation of hepatic AMP-activated protein kinase and liver kinase B1 is increased after a single oral dose of green tea extract to mice." Nutr Res **32**(12): 985-990.

We have previously shown that green and black tea extracts increase the phosphorylation of AMP-activated protein kinase (AMPK) and HMG-CoA reductase in rat hepatoma cells in culture, concomitant with a decrease in cholesterol synthesis. In the present study, we evaluated the ability of a single oral dose of green or black tea extract to promote the phosphorylation of AMPK, liver kinase B1 (LKB1, an AMPK-kinase), and HMG-CoA reductase in mouse liver. Green tea extract administered by gavage at 50 and 100 mg/kg caused a 2- to 3-fold increase in hepatic AMPK phosphorylation at 3 and 6 hours after dosing and a 1.5- to 2-fold increase in LKB1 phosphorylation at these same time points. The phosphorylation of HMG-CoA reductase at these and later time points was not significantly increased. Black tea administered by gavage at up to 250 mg/kg was ineffective in increasing hepatic AMPK phosphorylation. Both green and black tea extracts increased LKB1 phosphorylation in hepatoma cells in culture at 15 mug/mL, and black tea also increased the phosphorylation of protein kinase A in hepatoma cells. These results suggest that compounds in both tea extracts activate AMPK by activating its upstream kinase, LKB1, and that black tea may do so by first activating protein kinase A, a known kinase for LKB1. Only green tea, at 50 and 100 mg/kg, was able to activate AMPK and LKB1 in mouse liver after oral dosing, suggesting that the polymerized catechins present in black tea do not reach the liver in sufficient concentration to affect AMPK activity.

Bauerly, K., et al. (2011). "Altering pyrroloquinoline quinone nutritional status modulates mitochondrial, lipid, and energy metabolism in rats." PLoS One **6**(7): e21779.

We have reported that pyrroloquinoline quinone (PQQ) improves reproduction, neonatal development, and mitochondrial function in animals by mechanisms that involve mitochondrial related cell signaling pathways. To extend these observations, the influence of PQQ on energy and lipid relationships and apparent protection against ischemia reperfusion injury are described herein. Sprague-Dawley rats were fed a nutritionally complete diet with PQQ added at either 0 (PQQ-) or 2 mg PQQ/Kg diet (PQQ+). Measurements included: 1) serum glucose and insulin, 2) total energy expenditure per metabolic body size (Wt(3/4)), 3) respiratory quotients (in the fed and fasted states), 4) changes in plasma lipids, 5) the relative mitochondrial amount in liver and heart, and 6) indices related to cardiac ischemia. For the latter, rats (PQQ- or PQQ+) were subjected to left anterior descending occlusions followed by 2 h of reperfusion to determine PQQ's influence on infarct size and myocardial tissue levels of malondialdehyde, an indicator of lipid peroxidation. Although no striking differences in serum glucose, insulin, and free fatty acid levels were observed, energy expenditure was lower in PQQ- vs. PQQ+ rats and energy expenditure (fed state) was correlated with the hepatic mitochondrial content. Elevations in plasma di- and triacylglyceride and beta-hydroxybutryic acid concentrations were also observed in PQQ- rats vs. PQQ+ rats. Moreover, PQQ administration (i.p. at 4.5 mg/kg BW for 3 days) resulted in a greater than 2-fold decrease in plasma triglycerides during a 6-hour fast than saline administration in a rat model of type 2 diabetes. Cardiac injury resulting from ischemia/reperfusion was more pronounced in PQQ- rats than in PQQ+ rats. Collectively, these data demonstrate that PQQ deficiency impacts a number of parameters related to normal mitochondrial function.

Baur, J. A., et al. (2006). "Resveratrol improves health and survival of mice on a high-calorie diet." Nature **444**(7117): 337-342.

Resveratrol (3,5,4'-trihydroxystilbene) extends the lifespan of diverse species including Saccharomyces cerevisiae, Caenorhabditis elegans and Drosophila melanogaster. In these organisms, lifespan extension is dependent on Sir2, a conserved deacetylase proposed to underlie the beneficial effects of caloric restriction. Here we show that resveratrol shifts the physiology of middle-aged mice on a high-calorie diet towards that of mice on a standard diet and significantly increases their survival. Resveratrol produces changes associated with longer lifespan, including increased insulin sensitivity, reduced insulin-like growth factor-1 (IGF-I) levels, increased AMP-activated protein kinase (AMPK) and peroxisome proliferator-activated receptor-gamma coactivator 1alpha (PGC-1alpha) activity, increased mitochondrial number, and improved motor function. Parametric analysis of gene set enrichment revealed that resveratrol opposed the effects of the high-calorie diet in 144 out of 153 significantly altered pathways. These data show that improving general health in mammals using small molecules is an attainable goal, and point to new approaches for treating obesity-related disorders and diseases of ageing.

Bayram, B., et al. (2012). "A diet rich in olive oil phenolics reduces oxidative stress in the heart of SAMP8 mice by induction of Nrf2-dependent gene expression." Rejuvenation Res **15**(1): 71-81.

A Mediterranean diet rich in olive oil has been associated with health benefits in humans. It is unclear if and to what extent olive oil phenolics may mediate these health benefits. In this study, we fed senescence-accelerated mouse-prone 8 (SAMP8, n=11 per group) semisynthetic diets with 10% olive oil containing either high (HP) or low amounts of olive oil phenolics (LP) for 4.5 months. Mice consuming the HP diet had significantly lower concentrations of the oxidative damage markers thiobarbituric acid-reactive substances and protein carbonyls in the heart, whereas proteasomal activity was similar in both groups. Nrf2-dependent gene expression may be impaired during the aging process. Therefore, we measured Nrf2 and its target genes glutathione-S-transferase (GST), gamma-glutamyl cysteine synthetase (gamma-GCS), nicotinamide adenine dinucleotide phosphate [NAD(P)H]:quinone oxidoreductase (NQO1), and paraoxonase-2 (PON2) in the hearts of these mice. Nrf2 as well as GST, gamma-GCS, NQO1, and PON2 mRNA levels were significantly higher in heart tissue of the HP as compared to the LP group. The HP-fed mice had significantly higher PON1 activity in serum compared to those receiving the LP diet. Furthermore, HP feeding increased relative SIRT1 mRNA levels. Additional mechanistic cell culture experiments were performed, and they suggest that the olive oil phenolic hydroxytyrosol present in the HP oil may be responsible for the induction of Nrf2-dependent gene expression and the increase in PON activity. In conclusion, a diet rich in olive oil phenolics may prevent oxidative stress in the heart of SAMP8 mice by modulating Nrf2-dependent gene expression.

Beevers, C. S., et al. (2009). "Curcumin disrupts the Mammalian target of rapamycin-raptor complex." Cancer Res **69**(3): 1000-1008.

Curcumin (diferuloylmethane), a polyphenol natural product of the plant Curcuma longa, is undergoing early clinical trials as a novel anticancer agent. However, the anticancer mechanism of curcumin remains to be elucidated. Recently, we have shown that curcumin inhibits phosphorylation of p70 S6 kinase 1 (S6K1) and eukaryotic initiation factor 4E (eIF4E) binding protein 1 (4E-BP1), two downstream effector molecules of the mammalian target of rapamycin complex 1 (mTORC1) in numerous cancer cell lines. This study was designed to elucidate the underlying mechanism. We observed that curcumin inhibited mTORC1 signaling not by inhibition of the upstream kinases, such as insulin-like growth factor 1 receptor (IGF-IR) and phosphoinositide-dependent kinase 1 (PDK1). Further, we found that curcumin inhibited mTORC1 signaling independently of protein phosphatase 2A (PP2A) or AMP-activated protein kinase AMPK-tuberous sclerosis complex (TSC). This is evidenced by the findings that curcumin was able to inhibit phosphorylation of S6K1 and 4E-BP1 in the cells pretreated with PP2A inhibitor (okadaic acid) or AMPK inhibitor (compound C), or in the cells expressing dominant-negative (dn) PP2A, shRNA to PP2A-A subunit, or dn-AMPKalpha. Curcumin did not alter the TSC1/2 interaction. Knockout of TSC2 did not affect curcumin inhibition of mTOR signaling. Finally, we identified that curcumin was able to dissociate raptor from mTOR, leading to inhibition of mTORC1 activity. Therefore, our data indicate that curcumin may represent a new class of mTOR inhibitor.

Ben-Dor, A., et al. (2005). "Carotenoids activate the antioxidant response element transcription system." Mol Cancer Ther **4**(1): 177-186.

Brito, P. M., et al. (2009). "Resveratrol inhibits the mTOR mitogenic signaling evoked by oxidized LDL in smooth muscle cells." Atherosclerosis **205**(1): 126-134.

OBJECTIVES: Smooth muscle cell (SMC) proliferation is a major feature in atherosclerosis, since it contributes to the formation of the fibrous cap, thus to plaque stability, but also to arterial stenosis and post-angioplasty restenosis. Among the various mitogenic signaling pathways involved in SMC proliferation, the mTOR pathway regulates both the cell cycle and cell growth. Resveratrol, a polyphenolic compound from grapes and red

wine, has potential anti-atherogenic and anti-cancer properties. This work was designed to investigate the activation of the mTOR pathway by the proatherogenic oxidized LDL (oxLDL) in SMC, and the potential inhibitory effect of resveratrol. RESULTS: mTOR and its downstream target p70S6 kinase are phosphorylated and activated by mitogenic concentrations of oxLDL (50 microg/ml), and are involved in SMC proliferation, as assessed by the inhibitory effect of the mTOR inhibitor rapamycin. The activation of mTOR signaling by oxLDL, requires the upstream activation of PI3K and Akt, as assessed by the inhibitory effect of the PI3K inhibitor Ly294002 on mTOR activation and DNA synthesis. Resveratrol blocked the oxLDL-induced phosphorylation and activation of the PI3K/Akt/mTOR/p70S6K pathway and strongly inhibited both the DNA synthesis and proliferation of SMC. This activity is independent of the anti-oxidant effect and of AMPK activation by resveratrol. CONCLUSION: These data indicate that the mTOR pathway is activated by oxLDL via PI3K/PDK1/Akt, and is required for SMC proliferation. Resveratrol blocks specifically this pathway, thereby inhibiting oxLDL-induced SMC proliferation. These data highlight a new property for resveratrol that could contribute to the general anti-atherogenic properties of this polyphenol.

Cai, H., et al. (2015). "Cancer chemoprevention: Evidence of a nonlinear dose response for the protective effects of resveratrol in humans and mice." Sci Transl Med **7**(298): 298ra117.

Resveratrol is widely promoted as a potential cancer chemopreventive agent, but a lack of information on the optimal dose prohibits rationally designed trials to assess efficacy. To challenge the assumption that "more is better," we compared the pharmacokinetics and activity of a dietary dose with an intake 200 times higher. The dose-response relationship for concentrations generated and the metabolite profile of [(14)C]-resveratrol in colorectal tissue of cancer patients helped us to define clinically achievable levels. In Apc(Min) mice (a model of colorectal carcinogenesis) that received a high-fat diet, the low resveratrol dose suppressed intestinal adenoma development more potently than did the higher dose. Efficacy correlated with activation of adenosine monophosphate-activated protein kinase (AMPK) and increased expression of the senescence marker p21. Nonlinear dose responses were observed for AMPK and mechanistic target of rapamycin (mTOR) signaling in mouse adenoma cells, culminating in autophagy and senescence. In human colorectal tissues exposed to low dietary concentrations of resveratrol ex vivo, we measured enhanced AMPK phosphorylation and autophagy. The expression of the cytoprotective NAD(P)H dehydrogenase, quinone 1 (NQO1) enzyme was also increased in tissues from cancer patients participating in our [(14)C]-resveratrol trial. These findings warrant a revision of developmental strategies for diet-derived agents designed to achieve cancer chemoprevention.

Chan, A. Y., et al. (2008). "Resveratrol inhibits cardiac hypertrophy via AMP-activated protein kinase and Akt." J Biol Chem **283**(35): 24194-24201.

Whereas studies involving animal models of cardiovascular disease demonstrated that resveratrol is able to inhibit hypertrophic growth, the mechanisms involved have not been elucidated. Because studies in cells other than cardiomyocytes revealed that AMP-activated protein kinase (AMPK) and Akt are affected by resveratrol, we hypothesized that resveratrol prevents cardiac myocyte hypertrophy via these two kinase systems. Herein, we demonstrate that resveratrol reduces phenylephrine-induced protein synthesis and cell growth in rat cardiac myocytes via alterations of intracellular pathways involved in controlling protein synthesis (p70S6 kinase and eukaryotic elongation factor-2). Additionally, we demonstrate that resveratrol negatively regulates the calcineurin-nuclear factor of activated T cells pathway thus modifying a critical component of the transcriptional mechanism involved in pathological cardiac hypertrophy. Our data also indicate that these effects of resveratrol are mediated via AMPK activation and Akt inhibition, and in the case of AMPK, is dependent on the presence of the AMPK kinase, LKB1. Taken together, our data suggest that resveratrol exerts anti-hypertrophic effects by activating AMPK via LKB1 and inhibiting Akt, thus suppressing protein synthesis and gene transcription.

Chen, C. C., et al. (2011). "Upregulation of NF-E2-related factor-2-dependent glutathione by carnosol provokes a cytoprotective response and enhances cell survival." Acta Pharmacol Sin **32**(1): 62-69.

Chen, H. W., et al. (2014). "Bioavailability of andrographolide and protection against carbon tetrachloride-induced oxidative damage in rats." Toxicol Appl Pharmacol **280**(1): 1-9.

Chen, M. L., et al. (2013). "Resveratrol attenuates vascular endothelial inflammation by inducing autophagy through the cAMP signaling pathway." Autophagy **9**(12): 2033-2045.

Inflammation participates centrally in all stages of atherosclerosis (AS), which begins with inflammatory changes in the endothelium, characterized by expression of the adhesion molecules. Resveratrol (RSV) is a naturally occurring phytoalexin that can attenuate endothelial inflammation; however, the exact mechanisms have not been thoroughly elucidated. Autophagy refers to the normal process of cell degradation of proteins and organelles, and is protective against certain inflammatory injuries. Thus, we intended to determine the role of autophagy in the antiinflammatory effects of RSV in human umbilical vein endothelial cells (HUVECs). We found

that RSV pretreatment reduced tumor necrosis factor ? (TNF/TNF?)-induced inflammation and increased MAP1LC3B2 (microtubule-associated protein 1 light chain 3 ? 2) expression and SQSTM1/p62 (sequestosome 1) degradation in a concentration-dependent manner. A bafilomycin A 1 (BafA1) challenge resulted in further accumulation of MAP1LC3B2 in HUVECs. Furthermore, autophagy inhibitors 3-methyladenine (3-MA), chloroquine as well as ATG5 and BECN1 siRNA significantly attenuated RSV-induced autophagy, which, subsequently, suppressed the downregulation of RSV-induced inflammatory factors expression. RSV also increased cAMP (cyclic adenosine monophosphate) content, the expression of PRKA (protein kinase A) and SIRT1 (sirtuin 1), as well as the activity of AMPK (AMP-activated protein kinase). RSV-induced autophagy in HUVECs was abolished in the presence of inhibitors of ADCY (adenylyl cyclase, KH7), PRKA (H-89), AMPK (compound C), or SIRT1 (nicotinamide and EX-527), as well as ADCY, PRKA, AMPK, and SIRT1 siRNA transfection, indicating that the effects of RSV on autophagy induction were dependent on cAMP, PRKA, AMPK and SIRT1. In conclusion, RSV attenuates endothelial inflammation by inducing autophagy, and the autophagy in part was mediated through the activation of the cAMP-PRKA-AMPK-SIRT1 signaling pathway.

Chen, S., et al. (2011). "Resveratrol inhibits cell differentiation in 3T3-L1 adipocytes via activation of AMPK." Can J Physiol Pharmacol **89**(11): 793-799.

Resveratrol (Res) is a natural polyphenolic compound with anti-inflammatory and antioxidant properties. Also, Res can inhibit lipogenesis and adipocyte differentiation. However, the underlying mechanisms of Res's functions remain largely unknown. AMP-activated protein kinase (AMPK) is a key player in adipocyte differentiation. Therefore, the purpose of our study was to determine the role played by AMPK in the Res-mediated regulation of adipocyte differentiation. Incubation of 3T3-L1 cells with Res confirmed that Res inhibited adipocyte differentiation. The phosphorylation of AMPKalpha was increased by Res in a dose-dependent manner, while total AMPKalpha levels were unchanged, and peroxisome proliferator-activated receptor gamma (PPARgamma), CCAAT-enhancer-binding protein alpha (C/EBPalpha), and sterol regulatory element-binding protein 1c (SREBP-1c) levels were decreased. Interestingly, pretreatment with AMPKalpha siRNA and Res promoted adipocyte differentiation, while the decrease of p-AMPKalpha increased PPARgamma, C/EBPalpha, and SREBP-1c protein expression. Our study shows that Res is capable of inhibiting lipogenesis and differentiation of 3T3-L1 adipocytes via activation of AMPK, suggesting its potential therapeutic application in the treatment or prevention of obesity.

Chen, W. L., et al. (2012). "alpha-Lipoic acid regulates lipid metabolism through induction of sirtuin 1 (SIRT1) and activation of AMP-activated protein kinase." Diabetologia **55**(6): 1824-1835.

AIMS/HYPOTHESIS: Sirtuin 1 (SIRT1) is a longevity-associated protein, which regulates energy metabolism and lifespan in response to nutrient deprivation. It has been proposed to be a therapeutic target for obesity and metabolic syndrome. We investigated whether alpha-lipoic acid (ALA) exerts a lipid-lowering effect through regulation of SIRT1 activation and production in C(2)C(12) myotubes. METHODS: ALA-stimulated AMP-activated protein kinase (AMPK), acetyl-CoA carboxylase (ACC), adipose triacylglycerol lipase (ATGL) and fatty acid synthase (FAS) production, as well as intracellular triacylglycerol accumulation and fatty acid beta-oxidation were analysed in the absence or presence of a SIRT1 inhibitor (nicotinamide), SIRT1 small interfering (si) RNA and an AMPK inhibitor (compound C) in C(2)C(12) myotubes. Mice with streptozotocin/nicotinamide-induced diabetes and db/db mice fed on a high-fat diet were used to study the ALA-mediated lipid-lowering effects in vivo. RESULTS: ALA increased the NAD(+)/NADH ratio to enhance SIRT1 activity and production in C(2)C(12) myotubes. ALA subsequently increased AMPK and ACC phosphorylation, leading to increased palmitate beta-oxidation and decreased intracellular triacylglycerol accumulation in C(2)C(12) myotubes. In cells treated with nicotinamide or transfected with SIRT1 siRNA, ALA-mediated AMPK/ACC phosphorylation, intracellular triacylglycerol accumulation and palmitate beta-oxidation were reduced, suggesting that SIRT1 is an upstream regulator of AMPK. ALA increased ATGL and suppressed FAS protein production in C(2)C(12) myotubes. Oral administration of ALA in diabetic mice fed on a high-fat diet and db/db mice dramatically reduced the body weight and visceral fat content. CONCLUSIONS/INTERPRETATION: ALA activates both SIRT1 and AMPK, which leads to lipid-lowering effects in vitro and in vivo. These findings suggest that ALA may have beneficial effects in the treatment of dyslipidaemia and obesity.

Cheng, Z., et al. (2006). "Berberine-stimulated glucose uptake in L6 myotubes involves both AMPK and p38 MAPK." Biochim Biophys Acta **1760**(11): 1682-1689.

Berberine is a plant alkaloid used in traditional Chinese medicine and has been reported to have antihyperglycemic activity in NIDDM patients. However, the molecular basis for this action is yet to be elucidated. Here we investigate the effects and signaling pathways of berberine on L6 rat skeletal muscles. Our study demonstrates that berberine stimulates glucose uptake in a time- and dose-dependent manner. Intriguingly, berberine-stimulated glucose uptake does not vary as insulin concentration increases, and could not be blocked by the PI 3-kinase inhibitor wortmannin. Berberine only weakly stimulates the phosphorylation of Akt/PKB, a key molecule in the insulin signaling pathway, but strongly promotes the phosphorylation of AMPK and p38 MAPK. The

effects of berberine are not a result of pro-oxidant action, but a consequence of an increased cellular AMP:ATP ratio. Moreover, berberine-stimulated glucose uptake is inhibited by the AMPK inhibitor Compound C and the p38 MAPK inhibitor SB202190. Inhibition of AMPK reduces p38 MAPK phosphorylation, suggesting that AMPK lies upstream of p38 MAPK. These results suggest that berberine circumvents insulin signaling pathways and stimulates glucose uptake through the AMP-AMPK-p38 MAPK pathway, which may account for the antihyperglycemic effects of this drug.

Chin, R. M., et al. (2014). "The metabolite alpha-ketoglutarate extends lifespan by inhibiting ATP synthase and TOR." Nature.

Metabolism and ageing are intimately linked. Compared with ad libitum feeding, dietary restriction consistently extends lifespan and delays age-related diseases in evolutionarily diverse organisms. Similar conditions of nutrient limitation and genetic or pharmacological perturbations of nutrient or energy metabolism also have longevity benefits. Recently, several metabolites have been identified that modulate ageing; however, the molecular mechanisms underlying this are largely undefined. Here we show that alpha-ketoglutarate (alpha-KG), a tricarboxylic acid cycle intermediate, extends the lifespan of adult Caenorhabditis elegans. ATP synthase subunit beta is identified as a novel binding protein of alpha-KG using a small-molecule target identification strategy termed drug affinity responsive target stability (DARTS). The ATP synthase, also known as complex V of the mitochondrial electron transport chain, is the main cellular energy-generating machinery and is highly conserved throughout evolution. Although complete loss of mitochondrial function is detrimental, partial suppression of the electron transport chain has been shown to extend C. elegans lifespan. We show that alpha-KG inhibits ATP synthase and, similar to ATP synthase knockdown, inhibition by alpha-KG leads to reduced ATP content, decreased oxygen consumption, and increased autophagy in both C. elegans and mammalian cells. We provide evidence that the lifespan increase by alpha-KG requires ATP synthase subunit beta and is dependent on target of rapamycin (TOR) downstream. Endogenous alpha-KG levels are increased on starvation and alpha-KG does not extend the lifespan of dietary-restricted animals, indicating that alpha-KG is a key metabolite that mediates longevity by dietary restriction. Our analyses uncover new molecular links between a common metabolite, a universal cellular energy generator and dietary restriction in the regulation of organismal lifespan, thus suggesting new strategies for the prevention and treatment of ageing and age-related diseases.

Choi, K. M., et al. (2013). "Green tomato extract attenuates high-fat-diet-induced obesity through activation of the AMPK pathway in C57BL/6 mice." J Nutr Biochem **24**(1): 335-342.

Obesity is a risk factor for numerous metabolic disorders. Recently, natural compounds that may be beneficial for improving obesity have received increasing attention. In this study, we investigated whether red and green tomato extracts attenuate high-fat-diet-induced obesity in C57BL/6 mice. The mice were maintained on a normal diet (ND) or high-fat diet (HFD) for 4 weeks and then fed ND, HFD, HFD plus 2% red tomato extract (RTE) or HFD plus 2% green tomato extract (GTE) for 13 weeks. The weekly food intakes among the groups were not significantly different. Body weight of mice fed HFD plus GTE was significantly decreased to the level of mice fed ND, but the body weight was only slightly reduced in mice fed HFD plus RTE. Epididymal adipose tissue and liver weights were significantly decreased in mice fed HFD plus GTE compared to those in HFD. Serum total cholesterol and low-density lipoprotein cholesterol levels in mice fed GTE were modestly reduced, and liver total cholesterol level was strongly decreased in HFD plus GTE-fed mice compared to that in HFD-fed mice. Adenosine-monophosphate-activated protein kinase (AMPK) and acetyl-CoA carboxylase phosphorylation in liver from HFD plus GTE-fed mice was significantly elevated, and HMG-CoA reductase expression was also significantly decreased. GTE strongly decreased the expression of peroxisome proliferator-activated receptor gamma, CCAAT/enhancer-binding protein alpha and perilipin in the adipose tissue of mice fed HFD plus GTE. Our results indicate that the antiobesity effects of GTE may be associated with activation of the AMPK pathway.

Chowanadisai, W., et al. (2010). "Pyrroloquinoline quinone stimulates mitochondrial biogenesis through cAMP response element-binding protein phosphorylation and increased PGC-1alpha expression." J Biol Chem **285**(1): 142-152.

Bioactive compounds reported to stimulate mitochondrial biogenesis are linked to many health benefits such increased longevity, improved energy utilization, and protection from reactive oxygen species. Previously studies have shown that mice and rats fed diets lacking in pyrroloquinoline quinone (PQQ) have reduced mitochondrial content. Therefore, we hypothesized that PQQ can induce mitochondrial biogenesis in mouse hepatocytes. Exposure of mouse Hepa1-6 cells to 10-30 microm PQQ for 24-48 h resulted in increased citrate synthase and cytochrome c oxidase activity, Mitotracker staining, mitochondrial DNA content, and cellular oxygen respiration. The induction of this process occurred through the activation of cAMP response element-binding protein (CREB) and peroxisome proliferator-activated receptor-gamma coactivator-1alpha (PGC-1alpha), a pathway known to regulate mitochondrial biogenesis. PQQ exposure stimulated phosphorylation of CREB at serine 133, activated the promoter of PGC-1alpha, and increased PGC-1alpha mRNA and protein expression. PQQ did

not stimulate mitochondrial biogenesis after small interfering RNA-mediated reduction in either PGC-1alpha or CREB expression. Consistent with activation of the PGC-1alpha pathway, PQQ increased nuclear respiratory factor activation (NRF-1 and NRF-2) and Tfam, TFB1M, and TFB2M mRNA expression. Moreover, PQQ protected cells from mitochondrial inhibition by rotenone, 3-nitropropionic acid, antimycin A, and sodium azide. The ability of PQQ to stimulate mitochondrial biogenesis accounts in part for action of this compound and suggests that PQQ may be beneficial in diseases associated with mitochondrial dysfunction.

Cimini, A., et al. (2013). "Cocoa powder triggers neuroprotective and preventive effects in a human Alzheimer's disease model by modulating BDNF signaling pathway." J Cell Biochem **114**(10): 2209-2220.

The molecular mechanisms linking Abeta to the onset of neurotoxicity are still largely unknown, but several lines of evidence point to reactive oxygen species, which are produced even under the effect of nanomolar concentrations of soluble Abeta-oligomers. The consequent oxidative stress is considered as the mediator of a cascade of degenerative events in many neurological disorders. Epidemiological studies indicate that dietary habits and antioxidants from diet can influence the incidence of neurodegenerative disorders such as Alzheimer's and Parkinson's diseases. In the recent years, a number of reviews have reported on neuroprotective effects of polyphenols in cell and animal models. However, the majority of these studies have focused only on the anti-oxidant properties of these compounds and less on the mechanism/s of action at cellular level. In this work we investigated the effect of cocoa polyphenolic extract on a human AD in vitro model. The results obtained, other than confirming the anti-oxidant properties of cocoa, demonstrate that cocoa polyphenols triggers neuroprotection by activating BDNF survival pathway, both on Abeta plaque treated cells and on Abeta oligomers treated cells, resulting in the counteraction of neurite dystrophy. On the light of the results obtained the use of cocoa powder as preventive agent for neurodegeneration is further supported.

Costa, M. S., et al. (2008). "Caffeine improves adult mice performance in the object recognition task and increases BDNF and TrkB independent on phospho-CREB immunocontent in the hippocampus." Neurochem Int **53**(3-4): 89-94.

Caffeine is one of the most psychostimulants consumed all over the world that usually presents positive effects on cognition. In this study, effects of caffeine on mice performance in the object recognition task were tested in different intertrial intervals. In addition, it was analyzed the effects of caffeine on brain derived neurotrophic factor (BDNF) and its receptor, TrkB, immunocontent to try to establish a connection between the behavioral finding and BDNF, one of the neurotrophins strictly involved in memory and learning process. CF1 mice were treated during 4 consecutive days with saline (0.9g%, i.p.) or caffeine (10mg/kg, i.p., equivalent dose corresponding to 2-3 cups of coffee). Caffeine treatment was interrupted 24h before the object recognition task analysis. In the test session performed 15min after training session, caffeine-treated mice recognized more efficiently both the familiar and the novel object. In the test session performed 90min and 24h after training session, caffeine did not change the time spent in the familiar object but increased the object recognition index, when compared to control group. Western blotting analysis of hippocampus from caffeine-treated mice revealed an increase in BDNF and TrkB immunocontent, compared to their saline-matched controls. Phospho-CREB immunocontent did not change with caffeine treatment. Our results suggest that acute treatment with caffeine improves recognition memory, and this effect may be related to an increase of the BDNF and TrkB immunocontent in the hippocampus.

Cui, W., et al. (2012). "Prevention of diabetic nephropathy by sulforaphane: possible role of Nrf2 upregulation and activation." Oxid Med Cell Longev **2012**: 821936.

Dai, C., et al. (2014). "Lycopene attenuates colistin-induced nephrotoxicity in mice via activating the Nrf2/HO-1 pathway." Antimicrob Agents Chemother.

Davis, J. N., et al. (1999). "Genistein inhibits NF-kappa B activation in prostate cancer cells." Nutr Cancer **35**(2): 167-174.

Prostate cancer is the second leading cause of cancer-related deaths in men in the United States. Epidemiological studies indicate that susceptibility to prostate cancer may be partly due to environmental influences, especially diet. An association has been shown between decreased prostate cancer risk and mortality with increased consumption of soy products, resulting in increased levels of isoflavones. We previously demonstrated that the soy isoflavone genistein inhibits cell growth and induces apoptosis in prostate cancer cells. To further elucidate the molecular mechanism by which genistein elicits its apoptotic effect, we investigated the role of a transcription factor, nuclear factor-kappa B (NF-kappa B), in the androgen-sensitive cell line LNCaP and the androgen-insensitive cell line PC3. Here we show that genistein decreases NF-kappa B DNA binding and abrogates NF-kappa B activation by DNA-damaging agents, H2O2 and tumor necrosis factor-alpha, in prostate cancer cells regardless of androgen sensitivity. Additionally, we have demonstrated that genistein reduces phosphorylation of the inhibitory protein I kappa B alpha and blocks the nuclear translocation of NF-kappa B,

prohibiting DNA binding and preventing NF-kappa B activation. These results provide a mechanism by which genistein induces apoptosis in prostate cancer cells: the inactivation of NF-kappa B. Furthermore, genistein's ability to abrogate NF-kappa B activation by DNA-damaging agents strongly supports genistein's role as a chemopreventive agent.

De Nicolo, S., et al. (2013). "Effects of olive polyphenols administration on nerve growth factor and brain-derived neurotrophic factor in the mouse brain." Nutrition **29**(4): 681-687.

OBJECTIVE: Polyphenols are chemicals derived from plants known to possess antioxidant and anti-inflammatory properties. High intake of fruit and vegetables is believed to be beneficial to human health. Various studies have suggested that dietary polyphenols may protect against cancer and cardiometabolic and neurodegenerative diseases. Nerve growth factor (NGF) and brain-derived neurotrophic factor (BDNF) are neurotrophins that play key roles in brain cell development, growth, and survival. The aim of this study was to investigate whether or not administration of olive (Olea europaea L.) polyphenols could have an effect on NGF and BDNF content and the expression of their receptors, TrkA and TrkB, respectively, in the mouse brain. METHODS: NGF and BDNF were measured by enzyme-linked immunosorbent assay. TrkA and TrkB were measured by Western blotting. RESULTS: We found NGF and BDNF elevation in the hippocampus and olfactory bulbs and a decrease in the frontal cortex and striatum. These data were associated with potentiated expression of TrkA and TrkB in the hippocampus and olfactory bulbs but no differences between groups in the striatum and frontal cortex. Polyphenols did not affect some behavioral mouse parameters associated with stressing situations. CONCLUSIONS: Altogether, this study shows that olive polyphenols in the mouse may increase the levels of NGF and BDNF in crucial areas of the limbic system and olfactory bulbs, which play a key role in learning and memory processes and in the proliferation and migration of endogenous progenitor cells present in the rodent brain.

Deng, C., et al. (2013). "alpha-Lipoic acid reduces infarct size and preserves cardiac function in rat myocardial ischemia/reperfusion injury through activation of PI3K/Akt/Nrf2 pathway." PLoS One **8**(3): e58371.

Do, G. M., et al. (2012). "Resveratrol ameliorates diabetes-related metabolic changes via activation of AMP-activated protein kinase and its downstream targets in db/db mice." Mol Nutr Food Res **56**(8): 1282-1291.

SCOPE: This study investigated the effects of resveratrol (RV) on diabetes-related metabolic changes in a spontaneous model of type 2 diabetes, as well as activation of AMP-activated protein kinase (AMPK) and downstream targets. METHODS AND RESULTS: C57BL/KsJ-db/db mice were fed a normal diet with RV (0.005% and 0.02%, w/w) or rosiglitazone (RG, 0.001%, w/w) for 6 weeks. Both doses of RV significantly decreased blood glucose, plasma free fatty acid, triglyceride, apo B/apo Acapital I, Ukrainian levels and increased plasma adiponectin levels. RV activated AMPK and downstream targets leading to decreased blood HbA1c levels, hepatic gluconeogenic enzyme activity, and hepatic glycogen, while plasma insulin levels, pancreatic insulin protein, and skeletal muscle GLUT4 protein were higher after RV supplementation. The high RV dose also significantly increased hepatic glycolytic gene expression and enzyme activity, along with skeletal muscle glycogen synthase protein expression, similar to RG. Furthermore, RV dose dependently decreased hepatic triglyceride content and phosphorylated I kappa B kinase (p-IKK) protein expression, while hepatic uncoupling protein (UCP) and skeletal muscle UCP expression were increased. CONCLUSION: RV potentiates improving glycemic control, glucose uptake, and dyslipidemia, as well as protecting against pancreatic beta-cell failure in a spontaneous type 2 diabetes model. Dietary RV has potential as an antidiabetic agent via activation of AMPK and its downstream targets.

Elangovan, S. and T. C. Hsieh (2008). "Control of cellular redox status and upregulation of quinone reductase NQO1 via Nrf2 activation by alpha-lipoic acid in human leukemia HL-60 cells." Int J Oncol **33**(4): 833-838.

alpha-Lipoic acid (LA) is a naturally-occurring micronutrient that has been actively investigated for the treatment and management of various chronic medical conditions including neurodegenerative diseases, diabetes and hepatic disorders. However, relatively few studies have examined the effects of LA as a chemopreventive agent, particularly in regard to its ability to modulate homeostasis of oxidoreductive state and to regulate detoxification enzymes such as quinone reductase NQO1 in LA-responsive cells. We tested the hypothesis that LA affects the intracellular redox status and induces NQO1 expression using the human promyelocytic HL-60 leukemia cells. We showed that treatment by LA maintains HL-60 cells in a relatively reduced state, supported by the dose/time-dependent increase in the activities of glutathione peroxidase and glutathione reductase and decrease in the activity of catalase. Moreover, LA significantly increased the activity and protein expression of NQO1. The induction of NQO1 was accompanied by the nuclear accumulation of transcription factor Nrf2, which was correlated with a decreased level of Nrf2 in the cytosol as well as the concomitant reduction in the expression of cytoplasmic repressor of Nrf2, Keap1.

Fernandez-Iglesias, A., et al. (2013). "DHA sensitizes FaO cells to tert-BHP-induced oxidative effects. Protective role of EGCG." Food Chem Toxicol **62**: 750-757.

Fischedick, J. T., et al. (2013). "Structure activity relationship of phenolic diterpenes from Salvia officinalis as activators of the nuclear factor E2-related factor 2 pathway." Bioorg Med Chem **21**(9): 2618-2622.

Fujita, H., et al. (2008). "Alpha-lipoic acid suppresses 6-hydroxydopamine-induced ROS generation and apoptosis through the stimulation of glutathione synthesis but not by the expression of heme oxygenase-1." Brain Res **1206**: 1-12.

We previously reported that the generation of reactive oxygen species (ROS) is the initial event in cell death induced by 6-hydroxydopamine (6-OHDA), an experimental model of Parkinsonism. Since recent studies suggested the important role of antioxidant activity of alpha-lipoic acid (LA) in the suppression of apoptosis of various types, we studied the effect on 6-OHDA-induced apoptosis of PC12 cells. Biochemical analysis revealed that LA suppressed the 6-OHDA-induced ROS generation, increase of caspase-like activity and chromatin condensation. The suppression of 6-OHDA-induced apoptosis by LA required pre-incubation of PC12 cells with LA for 12-24 h. LA increased the intracellular levels of heme oxygenase-1 (HO-1) and glutathione (GSH) and stimulated the expression of GSH synthesis-related genes such as cystine/glutamate antiporter and gamma-glutamylcysteine synthetase (gamma-GCS). However, Sn-mesoporphyrin IX, an inhibitor of HO-1, did not attenuate the LA-induced suppression of apoptosis. In contrast, buthionine sulfoximine, an inhibitor of gamma-GCS, attenuated the LA-induced suppression of ROS generation and chromatin condensation. In addition, a transcription factor Nrf2, which regulates the expression of antioxidant enzymes such as gamma-GCS, translocated to the nucleus by LA. These results suggested that LA suppressed the 6-OHDA induced-apoptosis by the increase in cellular glutathione through stimulation of the GSH synthesis system but not by the expression of HO-1.

Gauhar, R., et al. (2012). "Heat-processed Gynostemma pentaphyllum extract improves obesity in ob/ob mice by activating AMP-activated protein kinase." Biotechnol Lett **34**(9): 1607-1616.

Gynostemma pentaphyllum is widely used in Asian countries as a herbal medicine to treat dyslipidemia, type 2 diabetes and inflammation. An ethanol extract of G. pentaphyllum lessened obesity by activating AMP-activated protein kinase (AMPK). The levels of damulins A and B, components responsible for AMPK activation in the extract, were increased by autoclaving in a time-dependent manner. Heat-processed G. pentaphyllum extract, actiponin containing damulins A (0.93 %, w/w) and B (0.68 %, w/w), significantly stimulated fat oxidation and glucose uptake via AMPK activation in L6 myotube cells. Oral administration of actiponin to ob/ob mice for 8 weeks decreased body weight gain, liver weight, and blood cholesterol levels with AMPK activation in the soleus muscle. Our results demonstrate the beneficial effect of G. pentaphyllum on improving obesity and have elucidated the underlying molecular mechanisms.

Ghanim, H., et al. (2011). "A resveratrol and polyphenol preparation suppresses oxidative and inflammatory stress response to a high-fat, high-carbohydrate meal." J Clin Endocrinol Metab **96**(5): 1409-1414.

Granado-Serrano, A. B., et al. (2012). "Quercetin modulates Nrf2 and glutathione-related defenses in HepG2 cells: Involvement of p38." Chem Biol Interact **195**(2): 154-164.

Dietary flavonoid quercetin has been suggested as a cancer chemopreventive agent, but the mechanisms of action remain unclear. This study investigated the influence of quercetin on p38-MAPK and the potential regulation of the nuclear transcription factor erythroid-2p45-related factor (Nrf2) and the cellular antioxidant/detoxifying defense system related to glutathione (GSH) by p38 in HepG2 cells. Incubation of HepG2 cells with quercetin at a range of concentrations (5-50muM) for 4 or 18h induced a differential effect on the modulation of p38 and Nrf2 in HepG2 cells, 50muM quercetin showed the highest activation of p38 at 4h of treatment and values of p38 similar to those of control cells after 18 h of incubation, together with the inhibition of Nrf2 at both incubation times. Quercetin (50muM) induced a time-dependent activation of p38, which was in concert with a transient stimulation of Nrf2 to provoke its inhibition afterward. Quercetin also increased GSH content, mRNA levels of glutamylcysteine-synthetase (GCS) and expression and/or activity of glutathione-peroxidase, glutathione-reductase and GCS after 4h of incubation, and glutathione-S-transferase after 18h of exposure. Further studies with the p38 specific inhibitor SB203580 showed that the p38 blockage restored the inhibited Nrf2 transcription factor and the enzymatic expression and activity of antioxidant/detoxificant enzymes after 4h exposure. In conclusion, p38-MAPK is involved in the mechanisms of the cell response to quercetin through the modulation of Nrf2 and glutathione-related enzymes in HepG2 cells.

Guan, S. P., et al. (2013). "Andrographolide protects against cigarette smoke-induced oxidative lung injury via augmentation of Nrf2 activity." Br J Pharmacol **168**(7): 1707-1718.

Guo, H., et al. (2013). "Resveratrol protects HUVECs from oxidized-LDL induced oxidative damage by autophagy upregulation via the AMPK/SIRT1 pathway." Cardiovasc Drugs Ther **27**(3): 189-198.

PURPOSE: Resveratrol could induce basal autophagy through the activation of sirtuin. In this study, we investigated the effect of resveratrol on oxidative injury of human umbilical endothelial vein cells (HUVECs) induced by oxidized low-density lipoprotein (ox-LDL) and the role of autophagy in this effect. METHODS: HUVECs were exposed to 100 mg/L ox-LDL for 24 h to cause oxidative injury. The effect of different concentrations of resveratrol on oxidative damage in HUVECs treated with ox-LDL was evaluated by MTT assay and superoxide dismutase (SOD) activity test. The autophagic level in different groups was measured by the protein expression of microtubule-associated protein 1 light chain 3 (LC3) and sequestosome 1 (SQSTM1/P62). Autophagosomes were observed under electron microscope and fluorescence microscope (by MDC staining). The expression of silencing information regulator1 (Sirt1) and AMP activated protein kinasealpha1 (AMPK) was investigated by Western blot. Autophagy inhibitor 3-methyladenine (3-MA) and Sirt1 inhibitor 6-Chloro-2,3,4,9-tetrahydro-1H-Carbazole-1-carboxamide (EX527) were used to confirm the role of autophagy in this effect of resveratrol and the pathway involved. RESULTS: Resveratrol reversed the decreases in cell viability (72.9 +/- 1.7 % of the control group) and SOD activity (14.37 +/- 0.21 U/ml) caused by ox-LDL at 83.4 +/- 1.4 % of the control group and 16.41 +/- 0.27 U/ml respectively. This effect accompanied by upregulation of autophagy and increased protein expression of Sirt1 and AMPK phosphorylation on threonine 172 (p-AMPK). Both 3-MA and EX527 abolished the protective effect of resveratrol in cell viability, at 80.4 +/- 2.7 % and 73.9 +/- 1.1 % of the control group respectively. 3-MA inhibited autophagy activation without any change of Sirt1 expression at both the mRNA and protein level. EX527 suppressed the expression of Sirt1 and diminished the upregulation of autophagy. Addition of 3-MA or EX527 could not affect the protein level of p-AMPK. CONCLUSION: Resveratrol protected HUVECs from oxidative damage caused by ox-LDL. This effect was mediated by Sirt1-dependent autophagy via the AMPK/ Sirt1 pathway.

Han, J., et al. (2014). "(-)-Epigallocatechin gallate protects against cerebral ischemia-induced oxidative stress via Nrf2/ARE signaling." Neurochem Res **39**(7): 1292-1299.

Han, S. G., et al. (2012). "EGCG protects endothelial cells against PCB 126-induced inflammation through inhibition of AhR and induction of Nrf2-regulated genes." Toxicol Appl Pharmacol **261**(2): 181-188.

Hanneken, A., et al. (2006). "Flavonoids protect human retinal pigment epithelial cells from oxidative-stress-induced death." Invest Ophthalmol Vis Sci **47**(7): 3164-3177.

Howitz, K. T., et al. (2003). "Small molecule activators of sirtuins extend Saccharomyces cerevisiae lifespan." Nature **425**(6954): 191-196.

In diverse organisms, calorie restriction slows the pace of ageing and increases maximum lifespan. In the budding yeast Saccharomyces cerevisiae, calorie restriction extends lifespan by increasing the activity of Sir2 (ref. 1), a member of the conserved sirtuin family of NAD(+)-dependent protein deacetylases. Included in this family are SIR-2.1, a Caenorhabditis elegans enzyme that regulates lifespan, and SIRT1, a human deacetylase that promotes cell survival by negatively regulating the p53 tumour suppressor. Here we report the discovery of three classes of small molecules that activate sirtuins. We show that the potent activator resveratrol, a polyphenol found in red wine, lowers the Michaelis constant of SIRT1 for both the acetylated substrate and NAD(+), and increases cell survival by stimulating SIRT1-dependent deacetylation of p53. In yeast, resveratrol mimics calorie restriction by stimulating Sir2, increasing DNA stability and extending lifespan by 70%. We discuss possible evolutionary origins of this phenomenon and suggest new lines of research into the therapeutic use of sirtuin activators.

Huang, C. H., et al. (2009). "EGCG inhibits protein synthesis, lipogenesis, and cell cycle progression through activation of AMPK in p53 positive and negative human hepatoma cells." Mol Nutr Food Res **53**(9): 1156-1165.

In the previous studies, (-)-epigallocatechin-3-gallate (EGCG) has been shown to have anticarcinogenic effects via modulation in protein expression of p53. Using p53 positive Hep G2 and p53 negative Hep 3B cells, we found that treatment of EGCG resulted in dose-dependent inhibition of cellular proliferation, which suggests that the interaction of EGCG with p53 may not fully explain its inhibitory effect on proliferation. Caloric restriction (CR) reduces the incidence and progression of spontaneous and induced tumors in laboratory rodents. EGCG has multiple beneficial activities similar to those associated with CR. One key enzyme thought to be activated during CR is AMP-activated kinase (AMPK), a sensor of cellular energy levels. Here, we showed that EGCG activated AMPK in both p53 positive and negative human hepatoma cells. The activation of AMPK suppressed downstream substrates, such as mammalian target of rapamycin (mTOR) and eukaryotic initiation factor 4E-binding protein-1 (4E-BP1) and a general decrease in mRNA translation. Moreover, EGCG activated AMPK decreases the activity and/or expression of lipogenic enzymes, such as fatty acid synthase (FASN) and acetyl-CoA carboxylase (ACC). Interestingly, the decision between apoptosis and growth arrest following AMPK activation is greatly influenced by p53 status. In p53 positive Hep G2 cells, EGCG blocked the progression of cell cycle at G1 phase by inducing p53 expression and further up-regulating p21 expression. However, EGCG inducted apoptosis in p53 negative Hep 3B cells. Based on these results, we have demonstrated that EGCG has a potential to be a chemoprevention and anti-

lipogenesis agent for human hepatoma cells.

Hubbard, B. P., et al. (2013). "Evidence for a common mechanism of SIRT1 regulation by allosteric activators." Science **339**(6124): 1216-1219.

A molecule that treats multiple age-related diseases would have a major impact on global health and economics. The SIRT1 deacetylase has drawn attention in this regard as a target for drug design. Yet controversy exists around the mechanism of sirtuin-activating compounds (STACs). We found that specific hydrophobic motifs found in SIRT1 substrates such as PGC-1alpha and FOXO3a facilitate SIRT1 activation by STACs. A single amino acid in SIRT1, Glu(230), located in a structured N-terminal domain, was critical for activation by all previously reported STAC scaffolds and a new class of chemically distinct activators. In primary cells reconstituted with activation-defective SIRT1, the metabolic effects of STACs were blocked. Thus, SIRT1 can be directly activated through an allosteric mechanism common to chemically diverse STACs.

Huerta-Olvera, S. G., et al. (2010). "Alpha-lipoic acid regulates heme oxygenase gene expression and nuclear Nrf2 activation as a mechanism of protection against arsenic exposure in HepG2 cells." Environ Toxicol Pharmacol **29**(2): 144-149.

Oxidative stress is a known mechanism induced, among other things, by arsenic toxicity. As a response, the cell triggers the synthesis of antioxidant and stress response elements like glutathione and heme oxygenase. Alpha-lipoic acid (ALA) is a well-known antioxidant that confers protection to oxidative stress conditions. We analyzed the effect of ALA pretreatment on Nrf2-responsive gene expression of HepG2 cells exposed to As(3+). Cells were treated with 5mM ALA and 8h later exposed to 50muM As(3+) for 24h, analyzing MTT-activity, glutathione content, Nrf2 induction and antioxidant gene expression. As(3+) increased glutathione (154%), heme oxygenase, glutamate cystein ligase, modifier subunit and metallothionein (35-fold, 10-fold and 9-fold, respectively). ALA prevented the strong expression of heme oxygenase by As(3+) exposure (from 35- to 5-times of control cells), which correlated with the reduction of Nrf2 observed in As(3+) group. ALA pretreatment can down-modulate the response mediated by Nrf2 and provide protection to As(3+) exposed HepG2 cells.

Hwang, J. T., et al. (2008). "Resveratrol protects ROS-induced cell death by activating AMPK in H9c2 cardiac muscle cells." Genes Nutr **2**(4): 323-326.

Resveratrol, one of polyphenols derived from red wine, has been shown to protect against cell death, possibly through the association with several signaling pathways. Currently numerous studies indicate that cardiovascular diseases are linked to the release of intracellular reactive oxygen species (ROS) often generated in states such as ischemia/reperfusion injury. In the present study, we investigated whether resveratrol has the capability to control intracellular survival signaling cascades involving AMP-activated kinase (AMPK) in the inhibitory process of cardiac injury. We hypothesized that resveratrol may exert a protective effect on damage to heart muscle through modulating of the AMPK signaling pathway. We mimicked ischemic conditions by inducing cell death with H(2)O(2) in H9c2 muscle cells. In this experiment, resveratrol induced strong activation of AMPK and inhibited the occurrence of cell death caused by treatment with H(2)O(2). Under the same conditions, inhibition of AMPK using dominant negative AMPK constructs dramatically abolished the effect of resveratrol on cell survival in H(2)O(2)-treated cardiac muscle cells. These results indicate that resveratrol-induced cell survival is mediated by AMPK in H9c2 cells and may exert a novel therapeutic effect on oxidative stress induced in cardiac disorders.

Ito-Nagahata, T., et al. (2013). "Stilbene analogs of resveratrol improve insulin resistance through activation of AMPK." Biosci Biotechnol Biochem **77**(6): 1229-1235.

Resveratrol (RSV), 3,5,4'-trihydroxy-trans-stilbene, is known to have many beneficial physiological activities. We have synthesized several stilbene analogues and have reported that the hydroxyl group in the 4' position of RSV exhibited strong radical scavenging action. Using stilbene analogs, we investigated the structure of RSV to explain its protective effect against obesity and type 2 diabetes. All six analogs used in this study inhibited the differentiation of 3T3-L1 adipocytes. 3-Hydroxy-trans stilbene (3(OH)ST), and 3,4'-dihydroxy-trans stilbene (3,4'(OH)2ST) increased glucose uptake and induced adenosine monophosphate kinase (AMPK) phosphorylation in C2C12 myotubes independently of insulin. An in vivo study using mice fed high-fat diets indicated that 3(OH)ST was more effective than RSV in improving insulin resistance. In conclusion, RSV and its derivatives, particularly 3(OH)ST, inhibited adipocyte differentiation and enhanced glucose uptake in the myotubes, resulting in a reduction of obesity and an improvement in glucose tolerance in vivo.

Jeong, H. W., et al. (2009). "Berberine suppresses proinflammatory responses through AMPK activation in macrophages." Am J Physiol Endocrinol Metab **296**(4): E955-964.

Berberine (BBR) has been shown to improve several metabolic disorders, such as obesity, type 2 diabetes, and dyslipidemia, by stimulating AMP-activated protein kinase (AMPK). However, the effects of BBR on proinflammatory responses in macrophages are poorly understood. Here we show that BBR represses

proinflammatory responses through AMPK activation in macrophages. In adipose tissue of obese db/db mice, BBR treatment significantly downregulated the expression of proinflammatory genes such as TNF-alpha, IL-1beta, IL-6, monocyte chemoattractant protein-1 (MCP-1), inducible nitric oxide synthase (iNOS), and cyclooxygenase-2 (COX-2). Consistently, BBR inhibited LPS-induced expression of proinflammatory genes including IL-1beta, IL-6, iNOS, MCP-1, COX-2, and matrix metalloprotease-9 in peritoneal macrophages and RAW 264.7 cells. Upon various proinflammatory signals including LPS, free fatty acids, and hydrogen peroxide, BBR suppressed the phosphorylation of MAPKs, such as p38, ERK, and JNK, and the level of reactive oxygen species in macrophages. Moreover, these inhibitory effects of BBR on proinflammatory responses were abolished by AMPK inhibition via either compound C, an AMPK inhibitor, or dominant-negative AMPK, implying that BBR would downregulate proinflammatory responses in macrophages via AMPK stimulation.

Jia, L., et al. (2007). "Acrolein, a toxicant in cigarette smoke, causes oxidative damage and mitochondrial dysfunction in RPE cells: protection by (R)-alpha-lipoic acid." Invest Ophthalmol Vis Sci **48**(1): 339-348.

PURPOSE: To understand better the cell and molecular basis for the epidemiologic association between cigarette smoke, oxidant injury, and age-associated macular degeneration, the authors examined the effects of acrolein, a major toxicant in cigarette smoke, on oxidative mitochondrial damage in retinal pigment epithelial (RPE) cells and the reduction of this damage by lipoic acid. METHODS: Cultured human ARPE19 cells and primary cultures of human fetal (hf)RPE were treated with acrolein. The toxicity of acrolein and the protective effects of R-alpha-lipoic acid were examined with a variety of previously described techniques. RESULTS: Acute acrolein exposure exceeding 50 microM (24 hours) in ARPR19 cells caused toxicity, including decreases in cell viability, mitochondrial potential, GSH, antioxidant capacity, Nrf2 expression, enzyme activity (mitochondrial complexes I, II, III; superoxide dismutase; and glutathione peroxidase). Acute exposure also increased oxidant levels, protein carbonyls, and calcium. Continuous acrolein exposure over 8 or 32 days caused similar toxicity but from 10- to 100-fold lower doses (0.1-5 microM). Pretreatment with R-alpha-lipoic acid effectively protected ARPE-19 cells from acrolein toxicity. Primary hfRPE cells were comparable to the ARPE-19 cells in sensitivity to acrolein toxicity and lipoic acid protection. CONCLUSIONS: These results show that acrolein is a mitochondrial toxicant in RPE cells and that acrolein-induced oxidative mitochondrial dysfunction is reduced by lipoic acid. The similar sensitivity of the ARPE-19 and hfRPE cells suggests that both models are useful for studying RPE toxicity and protection. These experiments indicate that mitochondria-targeted antioxidants such as lipoic acid may be an effective strategy for reducing or preventing chronic oxidant-induced RPE degeneration in vivo from a variety of sources, including cigarette smoke.

Jiang, H., et al. (2009). "Resveratrol downregulates PI3K/Akt/mTOR signaling pathways in human U251 glioma cells." J Exp Ther Oncol **8**(1): 25-33.

Resveratrol (trans-3,4', 5-trihydroxystilbene) is a naturally occurring polyphenolic compound that has antiinflammatory, antioxidant, neuroprotective properties and acts as a chemopreventive agent. Resveratrol causes cell cycle arrest and induces apoptotic cell death in various types of cancer cells. In the current studies, the effect of resveratrol on phosphoinositide kinase-3 (PI3K)/protein kinase B (Akt)/mammalian target of rapamycin (mTOR) signaling pathway was examined in human U251 glioma cells. Resveratrol decreased both the expression and phosphorylation of Akt. Inhibitors of PI3K (LY294002) and Akt (SH-6) enhanced resveratrol-induced LDH release and caspase-3 activation. Resveratrol reduced phosphorylation of ribosomal protein S6 and the mTOR inhibitor rapamycin further enhanced resveratrol-induced cell death. These results suggest that the downregulation of PI3K/Akt/mTOR signaling pathways may be an important mediator in resveratrol-induced apoptosis in glioma cells.

Jiang, J., et al. (2012). "Epigallocatechin-3-gallate prevents TNF-alpha-induced NF-kappaB activation thereby upregulating ABCA1 via the Nrf2/Keap1 pathway in macrophage foam cells." Int J Mol Med **29**(5): 946-956.

Kang, C. H., et al. (2013). "Quercetin inhibits lipopolysaccharide-induced nitric oxide production in BV2 microglial cells by suppressing the NF-kappaB pathway and activating the Nrf2-dependent HO-1 pathway." Int Immunopharmacol **17**(3): 808-813.

Abnormal nitrosative stress-induced neuroinflammation is implicated in the pathogenesis of neurodegenerative diseases. Therefore, it has been thought that nitric oxide (NO) production is a good therapeutic target. In this sense, quercetin is a good chemopreventive component, because it has free radical-scavenging and anti-inflammatory activities. However, explicit mechanisms are not clear in the lipopolysaccharide (LPS)-stimulated BV2 microglial cell line. Here, we found that quercetin significantly suppressed LPS-induced NO production and inducible NO synthase (iNOS) expression. Notably, quercetin inhibited nuclear factor-kappaB (NF-kappaB) activation by inhibiting degradation of the inhibitor of kappa Balpha (IkappaBalpha) in LPS-stimulated BV2 microglial cells corresponding to the inhibitory effect of specific NF-kappaB inhibitors, namely proteasome inhibitor I (PSI) and MG132. Quercetin caused significant increases in the levels of heme oxgenase-1 (HO-1) mRNA and protein. Notably, treatment with an HO-1 inducer, cobalt protoporphyrin (CoPP), significantly diminished LPS-

stimulated NO production. Additionally, quercetin induced the specific DNA-binding activity of nuclear factor-2-erythroid 2-related factor 2 (Nrf2), and siRNA-mediated knockdown of Nrf2 expression reduced the inhibitory effect of quercetin on LPS-stimulated NO production by inhibiting HO-1 expression, indicating that quercetin regulated NO production by inducing Nrf2-mediated HO-1 expression. Therefore, quercetin has the potential to decrease nitrosative stress by suppressing NF-kappaB activation and inducing Nrf2-mediated HO-1 expression.

Kang, S. I., et al. (2012). "Petalonia binghamiae extract and its constituent fucoxanthin ameliorate high-fat diet-induced obesity by activating AMP-activated protein kinase." J Agric Food Chem **60**(13): 3389-3395.

In this study, we investigated the antiobesity properties of Petalonia binghamiae extract (PBE) in mice in which obesity was induced with a high-fat diet (HFD). PBE administration (150 mg/kg/day) for 70 days decreased body weight gain, adipose tissue weight, and the serum triglyceride level in mice fed a HFD. PBE reduced serum levels of glutamic pyruvic transaminase and glutamic oxaloacetic transaminase as well as the accumulation of lipid droplets in the liver. PBE restored the HFD-induced decrease in phosphorylation of AMP-activated protein kinase (AMPK) and acetyl-CoA carboxylase (ACC) in epididymal adipose tissue. PBE increased the phosphorylation of AMPK and ACC and decreased the expression of SREBP1c in mature 3T3-L1 adipocytes. In addition, we further explored the active compound responsible for AMPK activation by PBE in 3T3-L1 adipocytes. Fucoxanthin isolated from PBE increased the phosphorylation of AMPK and ACC with increasing LKB1 phosphorylation in mature 3T3-L1 adipocytes. Taken together, these data suggest that PBE (or fucoxanthin) exert improving effects on HFD-induced obesity by promoting beta-oxidation and reducing lipogenesis.

Kim, M. S., et al. (2004). "Anti-obesity effects of alpha-lipoic acid mediated by suppression of hypothalamic AMP-activated protein kinase." Nat Med **10**(7): 727-733.

AMP-activated protein kinase (AMPK) functions as a fuel sensor in the cell and is activated when cellular energy is depleted. Here we report that alpha-lipoic acid (alpha-LA), a cofactor of mitochondrial enzymes, decreases hypothalamic AMPK activity and causes profound weight loss in rodents by reducing food intake and enhancing energy expenditure. Activation of hypothalamic AMPK reverses the effects of alpha-LA on food intake and energy expenditure. Intracerebroventricular (i.c.v.) administration of glucose decreases hypothalamic AMPK activity, whereas inhibition of intracellular glucose utilization through the administration of 2-deoxyglucose increases hypothalamic AMPK activity and food intake. The 2-deoxyglucose-induced hyperphagia is reversed by inhibiting hypothalamic AMPK. Our findings indicate that hypothalamic AMPK is important in the central regulation of food intake and energy expenditure and that alpha-LA exerts anti-obesity effects by suppressing hypothalamic AMPK activity.

Kim, W. S., et al. (2009). "Berberine improves lipid dysregulation in obesity by controlling central and peripheral AMPK activity." Am J Physiol Endocrinol Metab **296**(4): E812-819.

AMP-activated protein kinase (AMPK) plays an important role in regulating whole body energy homeostasis. Recently, it has been demonstrated that berberine (BBR) exerts antiobesity and antidiabetic effects in obese and diabetic rodent models through the activation of AMPK in peripheral tissues. Here we show that BBR improves lipid dysregulation and fatty liver in obese mice through central and peripheral actions. In obese db/db and ob/ob mice, BBR treatment reduced liver weight, hepatic and plasma triglyceride, and cholesterol contents. In the liver and muscle of db/db mice, BBR promoted AMPK activity and fatty acid oxidation and changed expression of genes involved in lipid metabolism. Additionally, intracerebroventricular administration of BBR decreased the level of malonyl-CoA and stimulated the expression of fatty acid oxidation genes in skeletal muscle. Together, these data suggest that BBR would improve fatty liver in obese subjects, which is probably mediated not only by peripheral AMPK activation but also by neural signaling from the central nervous system.

Kim, Y. S., et al. (2013). "Cytoprotective effect of alpha-lipoic acid on paraquat-exposed human bronchial epithelial cells via activation of nuclear factor erythroid related factor-2 pathway." Biol Pharm Bull **36**(5): 802-811.

Alpha-lipoic acid (LA), a metabolic antioxidant, is a natural compound and its biological function has been well studied in various human diseases. The present study was designed to investigate the cytoprotective effect and the molecular mechanisms of LA in paraquat (PQ)-induced oxidative stress injury using BEAS-2B human bronchial epithelial cells. LA co-treatment prevented PQ-induced BEAS-2B cell death. LA also prevented PQ-induced increases in total reactive oxygen species (ROS), lactate dehydrogenase (LDH) and malondialdehyde (MDA). LA also increased the expression of detoxifying phase II enzyme encoding genes and antioxidant genes including HO-1, NQO1, CAT, GPX3 and GPX4, resulting in the attenuation of the decreases of antioxidants during PQ-induced oxidative stress. Nuclear factor erythroid related factor 2 (Nrf2) was induced by LA. Additionally, translocation of Nrf2 from the cytoplasm to the nucleus was promoted by LA treatment. While LA was responsible for the upregulation of Nrf2, it also activated and up-regulated the downstream proteins heme oxygenase-1 (HO-1) and reduced nicotinamide adenine dinucleotide phosphate (NAD(P)H) quinone oxidoreductase 1 (NQO1). The data collectively suggest that the beneficial effect of LA involving the activation of cytoprotective antioxidant genes make

LA a potential candidate in the prevention of PQ-induced oxidative stress-related bronchial cell death, pending clinically relevant studies.

Kluth, D., et al. (2007). "Modulation of pregnane X receptor- and electrophile responsive element-mediated gene expression by dietary polyphenolic compounds." Free Radic Biol Med **42**(3): 315-325.

Kong, D., et al. (2008). "Mammalian target of rapamycin repression by 3,3'-diindolylmethane inhibits invasion and angiogenesis in platelet-derived growth factor-D-overexpressing PC3 cells." Cancer Res **68**(6): 1927-1934.

Platelet-derived growth factor-D (PDGF-D) is a newly recognized growth factor known to regulate many cellular processes, including cell proliferation, transformation, invasion, and angiogenesis. Recent studies have shown that PDGF-D and its cognate receptor PDGFR-beta are expressed in prostate tumor tissues, suggesting that PDGF-D might play an important role in the development and progression of prostate cancer. However, the biological role of PDGF-D in tumorigenesis remains elusive. In this study, we found that PDGF-D-overexpressing PC3 cells (PC3 cells stably transfected with PDGF-D cDNA and referred to as PC3 PDGF-D) exhibited a rapid growth rate and enhanced cell invasion that was associated with the activation of mammalian target of rapamycin (mTOR) and reduced Akt activity. Rapamycin repressed mTOR activity and concomitantly resulted in the activation of Akt, which could attenuate the therapeutic effects of mTOR inhibitors. In contrast, B-DIM (BR-DIM from Bioresponse, Inc.; a chemopreventive agent) significantly inhibited both mTOR and Akt in PC3 PDGF-D cells, which were correlated with decreased cell proliferation and invasion. Moreover, conditioned medium from PC3 PDGF-D cells significantly increased the tube formation of human umbilical vein endothelial cells, which was inhibited by B-DIM treatment concomitant with reduced full-length and active form of PDGF-D. Our results suggest that B-DIM could serve as a novel and efficient chemopreventive and/or therapeutic agent by inactivation of both mTOR and Akt activity in PDGF-D-overexpressing prostate cancer.

Koriyama, Y., et al. (2013). "Protective effect of lipoic acid against oxidative stress is mediated by Keap1/Nrf2-dependent heme oxygenase-1 induction in the RGC-5 cellline." Brain Res **1499**: 145-157.

Krajka-Kuzniak, V., et al. (2014). "Hawthorn (Crataegus oxyacantha L.) bark extract regulates antioxidant response element (ARE)-mediated enzyme expression via Nrf2 pathway activation in normal hepatocyte cell line." Phytother Res **28**(4): 593-602.

Hawthorn (Crataegus oxyacantha L.), a plant used in traditional medicine, is a rich source of procyanidins which have been reported to exhibit antioxidant and anti-carcinogenic activity. In this study, we assessed the effect of hawthorn bark extract (HBE) on Nrf2 pathway activation in THLE-2 and HepG2 cells. Treatment with 1.1 microg/mL, 5.5 microg/mL and 11 microg/mL of HBE resulted in the translocation of Nrf2 from the cytosol to the nucleus in both cell lines; however, the accumulation of phosphorylated Nrf2 was observed only in THLE-2. Accordingly, treatment of cells with HBE was associated with an increase in the mRNA and protein level of such Nrf2-dependent genes as glutathione S-transferases (GSTA, GSTP, GSTM, GSTT), NAD(P)H:quinone oxidoreductase 1 (NQO1) and heme oxygenase-1 (HO-1) (0.2-1.1-fold change, $p < 0.05$), however, only in normal THLE-2 hepatocytes. The induction of NQO1 correlated with an increased level of p53 (0.21-0.42-fold change, $p < 0.05$). These effects may be related to induction of phosphorylation of upstream ERK and JNK kinases. Collectively, the results suggest that the Nrf2/ARE pathway may play an important role in the regulation of procyanidin-mediated antioxidant/detoxifying effects in hepatocytes, and this may explain the hepatoprotective and chemopreventive properties of these phytochemicals.

Lee, J. C., et al. (2014). "Andrographolide exerts anti-hepatitis C virus activity by up-regulating haeme oxygenase-1 via the p38 MAPK/Nrf2 pathway in human hepatoma cells." Br J Pharmacol **171**(1): 237-252.

Lee, W. J., et al. (2005). "Alpha-lipoic acid prevents endothelial dysfunction in obese rats via activation of AMP-activated protein kinase." Arterioscler Thromb Vasc Biol **25**(12): 2488-2494.

OBJECTIVE: Lipid accumulation in vascular endothelial cells may play an important role in the pathogenesis of atherosclerosis in obese subjects. We showed previously that alpha-lipoic acid (ALA) activates AMP-activated protein kinase (AMPK) and reduces lipid accumulation in skeletal muscle of obese rats. Here, we investigated whether ALA improves endothelial dysfunction in obese rats by activating AMPK in endothelial cells. METHODS AND RESULTS: Endothelium-dependent vascular relaxation was impaired, and the number of apoptotic endothelial cells was higher in the aorta of obese rats compared with control rats. In addition, triglyceride and lipid peroxide levels were higher, and NO synthesis was lower. Administration of ALA improved all of these abnormalities. AMPK activity was lower in aortic endothelium of obese rats, and ALA normalized it. Incubation of human aortic endothelial cells with ALA activated AMPK and protected cells from linoleic acid-induced apoptosis. Dominant-negative AMPK inhibited the antiapoptotic effects of ALA. CONCLUSIONS: Reduced AMPK activation may play an important role in the genesis of endothelial dysfunction in obese rats. ALA improves vascular

dysfunction by normalizing lipid metabolism and activating AMPK in endothelial cells.

Lee, W. J., et al. (2005). "Alpha-lipoic acid increases insulin sensitivity by activating AMPK in skeletal muscle." Biochem Biophys Res Commun **332**(3): 885-891.

Triglyceride accumulation in skeletal muscle contributes to insulin resistance in obesity. We recently showed that alpha-lipoic acid (ALA) reduces body weight and prevents the development of diabetes in diabetes-prone obese rats by reducing triglyceride accumulation in non-adipose tissues. AMP-activated protein kinase (AMPK) is a major regulator of cellular energy metabolism. We examined whether ALA lowers triglyceride accumulation in skeletal muscle by activating AMPK. Alpha2-AMPK activity was decreased in obese rats compared to control rats. Administration of ALA to obese rats increased insulin-stimulated glucose disposal in whole body and in skeletal muscle. ALA also increased fatty acid oxidation and activated AMPK in skeletal muscle. Adenovirus-mediated administration of dominant negative AMPK into skeletal muscle prevented the ALA-induced increases in fatty acid oxidation and insulin-stimulated glucose uptake. These results suggest that ALA-induced improvement of insulin sensitivity is mediated by activation of AMPK and reduced triglyceride accumulation in skeletal muscle.

Lee, Y. S., et al. (2006). "Berberine, a natural plant product, activates AMP-activated protein kinase with beneficial metabolic effects in diabetic and insulin-resistant states." Diabetes **55**(8): 2256-2264.

Berberine has been shown to have antidiabetic properties, although its mode of action is not known. Here, we have investigated the metabolic effects of berberine in two animal models of insulin resistance and in insulin-responsive cell lines. Berberine reduced body weight and caused a significant improvement in glucose tolerance without altering food intake in db/db mice. Similarly, berberine reduced body weight and plasma triglycerides and improved insulin action in high-fat-fed Wistar rats. Berberine downregulated the expression of genes involved in lipogenesis and upregulated those involved in energy expenditure in adipose tissue and muscle. Berberine treatment resulted in increased AMP-activated protein kinase (AMPK) activity in 3T3-L1 adipocytes and L6 myotubes, increased GLUT4 translocation in L6 cells in a phosphatidylinositol 3' kinase-independent manner, and reduced lipid accumulation in 3T3-L1 adipocytes. These findings suggest that berberine displays beneficial effects in the treatment of diabetes and obesity at least in part via stimulation of AMPK activity.

Lian, F. and X. D. Wang (2008). "Enzymatic metabolites of lycopene induce Nrf2-mediated expression of phase II detoxifying/antioxidant enzymes in human bronchial epithelial cells." Int J Cancer **123**(6): 1262-1268.

Liang, K. W., et al. (2008). "Berberine inhibits platelet-derived growth factor-induced growth and migration partly through an AMPK-dependent pathway in vascular smooth muscle cells." Eur J Pharmacol **590**(1-3): 343-354.

Platelet-derived growth factor (PDGF) is released from vascular smooth muscle cells (VSMCs), endothelial cells, or macrophages after percutaneous coronary intervention and is related with neointimal proliferation and restenosis. Berberine is a well-known component of the Chinese herb medicine Huanglian (Coptis chinensis), and is capable of inhibiting growth and endogenous PDGF synthesis in VSMCs after in vitro mechanical injury. We analyzed the effects of berberine on VSMC growth, migration, and signaling events after exogenous PDGF stimulation in vitro in order to mimic a post-angioplasty PDGF shedding condition. Pretreatment of VSMCs with berberine inhibited PDGF-induced proliferation. Berberine significantly suppressed PDGF-stimulated Cyclin D1/D3 and Cyclin-dependent kinase (Cdk) gene expression. Moreover, berberine increased the activity of AMP-activated protein kinase (AMPK), which led to phosphorylation activation of p53 and increased protein levels of the Cdk inhibitor p21(Cip1). Compound C, an AMPK inhibitor, partly but significantly attenuated berberine-elicited growth inhibition. In addition, stimulation of VSMCs with PDGF led to a transient increase in GTP-bound, active form of Ras, Cdc42 and Rac1, as well as VSMC migration. However, pretreatment with berberine significantly inhibited PDGF-induced Ras, Cdc42 and Rac1 activation and cell migration. Co-treatment with farnesyl pyrophosphate and geranylgeranyl pyrophosphate drastically reversed berberine-mediated anti-proliferative and migratory effects in VSMCs. Based on these findings, we conclude that berberine inhibited PDGF-induced VSMC growth via activation of AMPK/p53/p21(Cip1) signaling while inactivating Ras/Rac1/Cyclin D/Cdks and suppressing PDGF-stimulated migration via inhibition of Rac1 and Cdc42. These observations offer a molecular explanation for the anti-proliferative and anti-migratory properties of berberine.

Liang, L., et al. (2013). "Dihydroquercetin (DHQ) induced HO-1 and NQO1 expression against oxidative stress through the Nrf2-dependent antioxidant pathway." J Agric Food Chem **61**(11): 2755-2761.

Dihydroquercetin (DHQ) is a well-known antioxidant agent. In the present investigation, we reported for the first time that DHQ stimulates the expression of phase II detoxifying enzymes through the Nrf2-dependent signaling pathway. The IC50 values of DHQ for reduction of 2,2-diphenyl-1-picrylhydrazol (DPPH), reducing power assay, lipid peroxidation assay, and xanthine oxidase inhibition were 5.96, 4.31, 2.03, and 13.24 muM, respectively. DHQ possessed considerable protective activity from oxidative DNA damage. A luciferase reporter assay also demonstrated that DHQ-activated signaling resulted in the increased transcriptional activity of Nrf2 through binding

to the ARE (antioxidant response element) enhancer sequence. Furthermore, Western blotting and luciferase assay revealed DHQ activated ERK1/2, Akt, and JNK signaling pathways, subsequently leading to Nrf2 nuclear translocation. DHQ upregulated the Nrf2-related antioxidant genes heme oxygenase-1 (HO-1), NAD(P)H quinone oxidoreductase-1 (NQO1), and glutamate-cysteine ligase modifier subunits. Inhibition of Nrf2 by siRNA reduced DHQ-induced upregulation of these antioxidant genes.

Lii, C. K., et al. (2010). "Sulforaphane and alpha-lipoic acid upregulate the expression of the pi class of glutathione S-transferase through c-jun and Nrf2 activation." J Nutr **140**(5): 885-892.

The anticarcinogenic effect of dietary organosulfur compounds has been partly attributed to their modulation of the activity and expression of phase II detoxification enzymes. Our previous studies indicated that garlic allyl sulfides upregulate the expression of the pi class of glutathione S-transferase (GSTP) through the activator protein-1 pathway. Here, we examined the modulatory effect of sulforaphane (SFN) and alpha-lipoic acid (LA) or dihydrolipoic acid (DHLA) on GSTP expression in rat Clone 9 liver cells. Cells were treated with LA or DHLA (50-600 micromol/L) or SFN (0.2-5 micromol/L) for 24 h. Immunoblots and real-time PCR showed that SFN, LA, and DHLA dose dependently induced GSTP protein and mRNA expression. Compared with the induction by the garlic organosulfur compound diallyl trisulfide (DATS), the effectiveness was in the order of SFN > DATS > LA = DHLA. The increase in GSTP enzyme activity in cells treated with 5 micromol/L SFN, 50 micromol/L DATS, and 600 micromol/L LA and DHLA was 172, 75, 122, and 117%, respectively ($P < 0.05$). A reporter assay showed that the GSTP enhancer I (GPEI) was required for GSTP induction by the organosulfur compounds. Electromobility gel shift assays showed that the DNA binding of GPEI to nuclear proteins reached a maximum at 0.5-1 h after SFN, LA, and DHLA treatment. Super-shift assay revealed that the transcription factors c-jun and nuclear factor erythroid-2 related factor 2 (Nrf2) were bound to GPEI. These results suggest that SFN and LA in either its oxidized or reduced form upregulate the transcription of the GSTP gene by activating c-jun and Nrf2 binding to the enhancer element GPEI.

Lin, Y. C., et al. (2013). "The protective effect of alpha-lipoic Acid in lipopolysaccharide-induced acute lung injury is mediated by heme oxygenase-1." Evid Based Complement Alternat Med **2013**: 590363.

Linnewiel, K., et al. (2009). "Structure activity relationship of carotenoid derivatives in activation of the electrophile/antioxidant response element transcription system." Free Radic Biol Med **47**(5): 659-667.

Liu, P. L., et al. (2014). "Epigallocatechin gallate attenuates proliferation and oxidative stress in human vascular smooth muscle cells induced by interleukin-1beta via heme oxygenase-1." Mediators Inflamm **2014**: 523684.

Liu, S., et al. (2012). "Heme oxygenase-1 mediates the protective role of quercetin against ethanol-induced rat hepatocytes oxidative damage." Toxicol In Vitro **26**(1): 74-80.

Quercetin, one of the most widely distributed flavonoids in plants, possesses strong free radical scavenging ability and potent hepatoprotective effects. However, the protective effect and mechanism of quercetin on ethanol-induced oxidative damage in hepatocytes remain unclear. In this study, primary rat hepatocytes were incubated with ethanol and quercetin in the presence or absence of ZnPP 9, an antagonist of HO-1 induction. The ethanol-induced hepatotoxicity was found to be greatly diminished by pre-treatment of quercetin and this hepatoprotective effect could be partly blocked by ZnPP 9. This study also showed that quercetin significantly stimulated HO-1 expression at both mRNA and protein levels, then subsequently induced HO-1 activity. To further study the signaling pathways underlying quercetin-induced HO-1 up-regulation, HO-1 expression and activity in cytosolic microsomal fractions and Nrf2 expression in nuclear fractions were analyzed following quercetin or/and MAPK inhibitor(s) as well as PI3K inhibitor incubation for primary rat hepatocytes. These results indicated that ERK was required to induce HO-1 expression in rat hepatocytes. In summary, these data suggested that quercetin attenuates ethanol-induced oxidative stress through a pathway which involves ERK activation and HO-1 upregulation.

Liu, Z., et al. (2007). "Hydroxytyrosol protects retinal pigment epithelial cells from acrolein-induced oxidative stress and mitochondrial dysfunction." J Neurochem **103**(6): 2690-2700.

Hydroxytyrosol (HTS) is a natural polyphenol abundant in olive oil. Increasing evidence indicates HTS has beneficial effect on human health for preventing various diseases. In the present study, we investigated the protective effects of HTS on acrolein-induced toxicity in human retinal pigment epithelial cell line, ARPE-19, a cellular model of smoking- and age-related macular degeneration. Acrolein, a major component of the gas phase cigarette smoke and also a product of lipid peroxidation in vivo, at 75 mumol/L for 24 h caused significant loss of cell viability, oxidative damage (increase in oxidant generation and oxidative damage to proteins and DNA, decrease in antioxidants and antioxidant enzymes, and also inactivation of the Keap1/Nrf2 pathway), and mitochondrial dysfunction (decrease in membrane potential, activities of mitochondrial complexes, viable

mitochondria, oxygen consumption, and factors for mitochondrial biogenesis, and increase in calcium). Pre-treatment with HTS dose dependently and also time dependently protected the ARPE-19 cells from acrolein-induced oxidative damage and mitochondrial dysfunction. A short-term pre-treatment with HTS (48 h) required > 75 mumol/L for showing protection while a long-term pre-treatment (7 days) showed protective effect from 5 mumol/L on. The protective effect of HTS in this model was as potent as that of established mitochondria-targeting antioxidant nutrients. These results suggest that HTS is also a mitochondrial-targeting antioxidant nutrient and that dietary administration of HTS may be an effective measure in reducing and or preventing cigarette smoke-induced or age-related retinal pigment epithelial degeneration, such as age-associated macular degeneration.

Lorente-Cebrian, S., et al. (2009). "Eicosapentaenoic acid stimulates AMP-activated protein kinase and increases visfatin secretion in cultured murine adipocytes." Clin Sci (Lond) **117**(6): 243-249.

Visfatin is an adipokine highly expressed in visceral AT (adipose tissue) of humans and rodents, the production of which seems to be dysregulated in excessive fat accumulation and conditions of insulin resistance. EPA (eicosapentaenoic acid), an n-3 PUFA (polyunsaturated fatty acid), has been demonstrated to exert beneficial effects in obesity and insulin resistance conditions, which have been further linked to its reported ability to modulate adipokine production by adipocytes. TNF-alpha (tumour necrosis factor-alpha) is a pro-inflammatory cytokine whose production is increased in obesity and is involved in the development of insulin resistance. Control of adipokine production by some insulin-sensitizing compounds has been associated with the stimulation of AMPK (AMP-activated protein kinase). The aim of the present study was to examine in vitro the effects of EPA on visfatin production and the potential involvement of AMPK both in the absence or presence of TNF-alpha. Treatment with the pro-inflammatory cytokine TNF-alpha (1 ng/ml) did not modify visfatin gene expression and protein secretion in primary cultured rat adipocytes. However, treatment of these primary adipocytes with EPA (200 mumol/l) for 24 h significantly increased visfatin secretion ($P<0.001$) and mRNA gene expression ($P<0.05$). Moreover, the stimulatory effect of EPA on visfatin secretion was prevented by treatment with the AMPK inhibitor Compound C, but not with the PI3K (phosphoinositide 3-kinase) inhibitor LY294002. Similar results were observed in 3T3-L1 adipocytes. Moreover, EPA strongly stimulated AMPK phosphorylation alone or in combination with TNF-alpha in 3T3-L1 adipocytes and pre-adipocytes. The results of the present study suggest that the stimulatory action of EPA on visfatin production involves AMPK activation in adipocytes.

Louhelainen, M., et al. (2006). "Lipoic acid supplementation prevents cyclosporine-induced hypertension and nephrotoxicity in spontaneously hypertensive rats." J Hypertens **24**(5): 947-956.

BACKGROUND: Cyclosporine (CsA) has significantly improved long-term survival after organ transplantations. Hypertension and nephrotoxicity are common side effects during CsA treatment and are aggravated by high salt intake. OBJECTIVE: To examine whether lipoic acid (LA), a natural antioxidant that scavenges reactive oxygen species and regenerates/recycles endogenous antioxidants, could prevent CsA-induced hypertension and nephrotoxicity. METHODS: Six-week-old spontaneously hypertensive rats (SHR) on a high-sodium diet (NaCl 6%) received CsA [5 mg/kg subcutaneously (s.c.)] alone or in combination with LA (0.5% w/w) for 6 weeks. Blood pressure, arterial functions, and tissue morphology were determined. Immunohistochemistry, quantitative reverse transcriptase-polymerase chain reaction (RT-PCR) and high-pressure liquid chromatography were used for kidney and heart samples. RESULTS: CsA induced severe hypertension, cardiac hypertrophy, endothelial dysfunction, and pronounced albuminuria. Histologically, the kidneys showed severe thickening of the media of the afferent arteries with fibrinoid necrosis, perivascular monocyte/macrophage infiltration and nitrotyrosine overexpression. CsA induced the expression of fibrogenic connective tissue growth factor both in the heart and kidneys. The detrimental effects of CsA were associated with upregulation of myocardial atrial natriuretic peptide (ANP) mRNA expression, paradoxical activation of the renin-angiotensin system (RAS), induction of renal reduced nicotinamide-adenine dinucleotide phosphate (NADPH) oxidase, and overexpression of oxidative stress-induced transcription factor NRF2. LA lowered blood pressure, ameliorated cardiac hypertrophy and endothelial dysfunction, and totally normalized albuminuria. In LA-treated rats, renal and cardiac morphologies were indistinguishable from those of SHR controls. CsA-induced myocardial ANP and connective tissue growth factor (CTGF) mRNA overexpression, RAS activation, NADPH oxidase induction, and NRF2 overexpression were prevented by LA. LA induced the mRNA expression of gamma-glutamylcysteine ligase, the rate-limiting enzyme in glutathione synthesis, and markedly increased hepatic cysteine and glutathione concentrations. CONCLUSIONS: Our findings suggest a salutary role for lipoic acid supplementation in the prevention of CsA-induced hypertension and nephrotoxicity, and underscore the importance of increased oxidative stress in the pathogenesis of CsA toxicity.

Lu, C. Y., et al. (2014). "Andrographolide inhibits TNFalpha-induced ICAM-1 expression via suppression of NADPH oxidase activation and induction of HO-1 and GCLM expression through the PI3K/Akt/Nrf2 and PI3K/Akt/AP-1 pathways in human endothelial cells." Biochem Pharmacol **91**(1): 40-50.

Ma, X., et al. (2010). "Berberine-induced activation of 5'-adenosine monophosphate-activated protein kinase and glucose transport in rat skeletal muscles." Metabolism **59**(11): 1619-1627.

Berberine (BBR) is the main alkaloid of Coptis chinensis, which has been used as a folk medicine to treat diabetes mellitus in Asian countries. We explored the possibility that 5'-adenosine monophosphate-activated protein kinase (AMPK) is involved in metabolic enhancement by BBR in skeletal muscle, the important tissue for glucose metabolism. Isolated rat epitrochlearis and soleus muscles were incubated in a buffer containing BBR, and activation of AMPK and related events were examined. In response to BBR treatment, the Thr(172) phosphorylation of the catalytic alpha-subunit of AMPK, an essential step for full kinase activation, increased in a dose- and time-dependent manner. Ser(79) phosphorylation of acetyl-coenzyme A carboxylase, an intracellular substrate of AMPK, increased correspondingly. Analysis of isoform-specific AMPK activity revealed that BBR activated both the alpha1 and alpha2 isoforms of the catalytic subunit. This increase in enzyme activity was associated with an increased rate of 3-O-methyl-d-glucose transport in the absence of insulin and with phosphorylation of AS160, a signaling intermediary leading to glucose transporter 4 translocation. The intracellular energy status estimated from the phosphocreatine concentration was decreased by BBR. These results suggest that BBR acutely stimulates both AMPKalpha1 and AMPKalpha2 and insulin-independent glucose transport in skeletal muscle with a reduction of the intracellular energy status.

Magbanua, M. J., et al. (2011). "Gene expression and biological pathways in tissue of men with prostate cancer in a randomized clinical trial of lycopene and fish oil supplementation." PLoS One **6**(9): e24004.

BACKGROUND: Studies suggest that micronutrients may modify the risk or delay progression of prostate cancer; however, the molecular mechanisms involved are poorly understood. We examined the effects of lycopene and fish oil on prostate gene expression in a double-blind placebo-controlled randomized clinical trial. METHODS: Eighty-four men with low risk prostate cancer were stratified based on self-reported dietary consumption of fish and tomatoes and then randomly assigned to a 3-month intervention of lycopene (n = 29) or fish oil (n = 27) supplementation or placebo (n = 28). Gene expression in morphologically normal prostate tissue was studied at baseline and at 3 months via cDNA microarray analysis. Differential gene expression and pathway analyses were performed to identify genes and pathways modulated by these micronutrients. RESULTS: Global gene expression analysis revealed no significant individual genes that were associated with high intake of fish or tomato at baseline or after 3 months of supplementation with lycopene or fish oil. However, exploratory pathway analyses of rank-ordered genes (based on p-values not corrected for multiple comparisons) revealed the modulation of androgen and estrogen metabolism in men who routinely consumed more fish ($p = 0.029$) and tomato ($p = 0.008$) compared to men who ate less. In addition, modulation of arachidonic acid metabolism ($p = 0.01$) was observed after 3 months of fish oil supplementation compared with the placebo group; and modulation of nuclear factor (erythroid derived-2) factor 2 or Nrf2-mediated oxidative stress response for either supplement versus placebo (fish oil: $p = 0.01$, lycopene: $p = 0.001$). CONCLUSIONS: We did not detect significant individual genes associated with dietary intake and supplementation of lycopene and fish oil. However, exploratory analyses revealed candidate in vivo pathways that may be modulated by these micronutrients. TRIAL REGISTRATION: ClinicalTrials.gov NCT00402285.

Mansfeld, J., et al. (2015). "Branched-chain amino acid catabolism is a conserved regulator of physiological ageing." Nat Commun **6**: 10043.

Ageing has been defined as a global decline in physiological function depending on both environmental and genetic factors. Here we identify gene transcripts that are similarly regulated during physiological ageing in nematodes, zebrafish and mice. We observe the strongest extension of lifespan when impairing expression of the branched-chain amino acid transferase-1 (bcat-1) gene in C. elegans, which leads to excessive levels of branched-chain amino acids (BCAAs). We further show that BCAAs reduce a LET-363/mTOR-dependent neuro-endocrine signal, which we identify as DAF-7/TGFbeta, and that impacts lifespan depending on its related receptors, DAF-1 and DAF-4, as well as ultimately on DAF-16/FoxO and HSF-1 in a cell-non-autonomous manner. The transcription factor HLH-15 controls and epistatically synergizes with BCAT-1 to modulate physiological ageing. Lastly and consistent with previous findings in rodents, nutritional supplementation of BCAAs extends nematodal lifespan. Taken together, BCAAs act as periphery-derived metabokines that induce a central neuro-endocrine response, culminating in extended healthspan.

Marina, R., et al. (2015). "Hepatic Nrf2 expression is altered by quercetin supplementation in X-irradiated rats." Mol Med Rep **11**(1): 539-546.

Whole-body irradiation has been associated with liver function alterations. Ionizing radiation exposure increases oxidative stress and antioxidants can activate transcription of antioxidant target genes. In the present study, modifications of the liver antioxidant system were evaluated at 7 and 30 days following sub-lethal whole-body X-irradiation in male Wistar rats, which were intragastrically supplemented with quercetin or control solvent for 4 days prior to and 6 days following irradiation. Animal groups were as follows: CS, control, solvent-supplemented;

CQ, control, quercetin-supplemented; RS, irradiated, solvent-supplemented; and RQ, irradiated, quercetin-supplemented. After 7 days, liver tissue from RS animals demonstrated marked hydropic panlobular degeneration with Mallory bodies in ballooning hepatocytes. These changes were mostly reversed in RQ rats. Lipid peroxidation in addition to copper/zinc superoxide dismutase (Cu/Zn-SOD), nuclear factor (erythroid-derived 2)-like 2 (Nrf2) and Kelch-like ECH-associated protein 1 (Keap1) protein expression levels were all increased by X-irradiation, but significantly decreased by quercetin supplementation. Catalase (CAT) and NAD(P)H: quinone oxidoreductase 1 (NQO1) expression levels remained high in irradiated rats regardless of quercetin supplementation. After 30 days, the liver from RS animals had small portal infiltrates and diffuse cytoplasmic vacuolization, with reduced lipid peroxidation and reduced expression levels of CAT, NQO1, Nrf2 and Keap1, but consistently elevated Cu/Zn-SOD expression. RQ animals indicated reduced expression levels of Nrf2 and Keap1 30 days after irradiation. The present study demonstrated a quercetin-induced reduction of the oxidative stress-associated increase in Nrf2 expression that may be useful for preventing cancer cell survival in response to ionizing radiation exposure.

Marrot, L., et al. (2008). "The significance of Nrf2 pathway in (photo)-oxidative stress response in melanocytes and keratinocytes of the human epidermis." Pigment Cell Melanoma Res **21**(1): 79-88.

The expression of genes encoding antioxidant and/or phase 2 detoxifying enzymes can be enhanced in response to various environmental stresses. The main transcription factor involved in this response is nuclear factor erythroid 2-related factor 2 (Nrf2). Nrf2 activity is negatively regulated by the protein Kelch-like-Ech-associated-protein 1 (Keap1). While the roles of Nrf2 and phase 2 genes in chemoprevention of carcinogenesis have been well described; only few studies have dealt with their role in skin cancer. Normal human keratinocytes (NHK) and melanocytes (NHM) were treated by chemical inducers of the Nrf2 pathway or by small interfering RNAs (siRNA) used to knock down Keap1 mRNA. The above treatments resulted in significant stimulation of NQO-1 (NADPH-Quinone-Oxidoreductase 1) gene expression. GCL (gamma-Glutamyl-cysteinyl-ligase) gene was also induced but interestingly increased mRNA encoding the catalytic, heavy subunit GCLC was mainly stimulated in NHK, whereas the mRNA encoding the modifier, light subunit GCLM was mostly induced in NHM. HO-1 (Heme Oxygenase 1) gene induction was relatively strong in NHM, but generally absent in NHK, except when the cells were subjected to cytotoxic doses of the above chemicals. Exposure to solar UV (UVB + UVA, 300-400 nm) or to UVA alone (320-400 nm) confirmed this trend, but interestingly, at doses where cell growth reduction was comparable, UVA was generally more efficient than solar UV in inducing phase 2 genes. When siRNAs directed against Nrf2 were used, a strong down-regulation of NQO-1 expression was observed in both, NHM and NHK, whereas reduction of HO-1 expression was mainly detected in NHM. To our knowledge, this is the first study comparing phase 2 gene modulation in NHK and NHM. The results hereby presented should contribute to a better understanding of the molecular mechanisms involved in skin adaptation to environmental stress.

Martin, D., et al. (2004). "Regulation of heme oxygenase-1 expression through the phosphatidylinositol 3-kinase/Akt pathway and the Nrf2 transcription factor in response to the antioxidant phytochemical carnosol." J Biol Chem **279**(10): 8919-8929.

Martin, M. A., et al. (2010). "Hydroxytyrosol induces antioxidant/detoxificant enzymes and Nrf2 translocation via extracellular regulated kinases and phosphatidylinositol-3-kinase/protein kinase B pathways in HepG2 cells." Mol Nutr Food Res **54**(7): 956-966.

Hydroxytyrosol (HTy) is a natural polyphenol abundant in olive oil, which possesses multiple biological actions. Particularly, HTy has cytoprotective activity against oxidative-stress-induced cell damage, but the underlying mechanisms of action remain unclear. Here, we have investigated the molecular mechanism involved in the protection exerted by HTy on tert-butyl hydroperoxide-induced damage in human HepG2 liver cells. Treatment of HepG2 cells with HTy increased the expression and the activity of glutathione-related enzymes such as glutathione peroxidase, glutathione reductase and glutathione S-transferase. HTy also induced the nuclear transcription factor erythroid 2p45-related factor (Nrf2), a transcription factor implicated in the expression of several antioxidant/detoxificant enzymes. Moreover, two important signalling proteins involved in Nrf2 translocation, the protein kinase B and the extracellular regulated kinases, were also activated by HTy. Further studies with specific inhibitors confirmed that both molecular pathways are critical for the nuclear translocation of Nrf2, the increased enzyme expression and activity and the beneficial effect against oxidative stress induced by HTy. In conclusion, together with the inherent radical scavenging activity of HTy, our results provide an additional mechanism of action to prevent oxidative stress damage through the modulation of signalling pathways involved in antioxidant/detoxifying enzymes regulation.

Matsushima, M., et al. (2009). "Heme oxygenase-1 mediates the anti-allergic actions of quercetin in rodent mast cells." Inflamm Res **58**(10): 705-715.

OBJECTIVE AND DESIGN: We investigated the involvement of heme oxygenase (HO)-1 in the anti-allergic action of quercetin against degranulation of rat basophilic leukemia (RBL-2H3) cells, rat peritoneal mast cells, and

mouse bone marrow-derived mast cells. METHODS: The strength of allergic reaction was evaluated by the extent of degranulation in mast cells sensitized with various stimulants. The levels of HO-1, HO-2, and nuclear factor erythroid 2-related factor 2 (Nrf2) expressions were determined by quantitative RT-PCR, western blotting, or immunocytochemistry. RESULTS: Heme oxygenase activity was upregulated after short exposure to quercetin, followed by the induction of HO-1 expression after long exposure to quercetin. The inhibition of degranulation by quercetin was reversed using tin protoporphyrin IX (SnPP), an HO-1 inhibitor. HO-1 metabolites, bilirubin and CO, led to inhibit degranulation, and quercetin translocated Nrf2 from cytoplasm into nucleus in RBL-2H3 cells. CONCLUSION: These results strongly suggest that quercetin exerted anti-allergic actions via activation of Nrf2-HO-1 pathway.

Milenkovic, D., et al. (2014). "Dietary Flavanols Modulate the Transcription of Genes Associated with Cardiovascular Pathology without Changes in Their DNA Methylation State." PLoS One **9**(4): e95527.

BACKGROUND: In a recent intervention study, the daily supplementation with 200 mg monomeric and oligomeric flavanols (MOF) from grape seeds for 8 weeks revealed a vascular health benefit in male smokers. The objective of the present study was to determine the impact of MOF consumption on the gene expression profile of leukocytes and to assess changes in DNA methylation. METHODOLOGY/PRINCIPAL FINDINGS: Gene expression profiles were determined using whole genome microarrays (Agilent) and DNA methylation was assessed using HumanMethylation450 BeadChips (Illumina). MOF significantly modulated the expression of 864 genes. The majority of the affected genes are involved in chemotaxis, cell adhesion, cell infiltration or cytoskeleton organisation, suggesting lower immune cell adhesion to endothelial cells. This was corroborated by in vitro experiments showing that MOF exposure of monocytes attenuates their adhesion to TNF-alpha-stimulated endothelial cells. Nuclear factor kappa B (NF-kappaB) reporter gene assays confirmed that MOF decrease the activity of NF-kappaB. Strong inter-individual variability in the leukocytes' DNA methylation was observed. As a consequence, on group level, changes due to MOF supplementation could not be found. CONCLUSION: Our study revealed that an 8 week daily supplementation with 200 mg MOF modulates the expression of genes associated with cardiovascular disease pathways without major changes of their DNA methylation state. However, strong inter-individual variation in leukocyte DNA methylation may obscure the subtle epigenetic response to dietary flavanols. Despite the lack of significant changes in DNA methylation, the modulation of gene expression appears to contribute to the observed vascular health effect of MOF in humans.

Minokoshi, Y., et al. (2002). "Leptin stimulates fatty-acid oxidation by activating AMP-activated protein kinase." Nature **415**(6869): 339-343.

Leptin is a hormone secreted by adipocytes that plays a pivotal role in regulating food intake, energy expenditure and neuroendocrine function. Leptin stimulates the oxidation of fatty acids and the uptake of glucose, and prevents the accumulation of lipids in nonadipose tissues, which can lead to functional impairments known as "lipotoxicity". The signalling pathways that mediate the metabolic effects of leptin remain undefined. The 5'-AMP-activated protein kinase (AMPK) potently stimulates fatty-acid oxidation in muscle by inhibiting the activity of acetyl coenzyme A carboxylase (ACC). AMPK is a heterotrimeric enzyme that is conserved from yeast to humans and functions as a 'fuel gauge' to monitor the status of cellular energy. Here we show that leptin selectively stimulates phosphorylation and activation of the alpha2 catalytic subunit of AMPK (alpha2 AMPK) in skeletal muscle, thus establishing a previously unknown signalling pathway for leptin. Early activation of AMPK occurs by leptin acting directly on muscle, whereas later activation depends on leptin functioning through the hypothalamic-sympathetic nervous system axis. In parallel with its activation of AMPK, leptin suppresses the activity of ACC, thereby stimulating the oxidation of fatty acids in muscle. Blocking AMPK activation inhibits the phosphorylation of ACC stimulated by leptin. Our data identify AMPK as a principal mediator of the effects of leptin on fatty-acid metabolism in muscle.

Miodownik, C., et al. (2011). "Serum levels of brain-derived neurotrophic factor and cortisol to sulfate of dehydroepiandrosterone molar ratio associated with clinical response to L-theanine as augmentation of antipsychotic therapy in schizophrenia and schizoaffective disorder patients." Clin Neuropharmacol **34**(4): 155-160.

OBJECTIVES: L-Theanine (gamma-glutamylethylamide) augmentation to antipsychotic therapy ameliorates positive, activation, and anxiety symptoms in schizophrenia and schizoaffective disorder patients. This study examines the association between circulating levels of neurochemical indicators and the beneficial clinical effects of L-theanine augmentation. METHODS: Serum levels of neurochemical indicators such as brain-derived neurotrophic factor (BDNF), dehydroepiandrosterone (DHEA), its sulfate (DHEAS), cortisol, cholesterol, and insulin were monitored in 40 schizophrenia and schizoaffective disorder patients during an 8-week, double-blind, randomized, placebo-controlled trial with L-theanine (400 mg/d). Multiple regression analysis was applied for searching association between improvement in symptom scores and changes in circulating levels of neurochemical indicators for an 8-week trial. RESULTS: Regression models among L-theanine-treated patients indicate that circulating levels of BDNF and cortisol-to-DHEAS*100 molar ratio were significantly associated with the beneficial clinical effects of

L-theanine augmentation. Variability of serum BDNF levels accounted for 26.2% of the total variance in reduction of dysphoric mood and 38.2% in anxiety scores. In addition, the changes in cortisol-to-DHEAS*100 molar ratio accounted for 30% to 34% of the variance in activation factor and dysphoric mood scores and for 15.9% in anxiety scores. Regression models among placebo-treated patients did not reach significant level. CONCLUSIONS: These preliminary results indicate that circulating BDNF and cortisol-to-DHEAS*100 molar ratio may be involved in the beneficial clinical effects of L-theanine as augmentation of antipsychotic therapy in schizophrenia and schizoaffective disorder patients.

Miyamoto, N., et al. (2011). "Quercetin induces the expression of peroxiredoxins 3 and 5 via the Nrf2/NRF1 transcription pathway." Invest Ophthalmol Vis Sci **52**(2): 1055-1063.

PURPOSE: The flavonoids have potent antioxidant and free-radical scavenging properties and are beneficial in the prevention and treatment of ocular diseases including glaucoma. The authors have previously reported that antiglaucoma agents could transcriptionally activate the antioxidant protein peroxiredoxin (PRDX)2. The purpose of this study was to investigate whether quercetin can activate transcription factors and induce the expression of the PRDX family. METHODS: To demonstrate whether quercetin can transcriptionally induce the expression of the PRDX family, trabecular meshwork cells were treated with quercetin, and PRDX expression and transcription factors were both investigated by Western blot analysis, reporter assays, and siRNA strategies. Subsequently, cellular sensitivity to oxidative stress was determined. RESULTS: Expression of the PRDX3 and PRDX5 genes was induced by quercetin in a time- and dose-dependent manner. NRF1 transactivates the promoter activity of both PRDX3 and PRDX5 but not PRDX2 and PRDX4. Quercetin can also induce the expression of Nrf2 and NRF1 but not of Ets1, Ets2, or Foxo3a. Knockdown of NRF1 expression significantly reduced the expression of both PRDX3 and PRDX5. Reporter assays showed that NRF1 transactivated the promoter activity of both PRDX3 and PRDX5 and that the downregulation of NRF1 with siRNA repressed the promoter activity of both PRDX3 and PRDX5. Furthermore, the downregulation of NRF1, PRDX3, and PRDX5 renders trabecular meshwork cells sensitive to hydrogen peroxide. Finally, NRF1 activation by quercetin was completely abolished by the knockdown of Nrf2. CONCLUSIONS: Quercetin upregulates the antioxidant peroxiredoxins through the activation of the Nrf2/NRF1 transcription pathway and protects against oxidative stress-induced ocular disease.

Moghbelinejad, S., et al. (2014). "Rutin activates the MAPK pathway and BDNF gene expression on beta-amyloid induced neurotoxicity in rats." Toxicol Lett **224**(1): 108-113.

Flavonoids are present in foods such as fruits and vegetables. A relationship between the consumption of flavonoid-rich foods and prevention of human disease including neurodegenerative disorders has been demonstrated. We assessed the effect of rutin (3,3',4',5,7-pentahydroxyflavone-3-rhamnoglucoside) on the mitogen-activated protein kinase (MAPK) pathway, memory retrieval and oxidative stress in rats injected with beta-amyloid (Abeta), which is implicated to have an important role in Alzheimer's disease (AD). Abeta was injected bilaterally in the deep frontal cortex of rat brain. Next, rutin and saline were injected (i.p.) for 3 weeks. In comparison to the control group, rutin significantly increased extracellular signal-regulated protein kinase 1 (ERK1), cAMP response element-binding protein (CREB) and brain-derived neurotrophic factor (BDNF) gene expression in the hippocampus of rats. Rutin (100 mg/kg) significantly increased memory retrieval compared to the control group. Malondialdehyde (MDA) level in the hippocampus of the rutin group was significantly lower than those in the control group. The content of sulfhydryl groups in the rutin group was higher than that in the control group. The findings show a possibility that rutin may have beneficial effects against neurotoxicity of Abeta on memory in rats.

Mouchiroud, L., et al. (2013). "The NAD(+)/Sirtuin Pathway Modulates Longevity through Activation of Mitochondrial UPR and FOXO Signaling." Cell **154**(2): 430-441.

NAD(+) is an important cofactor regulating metabolic homeostasis and a rate-limiting substrate for sirtuin deacylases. We show that NAD(+) levels are reduced in aged mice and Caenorhabditis elegans and that decreasing NAD(+) levels results in a further reduction in worm lifespan. Conversely, genetic or pharmacological restoration of NAD(+) prevents age-associated metabolic decline and promotes longevity in worms. These effects are dependent upon the protein deacetylase sir-2.1 and involve the induction of mitonuclear protein imbalance as well as activation of stress signaling via the mitochondrial unfolded protein response (UPR(mt)) and the nuclear translocation and activation of FOXO transcription factor DAF-16. Our data suggest that augmenting mitochondrial stress signaling through the modulation of NAD(+) levels may be a target to improve mitochondrial function and prevent or treat age-associated decline.

Murakami, T. and M. Furuse (2010). "The impact of taurine- and beta-alanine-supplemented diets on behavioral and neurochemical parameters in mice: antidepressant versus anxiolytic-like effects." Amino Acids **39**(2): 427-434.

Taurine, a substrate of taurine transporter, has functions as a neuromodulator and antioxidant and beta-alanine, a taurine transporter inhibitor, has a role as a neurotransmitter in the brain, and they were expected to be involved in depression-like behavior and antidepressant treatment. These facts aroused our interest in new

capabilities of taurine and beta-alanine. Thus, to investigate the effects of chronic ingestion of taurine- (22.5 mmol/kg diet) supplemented diet and beta-alanine- (22.5 mmol/kg diet) supplemented diet under acute stressful conditions, behavioral changes and brain metabolites were compared with mice fed a control diet. In the open field test, no significant difference was observed in locomotor activity among groups. In the elevated plus-maze test, however, significant increases in the percentage of time spent and entries in the open arms were observed in the beta-alanine-supplemented diet fed group compared to both controls and animals fed with taurine-supplemented diet. Moreover, a significant decrease in the duration of immobility was observed in the taurine-supplemented diet group in the forced swimming test compared to both controls and animals fed with beta-alanine-supplemented diet. Taurine-supplemented diet increased taurine and L: -arginine concentrations in the hypothalamus. In contrast, beta-alanine-supplemented diet decreased the concentration of 5-hydroxyindoleacetic acid, a major metabolite of serotonin, in the hypothalamus. Beta-alanine-supplemented diet also increased carnosine (beta-alanyl-L: -histidine) concentration in the cerebral cortex and hypothalamus, and brain-derived neurotrophic factor concentration in the hippocampus. These results suggested that taurine-supplemented diet had an antidepressant-like effect and beta-alanine-supplemented diet had an anxiolytic-like effect.

Muthumani, M. and S. M. Prabu (2012). "Silibinin potentially protects arsenic-induced oxidative hepatic dysfunction in rats." Toxicol Mech Methods **22**(4): 277-288.

Arsenic (As) compounds are reported as environmental toxicants and human carcinogens. Exposure to arsenic imposes a big health issue worldwide. Silibinin (SB) is a major flavonolignan compound of silimarin and is found in milk thistle of Silybum marianum. It has been reported that silibinin has antioxidant efficacy as metal chelators due to the orientation of its functional groups. However, it has not yet been explored in experimental animals. In view of this fact, the purpose of this study was to delineate the ameliorative role of silibinin against arsenic-induced hepatotoxicity in rats. Rats were orally treated with arsenic alone (5 mg/kg body weight (bw)/day) plus silibinin (75 mg/kg bw/day) for 4weeks. Hepatotoxicity was evaluated by the increased activities of serum hepatospecific enzymes namely aspartate transaminase, alanine transaminase, alkaline phosphatase, gamma glutamyl transferase, lactate dehydrogenase and total bilirubin along with increased elevation of lipid peroxidative markers, thiobarbituric acid reactive substances, lipid hydroperoxides, protein carbonyl content and conjugated dienes. The toxic effect of arsenic was also indicated by significantly decreased activities of membrane bound ATPases, enzymatic antioxidants like superoxide dismutase, catalase, glutathione peroxidase, glutathione-S-transferase, glutathione reductase and glucose-6-phosphate dehydrogenase along with nonenzymatic antioxidants like reduced glutathione, total sulfhydryl groups, vitamins C and E. Administration of silibinin exhibited a significant reversal of arsenic-induced toxicity in hepatic tissue. All these changes were supported by reduction of DNA damage in hepatocytes and histopathological observations of the liver. These results suggest that silibinin has a potential protective effect over arsenic-induced hepatotoxicity in rat.

Na, H. K., et al. (2008). "(-)-Epigallocatechin gallate induces Nrf2-mediated antioxidant enzyme expression via activation of PI3K and ERK in human mammary epithelial cells." Arch Biochem Biophys **476**(2): 171-177.

Nakamura, Y., et al. (2009). "A combination of indol-3-carbinol and genistein synergistically induces apoptosis in human colon cancer HT-29 cells by inhibiting Akt phosphorylation and progression of autophagy." Mol Cancer **8**: 100.

BACKGROUND: The chemopreventive effects of dietary phytochemicals on malignant tumors have been studied extensively because of a relative lack of toxicity. To achieve desirable effects, however, treatment with a single agent mostly requires high doses. Therefore, studies on effective combinations of phytochemicals at relatively low concentrations might contribute to chemopreventive strategies. RESULTS: Here we found for the first time that co-treatment with I3C and genistein, derived from cruciferous vegetables and soy, respectively, synergistically suppressed the viability of human colon cancer HT-29 cells at concentrations at which each agent alone was ineffective. The suppression of cell viability was due to the induction of a caspase-dependent apoptosis. Moreover, the combination effectively inhibited phosphorylation of Akt followed by dephosphorylation of caspase-9 or down-regulation of XIAP and survivin, which contribute to the induction of apoptosis. In addition, the co-treatment also enhanced the induction of autophagy mediated by the dephosphorylation of mTOR, one of the downstream targets of Akt, whereas the maturation of autophagosomes was inhibited. These results give rise to the possibility that co-treatment with I3C and genistein induces apoptosis through the simultaneous inhibition of Akt activity and progression of the autophagic process. This possibility was examined using inhibitors of Akt combined with inhibitors of autophagy. The combination effectively induced apoptosis, whereas the Akt inhibitor alone did not. CONCLUSION: Although in vivo study is further required to evaluate physiological efficacies and toxicity of the combination treatment, our findings might provide a new insight into the development of novel combination therapies/chemoprevention against malignant tumors using dietary phytochemicals.

Nath, S., et al. (2012). "Catechins protect neurons against mitochondrial toxins and HIV proteins via activation of

the BDNF pathway." J Neurovirol **18**(6): 445-455.

Currently, there is no effective treatment for neurological complications of infection with the human immunodeficiency virus that persists despite the use of combination antiretroviral therapy. A medium throughput assay was developed for screening neuroprotective compounds using primary mixed neuronal cells and mitochondrial toxin 3-nitropropionic acid. Using this assay, a library of 2,000 compounds was screened. Out of 256 compounds that showed variable degrees of neuroprotection, nine were related to epicatechin, a monomeric flavonoid found in cocoa and green tea leaves that readily crosses the blood-brain barrier. Hence, catechin, epicatechin, and the related compound, epigallocatechin gallate (EGCG) were further screened for their neuroprotective properties against HIV proteins Tat and gp120, and compared to those of resveratrol. Epicatechin and EGCG targets the brain-derived neurotrophic factor (BDNF) and its precursor proBDNF signaling pathways, normalizing both Tat-mediated increases in proapoptotic proBDNF and concomitant Tat-mediated decreases in the mature BDNF protein in hippocampal neurons. Epicatechin and epigallocatechin gallate were more potent than catechin or resveratrol as neuroprotectants. Due to its simpler structure and more efficient blood-brain barrier penetration properties, epicatechin might be the best therapeutic candidate for neurodegenerative diseases including HIV-associated neurocognitive disorders where oxidative stress is an important pathophysiological mechanism.

Nguyen, P. H., et al. (2011). "New dammarane-type glucosides as potential activators of AMP-activated protein kinase (AMPK) from Gynostemma pentaphyllum." Bioorg Med Chem **19**(21): 6254-6260.

AMP-activated protein kinase (AMPK) is a key sensor and regulator of glucose, lipid, and energy metabolism throughout the body. Activation of AMPK improves metabolic abnormalities associated with metabolic diseases including obesity and type-2 diabetes. The oriental traditional medicinal herbal plant, Gynostemma pentaphyllum, has shown a wide range of beneficial effects on glucose and lipid metabolism. In this study, we found that G. pentaphyllum contains two novel dammarane-type saponins designated as damulin A (1), 2alpha,3beta,12beta-trihydroxydammar-20(22)-E,24-diene-3-O-[beta-D-glucopyranosyl -(1-->2)-beta-D-glucopyranoside], and damulin B (2), 2alpha,3beta,12beta-trihydroxydammar-20,24-diene-3-O-[beta-D-glucopyranosyl-(1--> 2)-beta-D-glucopyranoside], that strongly activate AMPK in cultured L6 myotube cells. Damulins A and B also increased beta-oxidation and glucose uptake with increasing GluT4 translocation to the plasma membrane in L6 myotube cells. Taken together our results indicate that activation of AMPK by damulins A and B may contribute to beneficial effect of G. pentaphyllum on glucose and lipid metabolism.

Ogborne, R. M., et al. (2005). "Alpha-lipoic acid-induced heme oxygenase-1 expression is mediated by nuclear factor erythroid 2-related factor 2 and p38 mitogen-activated protein kinase in human monocytic cells." Arterioscler Thromb Vasc Biol **25**(10): 2100-2105.

OBJECTIVE: Heme oxygenase-1 (HO-1), the rate-limiting enzyme in heme catabolism, plays a protective role in the vascular system. HO-1 induction inhibits cytokine production in macrophages. Antioxidants induce HO-1 expression in various cell types. Alpha-lipoic acid (ALA), a thiol-containing dietary antioxidant, exhibits protective effects in vascular disease and induces anti-inflammatory effects in monocytes. This study examined the effects of ALA on HO-1 expression in human monocytic cells. METHODS AND RESULTS: ALA time and dose-dependently induced HO-1 mRNA expression in THP-1 cells, with peak expression at 4 hours and returning to baseline by 24 hours. This correlated with an increase in HO-1 protein expression. ALA stimulated translocation of the transcription factor nuclear factor-erythroid 2-related factor 2 (Nrf2) into the nucleus and binding to a human HO-1 antioxidant response element (ARE) by 30 minutes. A dominant-negative Nrf2 inhibitor reduced ALA-induced HO-1 mRNA expression by 66%. Pretreatment with SB203580, a p38 mitogen-activated protein kinase inhibitor, reduced ALA-induced HO-1 mRNA expression by 75% and inhibited ALA-induced Nrf2 binding to the HO-1 ARE. CONCLUSIONS: These results demonstrate that ALA induces HO-1 expression in THP-1 monocytic cells via Nrf2 and p38. Further studies are required to investigate whether the protective effects of ALA in monocytes are mediated by HO-1.

Park, S. H., et al. (2014). "Antiobesity effect of Gynostemma pentaphyllum extract (actiponin): a randomized, double-blind, placebo-controlled trial." Obesity (Silver Spring) **22**(1): 63-71.

OBJECTIVE: The effects of actiponin was investigated, a heat-processed Gynostemma pentaphyllum extract, on body weight, fat loss, and metabolic markers of Korean participants in a 12-week, randomized, double-blind, placebo-controlled clinical trial. DESIGN AND METHODS: Obese participants (BMI >/= 25 kg m(-2) and WHR >/= 0.90 for male or WHR >/= 0.85 for female) who had not been diagnosed with any disease and met the inclusion criteria were recruited for this study. The 80 subjects were randomly divided into actiponin (n = 40, 450 mg day(-1)) and placebo (n = 40) groups. Outcomes included measurement of efficacy (abdominal fat distribution, anthropometric parameters, and blood lipid profiles) and safety (adverse events, laboratory test results, electrocardiogram data, and vital signs). RESULTS: During 12-week of actiponin supplementation, total abdominal fat area, body weight, body fat mass, percent body fat, and BMI were significantly decreased ($P = 0.044$, $P < 0.05$,

P < 0.0001, P < 0.0001, and P < 0.05, respectively) in the actiponin group compared to the placebo group. No clinically significant changes in any safety parameter were observed. CONCLUSION: Our study revealed that actiponin is a potent antiobesity reagent that does not produce any significant adverse effects. These results suggest that actiponin supplementation may be effective for treating obese individuals.

Park, Y. S., et al. (2002). "Effect of an exo-polysaccharide from the culture broth of Hericium erinaceus on enhancement of growth and differentiation of rat adrenal nerve cells." Cytotechnology **39**(3): 155-162.

It was found that an exo-biopolymer (M.W. 1,000,000, molar ratio of 1.5:1.7:1.2:0.6:0.9, glucose:galactose:xylose:mannose:fructose, purity 99%) purified from the liquid culture broth of Hericium erinaceus mycelium enhanced the growth of rat adrenal nerve cells. The polymer also improved the extension of the neurites of PC12 cell. Its efficacy was found to be higher than those from known nerve growth factors such as Nerve Growth Factor (NGF) and Brain-Derived Nerve Factor (BDNF). The effect of two standards has not been observed above 0.1 (mg l(-1)) of supplementation; however, the polymer did show the effect of cell growth and neurite extension at up to 1.0 (mg l(-1)) of addition. While the polymer improved both cell growth and neurite extension, NGF and BDNF did only outgrowth of the neurites. Maximum cell density and length of the neurites were observed as 1.5x10(5) (viable cells ml(-1)) and 230 mum, respectively in adding 0.8 (mg l(-1)) of the biopolymer for 8 days cultivation. The control growth was observed only as 1.2x10(5) (viable cell ml(-1)) of maximum cell density and 140 mum of maximum length, respectively. It was also confirmed that the polymer reacted with the nerve cells within 30 min after adding the sample, compared to 80 min in adding two other growth factors. Number of neurite-bearing cells remained relatively steady in adding the polymer even when the cell growth started to be decreased. It was interesting that the polymer effectively delayed apoptosis of PC12 cells by dramatically reducing the ratio of apoptotic cells to 20% from 50% of the control.

Prabu, S. M. and M. Muthumani (2012). "Silibinin ameliorates arsenic induced nephrotoxicity by abrogation of oxidative stress, inflammation and apoptosis in rats." Mol Biol Rep **39**(12): 11201-11216.

Arsenic (As) is an environmental and industrial pollutant that affects various organs in human and experimental animals. Silibinin is a naturally occurring plant bioflavonoid found in the milk thistle of Silybum marianum, which has been reported to have a wide range of pharmacological properties. A body of evidence has accumulated implicating the free radical generation with subsequent oxidative stress in the biochemical and molecular mechanisms of As toxicity. Since kidney is the critical target organ of chronic As toxicity, we carried out this study to investigate the effects of silibinin on As-induced toxicity in the kidney of rats. In experimental rats, oral administration of sodium arsenite [NaAsO(2), 5 mg/(kg day)] for 4 weeks significantly induced renal damage which was evident from the increased levels of serum urea, uric acid, creatinine with a significant ($p < 0.05$) decrease in creatinine clearance. As also significantly decreased the levels of urea, uric acid and creatinine in urine. A markedly increased levels of lipid peroxidation markers (thiobarbituric acid reactive substances and lipid hydroperoxides) and protein carbonyl contents with significant ($p < 0.05$) decrease in non-enzymatic antioxidants (total sulfhydryl groups, reduced glutathione, vitamin C and vitamin E) and enzymatic antioxidants (superoxide dismutase, catalase, glutathione peroxidase and glutathione S-transferase), Glutathione metabolizing enzymes (glutathione reductase and glutathione-6-phosphate dehydrogenase) and membrane bound ATPases were also observed in As treated rats. Co-administration of silibinin (75 mg/kg day) along with As resulted in a reversal of As-induced biochemical changes in kidney accompanied by a significant decrease in lipid peroxidation and an increase in the level of renal antioxidant defense system. The histopathological and immunohistochemical studies in the kidney of rats also shows that silibinin (75 mg/kg day) markedly reduced the toxicity of As and preserved the normal histological architecture of the renal tissue, inhibited the caspase-3 mediated tubular cell apoptosis and decreased the NADPH oxidase, iNOS and NF-kappaB over expression by As and upregulated the Nrf2 expression in the renal tissue. The present study suggests that the nephroprotective potential of silibinin in As toxicity might be due to its antioxidant and metal chelating properties, which could be useful for achieving optimum effects in As-induced renal damage.

Price, N. L., et al. (2012). "SIRT1 is required for AMPK activation and the beneficial effects of resveratrol on mitochondrial function." Cell Metab **15**(5): 675-690.

Resveratrol induces mitochondrial biogenesis and protects against metabolic decline, but whether SIRT1 mediates these benefits is the subject of debate. To circumvent the developmental defects of germline SIRT1 knockouts, we have developed an inducible system that permits whole-body deletion of SIRT1 in adult mice. Mice treated with a moderate dose of resveratrol showed increased mitochondrial biogenesis and function, AMPK activation, and increased NAD(+) levels in skeletal muscle, whereas SIRT1 knockouts displayed none of these benefits. A mouse overexpressing SIRT1 mimicked these effects. A high dose of resveratrol activated AMPK in a SIRT1-independent manner, demonstrating that resveratrol dosage is a critical factor. Importantly, at both doses of resveratrol no improvements in mitochondrial function were observed in animals lacking SIRT1. Together these data indicate that SIRT1 plays an essential role in the ability of moderate doses of resveratrol to stimulate AMPK and improve mitochondrial function both in vitro and in vivo.

Ramyaa, P., et al. (2014). "Quercetin modulates OTA-induced oxidative stress and redox signalling in HepG2 cells - up regulation of Nrf2 expression and down regulation of NF-kappaB and COX-2." Biochim Biophys Acta **1840**(1): 681-692.

BACKGROUND: Ochratoxin A (OTA), a mycotoxin, causes extensive cell damage, affecting liver and kidney cells. OTA toxicity is fairly well characterized where oxidative stress is believed to play a role, however, the sequence of molecular events after OTA-exposure, have not been characterized in literature. Further, antidotes for alleviating the toxicity are sparsely reported. The aim of this study was to understand the sequence of some molecular mechanisms for OTA-induced toxicity and the cytoprotective effect of quercetin on OTA-induced toxicity. METHODS: Time course studies to evaluate the time of intracellular calcium release and ROS induction were carried out. The time of activation and induction of two key redox- sensitive transcription factors, NF-kappaB and Nrf-2 were determined by nuclear localization and expression respectively. The time of expression of inflammatory marker COX-2 was determined. Oxidative DNA damage by comet assay and micronucleus formation was studied. The ameliorative effect of quercetin on OTA-induced toxicity was also determined on all the above-mentioned parameters. RESULTS: OTA-induced calcium release, ROS generation and activated NF-kappaB nuclear translocation and expression. Pre-treatment with quercetin ameliorated ROS and calcium release as well as NF-kappaB induction and expression. Quercetin induced Nrf-2 nuclear translocation and expression. Quercetin's anti-inflammatory property was exhibited as it down regulated COX-2. Anti-genotoxic effect of quercetin was evident in prevention of DNA damage and micronucleus formation. CONCLUSION: Quercetin modulated OTA-induced oxidative stress and redox-signaling in HepG2 cells. GENERAL SIGNIFICANCE: The results of the study demonstrate for the first time that quercetin prevents OTA-induced toxicity in HepG2 cells.

Ramyaa, P. and V. V. Padma (2013). "Ochratoxin-induced toxicity, oxidative stress and apoptosis ameliorated by quercetin--modulation by Nrf2." Food Chem Toxicol **62**: 205-216.

Ochratoxin (OTA) is one of the most abundant food contaminating mycotoxins and is commonly present in the food chain. Many of the effects associated with OTA, appear to be mediated through oxidative stress. Although the toxicity of OTA is fairly well characterized, antidotes for alleviating the toxicity are sparsely reported. Dietary antioxidants have gained much importance in the recent years for their antioxidative and therapeutic properties. In the present study the therapeutic strategy was directed towards use of quercetin, a dietary antioxidant to combat OTA-induced toxicity in Vero cell line. Our results demonstrate that quercetin pre-treatment suppressed OTA-induced cytotoxicity and oxidative stress. It modulated OTA-induced alteration on the antioxidant defence through activation of Nrf2 pathway. Morphological studies by scanning electron microscopy (SEM) and cell cycle analysis indicated that quercetin prevented OTA-induced apoptosis. It also inhibited the activation of caspase cascade that leads to DNA fragmentation. Quercetin also exhibited antigenotoxic potential by attenuating OTA-induced DNA damage and micronucleus (MN) formation. The results of the study demonstrate for the first time that quercetin pre-treatment prevents OTA-induced oxidative stress and apoptosis in Vero cell line.

Reinke, A., et al. (2006). "Caffeine targets TOR complex I and provides evidence for a regulatory link between the FRB and kinase domains of Tor1p." J Biol Chem **281**(42): 31616-31626.

The target of rapamycin (TOR) kinase is an important regulator of growth in eukaryotic cells. In budding yeast, Tor1p and Tor2p function as part of two distinct protein complexes, TORC1 and TORC2, where TORC1 is specifically inhibited by the antibiotic rapamycin. Significant insight into TORC1 function has been obtained using rapamycin as a specific small molecule inhibitor of TOR activity. Here we show that caffeine acts as a distinct and novel small molecule inhibitor of TORC1: (i) deleting components specific to TORC1 but not TORC2 renders cells hypersensitive to caffeine; (ii) rapamycin and caffeine display remarkably similar effects on global gene expression; and (iii) mutations were isolated in Tor1p, a component specific to TORC1, that confers significant caffeine resistance both in vivo and in vitro. Strongest resistance requires two simultaneous mutations in TOR1, the first at either one of two highly conserved positions within the FRB (rapamycin binding) domain and a second at a highly conserved position within the ATP binding pocket of the kinase domain. Biochemical and genetic analyses of these mutant forms of Tor1p support a model wherein functional interactions between the FRB and kinase domains, as well as between the FRB domain and the TORC1 component Kog1p, regulate TOR activity as well as contribute to the mechanism of caffeine resistance.

Rendeiro, C., et al. (2013). "Dietary levels of pure flavonoids improve spatial memory performance and increase hippocampal brain-derived neurotrophic factor." PLoS One **8**(5): e63535.

Evidence suggests that flavonoid-rich foods are capable of inducing improvements in memory and cognition in animals and humans. However, there is a lack of clarity concerning whether flavonoids are the causal agents in inducing such behavioral responses. Here we show that supplementation with pure anthocyanins or pure flavanols for 6 weeks, at levels similar to that found in blueberry (2% w/w), results in an enhancement of spatial memory in 18 month old rats. Pure flavanols and pure anthocyanins were observed to induce significant improvements in

spatial working memory (p = 0.002 and p = 0.006 respectively), to a similar extent to that following blueberry supplementation (p = 0.002). These behavioral changes were paralleled by increases in hippocampal brain-derived neurotrophic factor (R = 0.46, p<0.01), suggesting a common mechanism for the enhancement of memory. However, unlike protein levels of BDNF, the regional enhancement of BDNF mRNA expression in the hippocampus appeared to be predominantly enhanced by anthocyanins. Our data support the claim that flavonoids are likely causal agents in mediating the cognitive effects of flavonoid-rich foods.

Riedl, M. A., et al. (2009). "Oral sulforaphane increases Phase II antioxidant enzymes in the human upper airway." Clin Immunol **130**(3): 244-251.

Romeo, L., et al. (2009). "The major green tea polyphenol, (-)-epigallocatechin-3-gallate, induces heme oxygenase in rat neurons and acts as an effective neuroprotective agent against oxidative stress." J Am Coll Nutr **28 Suppl**: 492S-499S.

Sahin, K., et al. (2010). "Epigallocatechin-3-gallate activates Nrf2/HO-1 signaling pathway in cisplatin-induced nephrotoxicity in rats." Life Sci **87**(7-8): 240-245.

Sahin, K., et al. (2010). "Nrf2/HO-1 signaling pathway may be the prime target for chemoprevention of cisplatin-induced nephrotoxicity by lycopene." Food Chem Toxicol **48**(10): 2670-2674.

Satoh, T., et al. (2008). "Carnosic acid protects neuronal HT22 Cells through activation of the antioxidant-responsive element in free carboxylic acid- and catechol hydroxyl moieties-dependent manners." Neurosci Lett **434**(3): 260-265.

Satoh, T., et al. (2013). "Nrf2/ARE-mediated antioxidant actions of pro-electrophilic drugs." Free Radic Biol Med **65**: 645-657.

Schaffer, S. and B. Halliwell (2011). "Comment on hydroxytyrosol induces proliferation and cytoprotection against oxidative injury in vascular endothelial cells: role of Nrf2 activation and HO-1 induction." J Agric Food Chem **59**(19): 10770-10771.

Schmeisser, K., et al. (2013). "Role of sirtuins in lifespan regulation is linked to methylation of nicotinamide." Nat Chem Biol **9**(11): 693-700.

Sirtuins, a family of histone deacetylases, have a fiercely debated role in regulating lifespan. In contrast with recent observations, here we find that overexpression of sir-2.1, the ortholog of mammalian SirT1, does extend Caenorhabditis elegans lifespan. Sirtuins mandatorily convert NAD(+) into nicotinamide (NAM). We here find that NAM and its metabolite, 1-methylnicotinamide (MNA), extend C. elegans lifespan, even in the absence of sir-2.1. We identify a previously unknown C. elegans nicotinamide-N-methyltransferase, encoded by a gene now named anmt-1, to generate MNA from NAM. Disruption and overexpression of anmt-1 have opposing effects on lifespan independent of sirtuins, with loss of anmt-1 fully inhibiting sir-2.1-mediated lifespan extension. MNA serves as a substrate for a newly identified aldehyde oxidase, GAD-3, to generate hydrogen peroxide, which acts as a mitohormetic reactive oxygen species signal to promote C. elegans longevity. Taken together, sirtuin-mediated lifespan extension depends on methylation of NAM, providing an unexpected mechanistic role for sirtuins beyond histone deacetylation.

Sgarbossa, A., et al. (2012). "Phenylpropanoid glycosides from plant cell cultures induce heme oxygenase 1 gene expression in a human keratinocyte cell line by affecting the balance of NRF2 and BACH1 transcription factors." Chem Biol Interact **199**(2): 87-95.

Phenylpropanoids have several highly significant biological properties in both plants and animals. Four phenylpropanoid glycosides (PPGs), verbascoside (VB), forsythoside B (FB), echinacoside (EC) and campneoside I (CP), were purified and tested for their capability to activate NRF2 and induce phase II cytoprotective enzymes in a human keratinocyte cell line (HaCaT). All four substances showed similar strong antioxidant and radical-scavenging activities as determined by diphenylpicrylhydrazyl assay. Furthermore, in HaCaT cells, FB and EC are strong activators of NRF2, the nuclear transcription factor regulating many phase II detoxifying and cytoprotective enzymes, such as heme oxygenase 1 (HMOX1). In HaCaT cells, FB and EC (200 muM) induced nuclear translocation of NRF2 protein after 24 h and reduced nuclear protein levels of BACH1, a repressor of the antioxidant response element. FB and EC greatly HMOX1 mRNA levels by more than 40-fold in 72 h. Cytoplasmic HMOX1 protein levels were also increased at 48 h after treatment. VB was less active compared to FB and EC, and CP was slightly active only at later times of treatment. We suggest that hydroxytyrosol (HYD) could be a potential bioactive metabolite of PPGs since HYD, in equimolar amounts to PGGs, is able to both activate HO-1

transcription and modify Nrf2/Bach1 nuclear protein levels. This is in agreement with the poor activity of CP, which contains a HYD moiety modified by an O-methyl group. In conclusion, FB and EC from plant cell cultures may provide long-lasting skin protection by induction of phase II cytoprotective capabilities.

Shen, G., et al. (2005). "Comparison of (-)-epigallocatechin-3-gallate elicited liver and small intestine gene expression profiles between C57BL/6J mice and C57BL/6J/Nrf2 (-/-) mice." Pharm Res **22**(11): 1805-1820.

Shen, W., et al. (2008). "R-alpha-lipoic acid and acetyl-L-carnitine complementarily promote mitochondrial biogenesis in murine 3T3-L1 adipocytes." Diabetologia **51**(1): 165-174.

AIMS/HYPOTHESIS: The aim of the study was to address the importance of mitochondrial function in insulin resistance and type 2 diabetes, and also to identify effective agents for ameliorating insulin resistance in type 2 diabetes. We examined the effect of two mitochondrial nutrients, R-alpha-lipoic acid (LA) and acetyl-L-carnitine (ALC), as well as their combined effect, on mitochondrial biogenesis in 3T3-L1 adipocytes. METHODS: Mitochondrial mass and oxygen consumption were determined in 3T3-L1 adipocytes cultured in the presence of LA and/or ALC for 24 h. Mitochondrial DNA and mRNA from peroxisome proliferator-activated receptor gamma and alpha (Pparg and Ppara) and carnitine palmitoyl transferase 1a (Cpt1a), as well as several transcription factors involved in mitochondrial biogenesis, were evaluated by real-time PCR or electrophoretic mobility shift (EMSA) assay. Mitochondrial complexes proteins were measured by western blot and fatty acid oxidation was measured by quantifying CO2 production from [1-14C]palmitate. RESULTS: Treatments with the combination of LA and ALC at concentrations of 0.1, 1 and 10 micromol/l for 24 h significantly increased mitochondrial mass, expression of mitochondrial DNA, mitochondrial complexes, oxygen consumption and fatty acid oxidation in 3T3L1 adipocytes. These changes were accompanied by an increase in expression of Pparg, Ppara and Cpt1a mRNA, as well as increased expression of peroxisome proliferator-activated receptor (PPAR) gamma coactivator 1 alpha (Ppargc1a), mitochondrial transcription factor A (Tfam) and nuclear respiratory factors 1 and 2 (Nrf1 and Nrf2). However, the treatments with LA or ALC alone at the same concentrations showed little effect on mitochondrial function and biogenesis. CONCLUSIONS/INTERPRETATION: We conclude that the combination of LA and ALC may act as PPARG/A dual ligands to complementarily promote mitochondrial synthesis and adipocyte metabolism.

Shi, Y., et al. (2013). "Quercetin protects rat dorsal root ganglion neurons against high glucose-induced injury in vitro through Nrf-2/HO-1 activation and NF-kappaB inhibition." Acta Pharmacol Sin **34**(9): 1140-1148.

AIM: To examine the effects of quercetin, a natural antioxidant, on high glucose (HG)-induced apoptosis of cultured dorsal root ganglion (DRG) neurons of rats. METHODS: DRG neurons exposed to HG (45 mmol/L) for 24 h were employed as an in vitro model of diabetic neuropathy. Cell viability, reactive oxygen species (ROS) level and apoptosis were determined. The expression of NF-small ka, CyrillicB, Ismall ka, CyrillicBalpha, phosphorylated Ismall ka, CyrillicBalpha and Nrf2 was examined using RT PCR and Western blot assay. The expression of hemeoxygenase-1 (HO-1), IL-6, TNF-alpha, iNOS, COX-2, and caspase-3 were also examined. RESULTS: HG treatment markedly increased DRG neuron apoptosis via increasing intracellular ROS level and activating the NF-kappaB signaling pathway. Co-treatment with quercetin (2.5, 5, and 10 mmol/L) dose-dependently decreased HG-induced caspase-3 activation and apoptosis. Quercetin could directly scavenge ROS and significantly increased the expression of Nrf-2 and HO-1 in DRG neurons. Quercetin also dose-dependently inhibited the NF-kappaB signaling pathway and suppressed the expression of iNOS, COX-2, and proinflammatory cytokines IL-6 and TNF-alpha. CONCLUSION: Quercetin protects rat DRG neurons against HG-induced injury in vitro through Nrf-2/HO-1 activation and NF-kappaB inhibition, thus may be beneficial for the treatment of diabetic neuropathy.

Shin, S. M., et al. (2009). "Resveratrol protects mitochondria against oxidative stress through AMP-activated protein kinase-mediated glycogen synthase kinase-3beta inhibition downstream of poly(ADP-ribose)polymerase-LKB1 pathway." Mol Pharmacol **76**(4): 884-895.

Arachidonic acid (AA, a proinflammatory fatty acid) in combination with iron promotes excess reactive oxygen species (ROS) production and exerts a deleterious effect on mitochondria. We have shown previously that activation of AMP-activated protein kinase (AMPK) protects hepatocytes from AA + iron-induced apoptosis. Resveratrol, a polyphenol in grapes, has beneficial effects mediated through SIRT1, LKB1, and AMPK. This study investigated the potential of resveratrol to protect against the mitochondrial impairment induced by AA + iron and the underlying mechanism for this cytoprotection. Resveratrol treatment inhibited apoptosis, ROS production, and glutathione depletion elicited by AA + iron in HepG2 cells. In addition, resveratrol attenuated superoxide generation in mitochondria and inhibited mitochondrial dysfunction induced by AA + iron. Overall, AMPK activation by resveratrol contributed to cell survival, as supported by the reversal of its restoration of mitochondrial membrane potential by either overexpression of a dominant-negative mutant of AMPKalpha or compound C treatment. Resveratrol increased inhibitory phosphorylation of glycogen synthase kinase-3beta (GSK3beta) downstream of AMPK, which contributed to mitochondrial protection and cell survival. Likewise, small interfering RNA knockdown of LKB1, an upstream kinase of AMPK, reduced the ability of resveratrol to protect cells from mitochondrial

dysfunction. Furthermore, this LKB1-dependent mitochondrial protection resulted from resveratrol's poly(ADP-ribose)polymerase activation, but not SIRT1 activation, as supported by the experiment using 3-aminobenzamide, a poly(ADP-ribose)polymerase inhibitor. Other polyphenols, such as apigenin, genistein, and daidzein, did not activate AMPK or protect mitochondria against AA + iron. Thus, resveratrol protects cells from AA + iron-induced ROS production and mitochondrial dysfunction through AMPK-mediated inhibitory phosphorylation of GSK3beta downstream of poly(ADP-ribose)polymerase-LKB1 pathway.

Shishodia, S. and B. B. Aggarwal (2004). "Guggulsterone inhibits NF-kappaB and IkappaBalpha kinase activation, suppresses expression of anti-apoptotic gene products, and enhances apoptosis." J Biol Chem **279**(45): 47148-47158.

Guggulsterone, derived from Commiphora mukul and used to treat obesity, diabetes, hyperlipidemia, atherosclerosis, and osteoarthritis, has been recently shown to antagonize the farnesoid X receptor and decrease the expression of bile acid-activated genes. Because activation of NF-kappaB has been closely linked with inflammatory diseases affected by guggulsterone, we postulated that it must modulate NF-kappaB activation. In the present study, we tested this hypothesis by investigating the effect of this steroid on the activation of NF-kappaB induced by inflammatory agents and carcinogens. Guggulsterone suppressed DNA binding of NF-kappaB induced by tumor necrosis factor (TNF), phorbol ester, okadaic acid, cigarette smoke condensate, hydrogen peroxide, and interleukin-1. NF-kappaB activation was not cell type-specific, because both epithelial and leukemia cells were inhibited. Guggulsterone also suppressed constitutive NF-kappaB activation expressed in most tumor cells. Through inhibition of IkappaB kinase activation, this steroid blocked IkappaBalpha phosphorylation and degradation, thus suppressing p65 phosphorylation and nuclear translocation. NF-kappaB-dependent reporter gene transcription induced by TNF, TNFR1, TRADD, TRAF2, NIK, and IKK was also blocked by guggulsterone but without affecting p65-mediated gene transcription. In addition, guggulsterone decreased the expression of gene products involved in anti-apoptosis (IAP1, xIAP, Bfl-1/A1, Bcl-2, cFLIP, and survivin), proliferation (cyclin D1 and c-Myc), and metastasis (MMP-9, COX-2, and VEGF); this correlated with enhancement of apoptosis induced by TNF and chemotherapeutic agents. Overall, our results indicate that guggulsterone suppresses NF-kappaB and NF-kappaB-regulated gene products, which may explain its anti-inflammatory activities.

Singh, D. K., et al. (2009). "Green and black tea extracts inhibit HMG-CoA reductase and activate AMP kinase to decrease cholesterol synthesis in hepatoma cells." J Nutr Biochem **20**(10): 816-822.

Recent studies have demonstrated that green and black tea consumption can lower serum cholesterol in animals and in man, and suppression of hepatic cholesterol synthesis is suggested to contribute to this effect. To evaluate this hypothesis, we measured cholesterol synthesis in cultured rat hepatoma cells in the presence of green and black tea extracts and selected components. Green and black tea decreased cholesterol synthesis by up to 55% and 78%, respectively, as measured by a 3-h incorporation of radiolabeled acetate. Inhibition was much less evident when radiolabeled mevalonate was used, suggesting that the inhibition was mediated largely at or above the level of HMG-CoA reductase. Both extracts directly inhibited HMG-CoA reductase when added to microsomal preparations, although the extent of inhibition was considerably less than the decrease in cholesterol synthesis observed in whole cells. As HMG-CoA reductase activity also can be decreased by enzyme phosphorylation by AMP kinase, the phosphorylation state of HMG-CoA reductase and AMP kinase, which is activated by phosphorylation, was determined in lysates from cells treated with tea extracts. Both extracts increased AMP-kinase phosphorylation and HMG-CoA reductase phosphorylation by 2.5- to 4-fold, but with different time courses: maximal phosphorylation with green tea was evident within 30 min of treatment, whereas with black tea phosphorylation was slower to develop, with maximal phosphorylation occurring > or =3 hours after treatment. These results suggest that both green and black tea decrease cholesterol synthesis in whole cells by directly inhibiting HMG-CoA reductase and by promoting its inactivation by AMP kinase.

Singh, D. K., et al. (2006). "Policosanol inhibits cholesterol synthesis in hepatoma cells by activation of AMP-kinase." J Pharmacol Exp Ther **318**(3): 1020-1026.

Policosanol is a mixture of long-chain primary alcohols that has been shown to decrease serum cholesterol in animals and in humans. The hypocholesterolemic effect results from a decrease in cholesterol synthesis by suppression of HMG-CoA reductase activity, but the mechanism of this suppression and the active components of policosanol have not been established. In the present study, we investigated the ability of policosanol and its principal components to inhibit cholesterol synthesis in cultured rat hepatoma cells. Maximal inhibition by policosanol yielded a 30% decrease in [(14)C]acetate incorporation without evidence of cellular toxicity. Octacosanol (C28, the major constituent of policosanol), heptacosanol (C27), and hexacosanol (C26) yielded smaller and statistically insignificant decreases in cholesterol synthesis, whereas triacontanol (1-hydroxytriacontane; C30) replicated the inhibition obtained with policosanol. At pharmacological concentrations (<5 microg/ml), policosanol and triacontanol decreased [(14)C]acetate incorporation into cholesterol without affecting the incorporation of [(14)C]mevalonate, indicating that these compounds act at or above HMG-CoA reductase.

Policosanol and triacontanol did not directly inhibit HMG-CoA reductase, and incubation of these compounds with hepatoma cells did not affect reductase enzyme levels. However, reductase activity was decreased by up to 55% in lysates prepared from these cells, suggesting that HMG-CoA reductase activity was down-regulated by policosanol treatment. Consistent with this hypothesis, a 3-fold increase in AMP-kinase phosphorylation was noted in policosanol-treated cells. Because AMP-kinase is activated by phosphorylation and is well established to suppress HMG-CoA reductase activity, these results suggest that policosanol or a metabolite decreases HMG-CoA reductase activity by activating AMP-kinase.

Skrobuk, P., et al. (2012). "Acute exposure to resveratrol inhibits AMPK activity in human skeletal muscle cells." Diabetologia **55**(11): 3051-3060.

AIMS/HYPOTHESIS: Recent studies have suggested resveratrol (RSV) as a new natural therapeutic agent to treat type 2 diabetes and lipid-induced insulin resistance. Here, we investigated whether RSV could reverse palmitate-induced insulin resistance in human primary muscle cells. METHODS: Myotubes obtained from six healthy men (54 +/- 3 years (mean +/- SE), BMI 25.0 +/- 1.7 kg/m(2), fasting plasma glucose concentration (fP-glucose) 5.47 +/- 0.09 mmol/l) were treated for 4 h with 100 mumol/l RSV and/or 0.2 mmol/l palmitate, and stimulated with or without 100 nmol/l insulin. Assays of glucose uptake, glycogen synthesis, palmitate oxidation, intracellular signalling and AMP-activated protein kinase (AMPK) activity were performed. RESULTS: RSV did not reverse palmitate-induced impairment of glucose metabolism. Surprisingly, RSV decreased glucose uptake and glycogen synthesis in human skeletal muscle cells. Palmitate oxidation and phosphorylation of AMPK and its downstream target acetyl-CoA carboxylase beta (ACCbeta) were inhibited by RSV, and RSV completely blocked the activity of AMPK isoform complexes alpha1/beta2/gamma1 and alpha2/beta2/gamma1 in in-vitro kinase activity assays. Endoplasmic reticulum (ER) stress was increased in response to RSV, as indicated by increased phosphorylation of eukaryotic initiation factor 2alpha (eIF2alpha) and increased expression of CCAAT/enhancer binding protein homologous protein (CHOP). CONCLUSIONS/INTERPRETATION: Acute exposure to RSV inhibits AMPK activity, fatty-acid oxidation and glucose metabolism in human myotubes.

Sriram, N., et al. (2009). "Epigallocatechin-3-gallate augments antioxidant activities and inhibits inflammation during bleomycin-induced experimental pulmonary fibrosis through Nrf2-Keap1 signaling." Pulm Pharmacol Ther **22**(3): 221-236.

Stites, T., et al. (2006). "Pyrroloquinoline quinone modulates mitochondrial quantity and function in mice." J Nutr **136**(2): 390-396.

When pyrroloquinoline quinone (PQQ) is added to an amino acid-based, but otherwise nutritionally complete basal diet, it improves growth-related variables in young mice. We examined PQQ and mitochondrial function based on observations that PQQ deficiency results in elevated plasma glucose concentrations in young mice, and PQQ addition stimulates mitochondrial complex 1 activity in vitro. PQQ-deficient weanling mice had a 20-30% reduction in the relative amount of mitochondria in liver; lower respiratory control ratios, and lower respiratory quotients than PQQ-supplemented mice (2 mg PQQ/kg diet). In mice from dams fed a conventional laboratory diet, but switched at weaning to the basal diet, plasma glucose, Ala, Gly, and Ser concentrations were elevated at 4 wk (PQQ- vs. PQQ+), but not at 8 wk. The relative mitochondrial content (ratio of mtDNA to nuclear DNA) also tended ($P<0.18$) to be lower (PQQ- vs. PQQ+) at 4 wk, but not at 8 wk. PQQ also counters the mitochondrial complex 1 inhibitor, diphenylene iodonium (DPI). Mice were gavaged with 0, 0.4, or 4 microg PQQ/g body weight (BW) daily for 14 d. At each PQQ level, DPI was injected (i.p.) at 0, 0.4, 0.8, or 1.6 microg DPI/g BW. The PQQ-deficient mice exposed to 0.4 or 4.0 microg DPI/g lost weight and had lower plasma glucose levels than PQQ-supplemented mice ($P<0.05$). In addition, fibroblasts took up (3)H-PQQ added to cell cultures, and cultured hepatocytes maintained mitochondrial PQQ concentrations similar to those observed in vivo. Collectively, these results indicate that dietary PQQ can influence mitochondrial amount and function, particularly in perinatal and weanling mice.

Suchankova, G., et al. (2005). "Dietary polyunsaturated fatty acids enhance hepatic AMP-activated protein kinase activity in rats." Biochem Biophys Res Commun **326**(4): 851-858.

Polyunsaturated fatty acids (PUFA) and a number of drugs (metformin, thiazolidinediones) and hormones (leptin, adiponectin) that activate AMP-activated protein kinase (AMPK) have been reported to improve insulin sensitivity. To determine whether PUFA activate AMPK, Sprague-Dawley rats were adapted to a 3h meal-feeding regimen using a fat-free diet (FFD) supplemented with fish oil (n-3) or triolein (n-9) for 7 days. No differences in hepatic AMPK activity were observed between the groups after 21h of fasting. On the other hand, hepatic AMPK phosphorylation was decreased in rats refed the FFD, the FFD+triolein, and the FFD+PUFA by 80%, 75%, and 50%, respectively, when assessed 2h after completion of a meal. In keeping with these changes, decreases in acetyl-CoA carboxylase phosphorylation and carnitine palmitoyl transferase-1 mRNA and increases in fatty acid synthase gene expression were greatest in rats fed the FFD and least in the PUFA-fed rats. The results indicate that dietary PUFA enhance hepatic AMPK activity in vivo, and implicate AMPK as a component of the nutrient-

sensing mechanism through which dietary fatty acids and especially PUFA influence the regulation of hepatic lipid metabolism and gene expression.

Suh, J. H., et al. (2004). "Decline in transcriptional activity of Nrf2 causes age-related loss of glutathione synthesis, which is reversible with lipoic acid." Proc Natl Acad Sci U S A **101**(10): 3381-3386.

Glutathione (GSH) significantly declines in the aging rat liver. Because GSH levels are partly a reflection of its synthetic capacity, we measured the levels and activity of gamma-glutamylcysteine ligase (GCL), the rate-controlling enzyme in GSH synthesis. With age, both the catalytic (GCLC) and modulatory (GCLM) subunits of GCL decreased by 47% and 52%, respectively (P < 0.005). Concomitant with lower subunit levels, GCL activity also declined by 53% (P < 0.05). Because nuclear factor erythroid2-related factor 2 (Nrf2) governs basal and inducible GCLC and GCLM expression by means of the antioxidant response element (ARE), we hypothesized that aging results in dysregulation of Nrf2-mediated GCL expression. We observed an approximately 50% age-related loss in total (P < 0.001) and nuclear (P < 0.0001) Nrf2 levels, which suggests attenuation in Nrf2-dependent gene transcription. By using gel-shift and supershift assays, a marked reduction in Nrf2/ARE binding in old vs. young rats was noted. To determine whether the constitutive loss of Nrf2 transcriptional activity also affects the inducible nature of Nrf2 nuclear translocation, old rats were treated with (R)-alpha-lipoic acid (LA; 40 mg/kg i.p. up to 48 h), a disulfide compound shown to induce Nrf2 activation in vitro and improve GSH levels in vivo. LA administration increased nuclear Nrf2 levels in old rats after 12 h. LA also induced Nrf2 binding to the ARE, and, consequently, higher GCLC levels and GCL activity were observed 24 h after LA injection. Thus, the age-related loss in GSH synthesis may be caused by dysregulation of ARE-mediated gene expression, but chemoprotective agents, like LA, can attenuate this loss.

Takahashi, T., et al. (2009). "Carnosic acid and carnosol inhibit adipocyte differentiation in mouse 3T3-L1 cells through induction of phase2 enzymes and activation of glutathione metabolism." Biochem Biophys Res Commun **382**(3): 549-554.

Tamaki, Y., et al. (2010). "Activated glutathione metabolism participates in protective effects of carnosic acid against oxidative stress in neuronal HT22 cells." Planta Med **76**(7): 683-688.

Tanigawa, S., et al. (2007). "Action of Nrf2 and Keap1 in ARE-mediated NQO1 expression by quercetin." Free Radic Biol Med **42**(11): 1690-1703.

Polyphenols are characterized by the presence of more than one phenolic group and are widely distributed in many fruits and vegetables. They possess antioxidant properties and interact with cellular defense systems through the antioxidant-responsive element/electrophile-responsive element (ARE/EpRE) although the precise mechanism by which polyphenols influence transcription factor complexes to target ARE is poorly understood. In the present study, we chose a typical polyphenol, quercetin, to investigate the mechanism in human HepG2 cells. Quercetin enhanced the ARE binding activity and Nrf2-mediated transcription activity. Molecular evidence revealed that quercetin not only up-regulated the expression of Nrf2 mRNA and protein, but also stabilized Nrf2 protein by inhibiting the ubiquitination and proteasomal turnover of Nrf2. At the same time, quercetin markedly reduced the level of Keap1 protein in posttranslational levels through the formation of modified Keap1 protein, rather than 26S proteasome-dependent degradation mechanisms, without affecting the dissociation of Keap1-Nrf2. Silencing Keap1 using Keap1 siRNA significantly increased the Nrf2-dependent ARE activity, whereas silencing Nrf2 using Nrf2 siRNA markedly reduced the ARE activity under both baseline and quercetin-induced conditions. Thus, we conclude that the pathway of quercetin-induced ARE activity involves up-regulation of Nrf2 through the regulation of both transcription and posttranscription sites and repression of Keap1 by affecting the posttranscription site, revealing some substantial differences between oxidative inducers. Thus, the findings provide an insight into the mechanisms underlying polyphenolic compounds in cytoprotection and cancer chemoprevention.

Tao, R., et al. (2007). "Pyrroloquinoline quinone preserves mitochondrial function and prevents oxidative injury in adult rat cardiac myocytes." Biochem Biophys Res Commun **363**(2): 257-262.

We investigated the ability of pyrroloquinoline quinone (PQQ) to confer resistance to acute oxidative stress in freshly isolated adult male rat cardiomyocytes. Fluorescence microscopy was used to detect generation of reactive oxygen species (ROS) and mitochondrial membrane potential (Deltapsi(m)) depolarization induced by hydrogen peroxide. H(2)O(2) caused substantial cell death, which was significantly reduced by preincubation with PQQ. H(2)O(2) also caused an increase in cellular ROS levels as detected by the fluorescent indicators CM-H2XRos and dihydroethidium. ROS levels were significantly reduced by a superoxide dismutase mimetic Mn (III) tetrakis (4-benzoic acid) porphyrin chloride (MnTBAP) or by PQQ treatment. Cyclosporine-A, which inhibits mitochondrial permeability transition, prevented H(2)O(2)-induced Deltapsi(m) depolarization, as did PQQ and MnTBAP. Our results provide direct evidence that PQQ reduces oxidative stress, mitochondrial dysfunction, and cell death in isolated adult rat cardiomyocytes. These findings provide new insight into the mechanisms of PQQ

action in the heart.

Tellez, L. A., et al. (2013). "A gut lipid messenger links excess dietary fat to dopamine deficiency." Science **341**(6147): 800-802.

Excessive intake of dietary fats leads to diminished brain dopaminergic function. It has been proposed that dopamine deficiency exacerbates obesity by provoking compensatory overfeeding as one way to restore reward sensitivity. However, the physiological mechanisms linking prolonged high-fat intake to dopamine deficiency remain elusive. We show that administering oleoylethanolamine, a gastrointestinal lipid messenger whose synthesis is suppressed after prolonged high-fat exposure, is sufficient to restore gut-stimulated dopamine release in high-fat-fed mice. Administering oleoylethanolamine to high-fat-fed mice also eliminated motivation deficits during flavorless intragastric feeding and increased oral intake of low-fat emulsions. Our findings suggest that high-fat-induced gastrointestinal dysfunctions play a key role in dopamine deficiency and that restoring gut-generated lipid signaling may increase the reward value of less palatable, yet healthier, foods.

Tennen, R. I., et al. (2012). "Finding a target for resveratrol." Cell **148**(3): 387-389.

Despite resveratrol's well-documented health benefits, its mechanism of action remains controversial. In particular, the direct molecular target of resveratrol has been elusive. Park et al. now show that resveratrol directly inhibits cAMP-dependent phosphodiesterases, triggering a cascade of events that converge on the important energy-sensing metabolic regulators AMPK, SIRT1, and PGC-1alpha.

Tsai, C. W., et al. (2011). "Carnosic acid induces the NAD(P)H: quinone oxidoreductase 1 expression in rat clone 9 cells through the p38/nuclear factor erythroid-2 related factor 2 pathway." J Nutr **141**(12): 2119-2125.

Tsai, P. Y., et al. (2011). "Epigallocatechin-3-gallate prevents lupus nephritis development in mice via enhancing the Nrf2 antioxidant pathway and inhibiting NLRP3 inflammasome activation." Free Radic Biol Med **51**(3): 744-754.

Tzeng, T. F., et al. (2012). "Emodin protects against high-fat diet-induced obesity via regulation of AMP-activated protein kinase pathways in white adipose tissue." Planta Med **78**(10): 943-950.

Emodin is an active herbal component traditionally used in China for treating a variety of diseases. The aim of this study was to examine the effect of emodin on the reducing lipid accumulation in white adipose tissue of high-fat diet-fed rats, and on the regulation of the expression of the genes involved in lipid metabolism to elucidate the mechanisms. After being fed a high-fat diet for two weeks, rats were dosed orally with emodin (20, 40, 80 mg/kg/day) or pioglitazone (20 mg/kg/day), once daily for eight weeks. Changes in body weight, feeding pattern, serum lipids, coronary artery risk index, and atherogenic index were investigated. Subcutaneous white adipose tissues were isolated for pathology histology and Western blot analyses. Changes of triglyceride accumulation in differentiated 3 T3-L1 adipocytes were also investigated. Emodin exhibited a significant concentration-dependent decrease in the intracellular accumulation of triglyceride in 3 T3-L1 adipocytes. Emodin (80 mg/kg/day) displayed similar characteristics to pioglitazone (20 mg/kg/day) in reducing body weight gain and plasma lipid levels as well as the coronary artery risk and atherogenic indices of high-fat diet-fed rats. Emodin also caused dose related reductions in epididymal white adipose tissue sizes in high-fat diet-fed rats. Emodin and pioglitazone enhanced the phosphorylation of AMP-activated protein kinase and its primary downstream targeting enzyme, acetyl-CoA carboxylase, upregulated gene expression of carnitine palmitoyl transferase 1, and downregulated sterol regulatory element binding protein 1 and fatty acid synthase protein levels in the epididymal white adipose tissue of high-fat diet-fed rats. Our findings suggest that emodin could attenuate lipid accumulation in white adipose tissue through AMP-activated protein kinase activation.

Tzeng, T. F., et al. (2013). "Reduction of lipid accumulation in white adipose tissues by Cassia tora (Leguminosae) seed extract is associated with AMPK activation." Food Chem **136**(2): 1086-1094.

Natural herbal medications may be one answer to the worldwide epidemic of obesity. This study examines the effects of Cassia seed ethanol extract (CSEE) upon lipid accumulation in white adipose tissue (WAT). CSEE exhibited a significant concentration-dependent decrease in the intracellular accumulation of trigycerides in 3T3-L1 adipocytes. After being fed a high-fat diet (HFD) for 2 weeks, rats were fed CSEE (100, 200 or 300 mg/kg) once daily for 8 weeks. CSEE caused dose-related reductions in body weight gain (as well as plasma lipid levels and epididymal WAT sizes in HFD-fed rats). CSEE enhanced the phosphorylation of AMP-activated protein kinase (AMPK) and its primary downstream targeting enzyme, acetyl-CoA carboxylase, up-regulated gene expression of carnitine palmitoyl transferase 1, and down-regulated sterol regulatory element-binding protein 1 and fatty acid synthase protein levels in epididymal WAT of HFD-fed rats. CSEE could attenuate lipid accumulation in WAT via AMPK signaling pathway activation.

Urizar, N. L., et al. (2002). "A natural product that lowers cholesterol as an antagonist ligand for FXR." Science

296(5573): 1703-1706.

Extracts of the resin of the guggul tree (Commiphora mukul) lower LDL (low-density lipoprotein) cholesterol levels in humans. The plant sterol guggulsterone [4,17(20)-pregnadiene-3,16-dione] is the active agent in this extract. We show that guggulsterone is a highly efficacious antagonist of the farnesoid X receptor (FXR), a nuclear hormone receptor that is activated by bile acids. Guggulsterone treatment decreases hepatic cholesterol in wild-type mice fed a high-cholesterol diet but is not effective in FXR-null mice. Thus, we propose that inhibition of FXR activation is the basis for the cholesterol-lowering activity of guggulsterone. Other natural products with specific biologic effects may modulate the activity of FXR or other relatively promiscuous nuclear hormone receptors.

Vingtdeux, V., et al. (2010). "AMP-activated protein kinase signaling activation by resveratrol modulates amyloid-beta peptide metabolism." J Biol Chem **285**(12): 9100-9113.

Alzheimer disease is an age-related neurodegenerative disorder characterized by amyloid-beta (Abeta) peptide deposition into cerebral amyloid plaques. The natural polyphenol resveratrol promotes anti-aging pathways via the activation of several metabolic sensors, including the AMP-activated protein kinase (AMPK). Resveratrol also lowers Abeta levels in cell lines; however, the underlying mechanism responsible for this effect is largely unknown. Moreover, the bioavailability of resveratrol in the brain remains uncertain. Here we show that AMPK signaling controls Abeta metabolism and mediates the anti-amyloidogenic effect of resveratrol in non-neuronal and neuronal cells, including in mouse primary neurons. Resveratrol increased cytosolic calcium levels and promoted AMPK activation by the calcium/calmodulin-dependent protein kinase kinase-beta. Direct pharmacological and genetic activation of AMPK lowered extracellular Abeta accumulation, whereas AMPK inhibition reduced the effect of resveratrol on Abeta levels. Furthermore, resveratrol inhibited the AMPK target mTOR (mammalian target of rapamycin) to trigger autophagy and lysosomal degradation of Abeta. Finally, orally administered resveratrol in mice was detected in the brain where it activated AMPK and reduced cerebral Abeta levels and deposition in the cortex. These data suggest that resveratrol and pharmacological activation of AMPK have therapeutic potential against Alzheimer disease.

Visioli, F., et al. (2000). "Olive phenol hydroxytyrosol prevents passive smoking-induced oxidative stress." Circulation **102**(18): 2169-2171.

Wagner, A. E., et al. (2012). "A combination of lipoic acid plus coenzyme Q10 induces PGC1alpha, a master switch of energy metabolism, improves stress response, and increases cellular glutathione levels in cultured C2C12 skeletal muscle cells." Oxid Med Cell Longev **2012**: 835970.

Skeletal muscle function largely depend on intact energy metabolism, stress response, and antioxidant defense mechanisms. In this study, we tested the effect of a combined supplementation of alpha-lipoic acid (LA) plus coenzyme Q10 (Q10) on PPARgamma-coactivator alpha (PGC1alpha) activity, expression of glutathione-related phase II enzymes and glutathione (GSH) levels in cultured C2C12 myotubes. Supplementation of myotubes with 250 mumol/L LA plus 100 mumol/L Q10 significantly increased nuclear levels of PGC1alpha, a master switch of energy metabolism and mitochondrial biogenesis. The increase of nuclear PGC1alpha was accompanied by an increase in PPARgamma transactivation, a downstream target of PGC1alpha, and an increase in mitochondrial transcription factor A mRNA centrally involved in mitochondrial replication and transcription. Furthermore, supplementation of myotubes with LA plus Q10 resulted in an increase of genes encoding proteins involved in stress response, GSH synthesis, and its recycling. In LA-plus-Q10-treated myotubes a significant 4-fold increase in GSH was evident. This increase in GSH was accompanied by increased nuclear Nrf2 protein levels, partly regulating gammaGCS and GST gene expression. Present data suggest that the combined supplementation of skeletal muscle cells with LA plus Q10 may improve energy homeostasis, stress response, and antioxidant defense mechanisms.

Wang, R., et al. (2010). "Curcumin produces neuroprotective effects via activating brain-derived neurotrophic factor/TrkB-dependent MAPK and PI-3K cascades in rodent cortical neurons." Prog Neuropsychopharmacol Biol Psychiatry **34**(1): 147-153.

Curcumin is a major constituent of curcuma longa, a traditional medicine used to manage mental disorders effectively in China. The neuroprotective effects of curcumin have been demonstrated in our previous studies. In the present research, we confirmed this effect by showing that curcumin application promoted the viability of cultured rodent cortical neurons. Moreover, when neurons were pretreated with tyrosine kinase B (TrkB) antibody, known to inhibit the activity of brain-derived neurotrophic factor (BDNF), the protective effect of curcumin was blocked. Additionally, treatment of curcumin increased BDNF and phosphor-TrkB and both of these enhancements can be suppressed by ERK and PI-3K inhibitors. The administration of curcumin led to increased levels of phosphor-ERK and AKT, which were each blocked by MAPK and PI-3K inhibitors. Furthermore, the curcumin-induced increase in phosphorylated cyclic AMP response element binding protein (CREB), which has been implicated as a possible mediator of antidepressant actions, was prevented by MAPK and PI-3K inhibitors.

Therefore, we hypothesize the neuroprotection of curcumin might be mediated via BDNF/TrkB-MAPK/PI-3K-CREB signaling pathway.

Wilkins, H. M., et al. (2014). "Oxaloacetate activates brain mitochondrial biogenesis, enhances the insulin pathway, reduces inflammation and stimulates neurogenesis." Hum Mol Genet **23**(24): 6528-6541.

Brain bioenergetic function declines in some neurodegenerative diseases, this may influence other pathologies and administering bioenergetic intermediates could have therapeutic value. To test how one intermediate, oxaloacetate (OAA) affects brain bioenergetics, insulin signaling, inflammation and neurogenesis, we administered intraperitoneal OAA, 1-2 g/kg once per day for 1-2 weeks, to C57Bl/6 mice. OAA altered levels, distributions or post-translational modifications of mRNA and proteins (proliferator-activated receptor-gamma coactivator 1alpha, PGC1 related co-activator, nuclear respiratory factor 1, transcription factor A of the mitochondria, cytochrome oxidase subunit 4 isoform 1, cAMP-response element binding, p38 MAPK and adenosine monophosphate-activated protein kinase) in ways that should promote mitochondrial biogenesis. OAA increased Akt, mammalian target of rapamycin and P70S6K phosphorylation. OAA lowered nuclear factor kappaB nucleus-to-cytoplasm ratios and CCL11 mRNA. Hippocampal vascular endothelial growth factor mRNA, doublecortin mRNA, doublecortin protein, doublecortin-positive neuron counts and neurite length increased in OAA-treated mice. (1)H-MRS showed OAA increased brain lactate, GABA and glutathione thereby demonstrating metabolic changes are detectable in vivo. In mice, OAA promotes brain mitochondrial biogenesis, activates the insulin signaling pathway, reduces neuroinflammation and activates hippocampal neurogenesis.

Wood, J. G., et al. (2004). "Sirtuin activators mimic caloric restriction and delay ageing in metazoans." Nature **430**(7000): 686-689.

Caloric restriction extends lifespan in numerous species. In the budding yeast Saccharomyces cerevisiae this effect requires Sir2 (ref. 1), a member of the sirtuin family of NAD+-dependent deacetylases. Sirtuin activating compounds (STACs) can promote the survival of human cells and extend the replicative lifespan of yeast. Here we show that resveratrol and other STACs activate sirtuins from Caenorhabditis elegans and Drosophila melanogaster, and extend the lifespan of these animals without reducing fecundity. Lifespan extension is dependent on functional Sir2, and is not observed when nutrients are restricted. Together these data indicate that STACs slow metazoan ageing by mechanisms that may be related to caloric restriction.

Wu, J., et al. (2002). "The hypolipidemic natural product guggulsterone acts as an antagonist of the bile acid receptor." Mol Endocrinol **16**(7): 1590-1597.

Ayurveda, the ancient Indian system of health care and medicine, has a well-organized materia medica in which plants form a dominant part. A key illustration of the exploitation of this knowledge toward the development of a modern drug is the isolation and characterization of two antihyperlipidemic compounds, Z-, and E-guggulsterone from the tree Commiphora mukul, the exudate of which has been traditionally used for mitigating lipid disorders. Here, we demonstrate that Z-guggulsterone and an analog, 80-574 currently in clinical trials, act as antagonists of the bile acid receptor (BAR), a member of the intracellular receptor superfamily. These compounds antagonize the activity of BAR in vitro, and in cell culture systems on promoters and endogenous target genes. In biochemical assays, they are able to displace coactivator peptides from the receptor in a dose-dependent manner. The mechanism by which they act as BAR antagonists is likely through their inability to recruit coactivator proteins, failure to release corepressor proteins from unliganded receptor, and ability to compete with BAR agonists to block coactivator recruitment. Our data suggest these compounds may mediate at least some of their effects via the BAR.

Wu, K. C., et al. (2014). "Screening of natural compounds as activators of the keap1-nrf2 pathway." Planta Med **80**(1): 97-104.

Xue, B., et al. (2012). "Omega-3 polyunsaturated fatty acids antagonize macrophage inflammation via activation of AMPK/SIRT1 pathway." PLoS One **7**(10): e45990.

Macrophages play a key role in obesity-induced inflammation. Omega-3 polyunsaturated fatty acids (omega-3 PUFAs) eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) exert anti-inflammatory functions in both humans and animal models, but the exact cellular signals mediating the beneficial effects are not completely understood. We previously found that two nutrient sensors AMP-activated protein kinase (AMPK) and SIRT1 interact to regulate macrophage inflammation. Here we aim to determine whether omega-3 PUFAs antagonize macrophage inflammation via activation of AMPK/SIRT1 pathway. Treatment of omega-3 PUFAs suppresses lipopolysaccharide (LPS)-induced cytokine expression in macrophages. Luciferase reporter assays, electrophoretic mobility shift assays (EMSA) and Chromatin immunoprecipitation (ChIP) assays show that treatment of macrophages with omega-3 PUFAs significantly inhibits LPS-induced NF-kappaB signaling. Interestingly, DHA also increases expression, phosphorylation and activity of the major isoform alpha1AMPK, which further leads to SIRT1 over-expression. More importantly, DHA mimics the effect of SIRT1 on deacetylation of the NF-kappaB subunit p65,

and the ability of DHA to deacetylate p65 and inhibit its signaling and downstream cytokine expression require SIRT1. In conclusion, omega-3 PUFAs negatively regulate macrophage inflammation by deacetylating NF-kappaB, which acts through activation of AMPK/SIRT1 pathway. Our study defines AMPK/SIRT1 as a novel cellular mediator for the anti-inflammatory effects of omega-3 PUFAs.

Yang, C. M., et al. (2012). "Apo-8'-lycopenal induces expression of HO-1 and NQO-1 via the ERK/p38-Nrf2-ARE pathway in human HepG2 cells." J Agric Food Chem **60**(6): 1576-1585.

Yang, Y., et al. (2014). "Alpha-lipoic acid improves high-fat diet-induced hepatic steatosis by modulating the transcription factors SREBP-1, FoxO1 and Nrf2 via the SIRT1/LKB1/AMPK pathway." J Nutr Biochem **25**(11): 1207-1217.

Understanding the mechanism by which alpha-lipoic acid supplementation has a protective effect upon nonalcoholic fatty liver disease in vivo and in vitro may lead to targets for preventing hepatic steatosis. Male C57BL/6J mice were fed a normal diet, high-fat diet or high-fat diet supplemented with alpha-lipoic acid for 24weeks. HepG2 cells were incubated with normal medium, palmitate or alpha-lipoic acid. The lipid-lowering effects were measured. The protein expression and distribution were analyzed by Western blot, immunoprecipitation and immunofluorescence, respectively. We found that alpha-lipoic acid enhanced sirtuin 1 deacetylase activity through liver kinase B1 and stimulated AMP-activated protein kinase. By activating the sirtuin 1/liver kinase B1/AMP-activated protein kinase pathway, the translocation of sterol regulatory element-binding protein-1 into the nucleus and forkhead box O1 into the cytoplasm was prevented. Alpha-lipoic acid increased adipose triacylglycerol lipase expression and decreased fatty acid synthase abundance. In in vivo and in vitro studies, alpha-lipoic acid also increased nuclear NF-E2-related factor 2 levels and downstream target amounts via the sirtuin 1 pathway. Alpha-lipoic acid eventually reduced intrahepatic and serum triglyceride content. The protective effects of alpha-lipoic acid on hepatic steatosis appear to be associated with the transcription factors sterol regulatory element-binding protein-1, forkhead box O1 and NF-E2-related factor 2.

Yao, P., et al. (2007). "Quercetin protects human hepatocytes from ethanol-derived oxidative stress by inducing heme oxygenase-1 via the MAPK/Nrf2 pathways." J Hepatol **47**(2): 253-261.

BACKGROUND/AIMS: Flavonoids, including quercetin, have been reported to have potent hepatoprotective effects, which may be associated with HO-1 induction. However, since the effect and signaling pathway of quercetin involved in HO-1 induction against alcoholic liver damage are still not fully understood, this is the target of the present study. METHODS: Human hepatocytes were incubated with ethanol (100 mM) and quercetin (10-200 microM), and cellular damage and HO-1 activity were measured. Nrf2 expression in cytosolic and nuclear fractions was studied following the incubation with MAPK inhibitor(s). RESULTS: Ethanol exposure resulted in a sustained glutathione depletion, malondialdehyde elevation, and evident release of cellular LDH and AST. Quercetin exerted a dose-dependent protective effect against alcoholic oxidative stress, and increased the EC50 of ethanol by approx. 40%, which is parallel to HO-1 induction with quercetin. Zinc protoporphyrin-9 abrogated the protective effect and dramatically enhanced ethanol cytotoxicity. SB203580 (p38 inhibitor) and especially PD98059 (ERK inhibitor) blocked quercetin-derived HO-1 induction and Nrf2 translocation, and subsequently inhibited the quercetin-related protection. CONCLUSIONS: HO-1 up-regulation by quercetin protected human hepatocytes from ethanol-induced oxidative stress. Among MAPK signaling pathways, p38 and ERK mediated quercetin-derived Nrf2 translocation into nuclei and subsequent induction of HO-1 activity, and the latter showed a stronger mediating effect.

Yao, R. Q., et al. (2012). "Quercetin attenuates cell apoptosis in focal cerebral ischemia rat brain via activation of BDNF-TrkB-PI3K/Akt signaling pathway." Neurochem Res **37**(12): 2777-2786.

Many studies have demonstrated that apoptosis play an important role in cerebral ischemic pathogenesis and may represent a target for treatment. Neuroprotective effect of quercetin has been shown in a variety of brain injury models including ischemia/reperfusion. It is not clear whether BDNF-TrkB-PI3K/Akt signaling pathway mediates the neuroprotection of quercetin, though there has been some reports on the quercetin increased brain-derived neurotrophic factor (BDNF) level in brain injury models. We therefore first examined the neurological function, infarct volume and cell apoptosis in quercetin treated middle cerebral artery occlusion (MCAO) rats. Then the protein expression of BDNF, cleaved caspase-3 and p-Akt were evaluated in either the absence or presence of PI3K inhibitor (LY294002) or tropomyosin receptor kinase B (TrkB) receptor antagonist (K252a) by immunohistochemistry staining and western blotting. Quercetin significantly improved neurological function, while it decreased the infarct volume and the number of TdT mediated dUTP nick end labeling positive cells in MCAO rats. The protein expression of BDNF, TrkB and p-Akt also increased in the quercetin treated rats. However, treatment with LY294002 or K252a reversed the quercetin-induced increase of BDNF and p-Akt proteins and decrease of cleaved caspase-3 protein in focal cerebral ischemia rats. These results demonstrate that quercetin can decrease cell apoptosis in the focal cerebral ischemia rat brain and the mechanism may be related to the activation of BDNF-TrkB-PI3K/Akt signaling pathway.

Young, R. L., et al. (2013). "Disordered control of intestinal sweet taste receptor expression and glucose absorption in type 2 diabetes." Diabetes **62**(10): 3532-3541.

We previously established that the intestinal sweet taste receptors (STRs), T1R2 and T1R3, were expressed in distinct epithelial cells in the human proximal intestine and that their transcript levels varied with glycemic status in patients with type 2 diabetes. Here we determined whether STR expression was 1) acutely regulated by changes in luminal and systemic glucose levels, 2) disordered in type 2 diabetes, and 3) linked to glucose absorption. Fourteen healthy subjects and 13 patients with type 2 diabetes were studied twice, at euglycemia (5.2 +/- 0.2 mmol/L) or hyperglycemia (12.3 +/- 0.2 mmol/L). Endoscopic biopsy specimens were collected from the duodenum at baseline and after a 30-min intraduodenal glucose infusion of 30 g/150 mL water plus 3 g 3-O-methylglucose (3-OMG). STR transcripts were quantified by RT-PCR, and plasma was assayed for 3-OMG concentration. Intestinal STR transcript levels at baseline were unaffected by acute variations in glycemia in healthy subjects and in type 2 diabetic patients. T1R2 transcript levels increased after luminal glucose infusion in both groups during euglycemia (+5.8 x 10(4) and +5.8 x 10(4) copies, respectively) but decreased in healthy subjects during hyperglycemia (-1.4 x 10(4) copies). T1R2 levels increased significantly in type 2 diabetic patients under the same conditions (+6.9 x 10(5) copies). Plasma 3-OMG concentrations were significantly higher in type 2 diabetic patients than in healthy control subjects during acute hyperglycemia. Intestinal T1R2 expression is reciprocally regulated by luminal glucose in health according to glycemic status but is disordered in type 2 diabetes during acute hyperglycemia. This defect may enhance glucose absorption in type 2 diabetic patients and exacerbate postprandial hyperglycemia.

Zerin, T., et al. (2013). "Quercetin reduces oxidative damage induced by paraquat via modulating expression of antioxidant genes in A549 cells." J Appl Toxicol **33**(12): 1460-1467.

Oxidative injury can occur in the lung through the generation of reactive oxygen species (ROS) via redox cycling owing to intentional or accidental ingestion of paraquat (PQ), a common herbicide. A wide array of phytochemicals has been shown to reduce cellular oxidative damage by modulating cytoprotective genes. Quercetin, a well-known flavonoid, has been reported to display cytoprotective effects by up-regulating certain cytoprotective genes. In this context, we investigated the effect of quercetin on PQ-induced cytotoxicity in alveolar A549 cells, modulation of antioxidant genes, activation of transcription factor-Nrf2 and its target HO-1 expression. Quercetin reduced PQ-induced cytotoxicity in A549 cells that was evaluated by both 3-(4,5-dimethylthiazol-2-yl)-2, 5-diphenyl-tetrazolium bromide (MTT) and lactate dehydrogenase (LDH) assays. Modulation of antioxidant genes was compared when cells were treated with PQ, quercetin and both using qRT-PCR. Activation of transcription factor-Nrf2 and induction of its target gene, HO-1 was demonstrated by western blot analysis. A remarkable reduction in the ROS level as well as an increase in the total cellular glutathione (GSH) level occurred when PQ-exposed cells were treated with quercetin. Our findings suggest that quercetin may be used to mitigate or minimize oxidative stress via reducing the generation of ROS.

Zhang, J. Q., et al. (2014). "Therapeutic detoxification of quercetin against carbon tetrachloride-induced acute liver injury in mice and its mechanism." J Zhejiang Univ Sci B **15**(12): 1039-1047.

This study observes the therapeutic detoxification of quercetin, a well-known flavonoid, against carbon tetrachloride (CCl4) induced acute liver injury in vivo and explores its mechanism. Quercetin decreased CCl4-increased serum activities of alanine and aspartate aminotransferases (ALT/AST) when orally taken 30 min after CCl4 intoxication. The results of a histological evaluation further evidenced the ability of quercetin to protect against CCl4-induced liver injury. Quercetin decreased the CCl4-increased malondialdehyde (MDA) and reduced the glutathione (GSH) amounts in the liver. It also reduced the enhanced immunohistochemical staining of the 4-hydroxynonenal (4-HNE) in the liver induced by CCl4. Peroxiredoxin (Prx) 1, 2, 3, 5, 6, thioredoxin reductase 1 and 2 (TrxR1/2), thioredoxin 1 and 2 (Trx1/2), nuclear factor erythroid 2-related factor 2 (Nrf2), and heme oxygenase-1 (HO-1) all play critical roles in maintaining cellular redox homeostasis. Real-time polymerase chain reaction (PCR) results demonstrated that quercetin reversed the decreased mRNA expression of all those genes induced by CCl4. In conclusion, our results demonstrate that quercetin ameliorates CCl4-induced acute liver injury in vivo via alleviating oxidative stress injuries when orally taken after CCl4 intoxication. This protection may be caused by the elevation of the antioxidant capacity induced by quercetin.

Zhang, Q., et al. (2006). "Green tea extract and (-)-epigallocatechin-3-gallate inhibit mast cell-stimulated type I collagen expression in keloid fibroblasts via blocking PI-3K/AkT signaling pathways." J Invest Dermatol **126**(12): 2607-2613.

Keloid, a chronic fibro-proliferative disease, exhibits distinctive histological features characterized by an abundant extracellular matrix stroma, a local infiltration of inflammatory cells including mast cells (MCs), and a milieu of enriched cytokines. Previous studies have demonstrated that co-culture with MCs stimulate type I collagen synthesis in fibroblasts, but the signaling mechanisms remain largely unknown. In this study, we investigated the

signaling pathways involved in MC-stimulated type I collagen synthesis and the effects of green tea extract (GTE) and its major catechin, (-)-epigallocatechin-3-gallate (EGCG), on collagen homeostasis in keloid fibroblasts. Our results showed that MCs significantly stimulated type I collagen expression in keloid fibroblasts, and the upregulation of type I collagen was significantly attenuated by blockade of phosphatidylinositol-3-kinase (PI-3K), mammalian target of rapamycin (mTOR), and p38 MAPK signaling pathways, but not by blockade of ERK1/2 pathway. Furthermore, GTE and EGCG dramatically inhibited type I collagen production possibly by interfering with the PI-3K/Akt/mTOR signaling pathway. Our findings suggest that interaction between MCs and keloid fibroblasts may contribute to excessive collagen accumulation in keloids and imply a therapeutic potential of green tea for the intervention and prevention of keloids and other fibrotic diseases.

Zhou, L., et al. (2008). "Berberine acutely inhibits insulin secretion from beta-cells through 3',5'-cyclic adenosine 5'-monophosphate signaling pathway." Endocrinology **149**(9): 4510-4518.

Berberine, a hypoglycemic agent, has recently been shown to activate AMP-activated protein kinase (AMPK) contributing to its beneficial metabolic effects in peripheral tissues. However, whether berberine exerts a regulatory effect on beta-cells via AMPK or other signaling pathways and counteracts glucolipotoxicity remains uncertain. In the present study, the impact of berberine on beta-cell function was investigated in vivo and in vitro. In high-fat-fed rats, berberine treatment for 6 wk significantly decreased plasma glucose and insulin levels before and after an oral glucose challenge along with the reduction of body weight and improvement of blood lipid profile. In accordance with the in vivo results, berberine acutely decreased glucose-stimulated insulin secretion (GSIS) and palmitate-potentiated insulin secretion in MIN6 cells and rat islets. However, pretreated with berberine for 24 h augmented the response of MIN6 cells and rat islets to glucose and attenuated the glucolipotoxicity. Berberine acutely increased AMPK activity in MIN6 cells. However, compound C, an AMPK inhibitor, completely reversed troglitazone-suppressed GSIS, not berberine-suppressed GSIS. Otherwise, berberine decreased cAMP-raising agent-potentiated insulin secretion in MIN6 cells and rat islets. These results suggest that the activation of AMPK is required for troglitazone-suppressed GSIS, whereas cAMP signaling pathway contributes, at least in part, to the regulatory effect of berberine on insulin secretion.

Zhu, B. Q., et al. (2006). "Comparison of pyrroloquinoline quinone and/or metoprolol on myocardial infarct size and mitochondrial damage in a rat model of ischemia/reperfusion injury." J Cardiovasc Pharmacol Ther **11**(2): 119-128.

The cardioprotective effectiveness of low-dose pyrroloquinoline quinone (PQQ, 3 mg/kg) was compared with metoprolol, a beta(1)-selective adrenoceptor antagonist. Rats underwent 30 minutes of left anterior descending coronary artery occlusion and 2 hours of reperfusion. Metoprolol and/or PQQ were given at the onset of reperfusion to mimic clinical treatment. Metoprolol and/or PQQ reduced infarct size and protected against ischemia-induced left ventricular dysfunction after 2 hours of reperfusion. Combined therapy augmented left ventricular developed pressure at the end of reperfusion. Metoprolol or PQQ alone enhanced mitochondrial respiratory ratios in ischemic and nonischemic myocardium. Although the PQQ/metoprolol combination therapy increased respiratory ratio values, the effects were small when compared with PQQ alone. Only PQQ decreased lipid peroxidation. Metoprolol and/or PQQ given at the onset of reperfusion reduce infarct size and improve cardiac function. Combination therapy further reduces infarct size. PQQ is superior to metoprolol in protecting mitochondria from ischemia/reperfusion oxidative damage.

Zhu, L., et al. (2010). "Hydroxytyrosol protects against oxidative damage by simultaneous activation of mitochondrial biogenesis and phase II detoxifying enzyme systems in retinal pigment epithelial cells." J Nutr Biochem **21**(11): 1089-1098.

Studies in this laboratory have previously shown that hydroxytyrosol, the major antioxidant polyphenol in olives, protects ARPE-19 human retinal pigment epithelial cells from oxidative damage induced by acrolein, an environmental toxin and endogenous end product of lipid oxidation, that occurs at increased levels in age-related macular degeneration lesions. A proposed mechanism for this is that protection by hydroxytyrosol against oxidative stress is conferred by the simultaneous activation of two critically important pathways, viz., induction of phase II detoxifying enzymes and stimulation of mitochondrial biogenesis. Cultured ARPE-19 cells were pretreated with hydroxytyrosol and challenged with acrolein. The protective effects of hydroxytyrosol on key factors of mitochondrial biogenesis and phase II detoxifying enzyme systems were examined. Hydroxytyrosol treatment simultaneously protected against acrolein-induced inhibition of nuclear factor-E2-related factor 2 (Nrf2) and peroxisome proliferator-activated receptor coactivator 1 alpha (PPARGC1alpha) in ARPE-19 cells. The activation of Nrf2 led to activation of phase II detoxifying enzymes, including gamma-glutamyl-cysteinyl-ligase, NADPH (nicotinamide adenine dinucleotide phosphate)-quinone-oxidoreductase 1, heme-oxygenase-1, superoxide dismutase, peroxiredoxin and thioredoxin as well as other antioxidant enzymes, while the activation of PPARGC1alpha led to increased protein expression of mitochondrial transcription factor A, uncoupling protein 2 and mitochondrial complexes. These results suggest that hydroxytyrosol is a potent inducer of phase II detoxifying enzymes and an enhancer of mitochondrial biogenesis. Dietary supplementation of hydroxytyrosol may contribute

to eye health by preventing the degeneration of retinal pigment epithelial cells induced by oxidative stress.

Zou, X., et al. (2012). "Stimulation of GSH synthesis to prevent oxidative stress-induced apoptosis by hydroxytyrosol in human retinal pigment epithelial cells: activation of Nrf2 and JNK-p62/SQSTM1 pathways." J Nutr Biochem **23**(8): 994-1006.

The Nrf2-Keap1 pathway is believed to be a critical regulator of the phase II defense system against oxidative stress. By activation of Nrf2, cytoprotective genes such as heme oxygenase-1 (HO-1), NAD(P)H:quinone oxidoreductase (NQO-1) and gamma-glutamyl-cysteine ligase (GCL) are induced. GCL-induced glutathione (GSH) production is believed to affect redox signaling, cell proliferation and death. We here report that tert-butyl hydroperoxide (t-BHP)-induced GSH reduction led to mitochondrial membrane potential loss and apoptosis in cultured human retinal pigment epithelial cells from the ARPE-19 cell line. Hydroxytyrosol (HT), a natural phytochemical from olive leaves and oil, was found to induce phase II enzymes and GSH, thus protect t-BHP-induced mitochondrial dysfunction and apoptosis. Depletion of GSH by buthionine-[S,R]-sulfoximine enhanced t-BHP toxicity and abolished HT protection. Overexpression of Nrf2 increased GSH content and efficiently protected t-BHP-induced mitochondrial membrane potential loss. Meanwhile, HT-induced GSH enhancement and induction of Nrf2 target gene (GCLc, GCLm, HO-1, NQO-1) messenger RNA (mRNA) were inhibited by Nrf2 knockdown, suggesting that HT increases GSH through Nrf2 activation. In addition, we found that HT was able to activate the PI3/Akt and mTOR/p70S6-kinase pathways, both of which contribute to survival signaling in stressed cells. However, the effect of HT was not inhibited by the PI3K inhibitor LY294002. Rather, c-Jun N-terminal kinase (JNK) activation was found to induce p62/SQSTM1 expression, which is involved in Nrf2 activation. Our study demonstrates that Nrf2 activation induced by the JNK pathway plays an essential role in the mechanism behind HT's strengthening of the antiapoptotic actions of the endogenous antioxidant system.

Zrelli, H., et al. (2011). "Hydroxytyrosol induces proliferation and cytoprotection against oxidative injury in vascular endothelial cells: role of Nrf2 activation and HO-1 induction." J Agric Food Chem **59**(9): 4473-4482.

Hydroxytyrosol (HT), a phenolic compound in olive oil and leaves, has been reported to prevent various human pathologies including cardiovascular diseases. This study investigated the effects of HT on proliferation and protection against oxidative stress-induced damage in vascular endothelial cells (VECs) and the molecular mechanism(s) involved. Treatment of VECs with HT increased cell proliferation, promoted wound repair, and protected cells against H(2)O(2) cytotoxicity through the activation of Akt and ERK1/2, but not p38 MAPK. HT increased the expression and nuclear translocation of nuclear factor-E2-related factor-2 (Nrf2). Nrf2 expression was attenuated by LY294002 and U0126, inhibitors of phosphatidylinositol-3-kinase and MEK1/2, respectively. Nrf2 siRNA decreased both proliferative and cytoprotective effects of HT and abrogated HO-1 induction. Moreover, HO-1 inhibition with HO-1 siRNA or zinc protoporphyrin IX significantly prevented HT-induced cell proliferation, cytoprotection, and reduction in intracellular reactive oxygen species (ROS), suggesting that HO-1 is involved in these HT functions. The findings demonstrate that HT positively regulates the antioxidant defense system in VECs through the activation of Nrf2 followed by cell proliferation and resistance to vascular injury. The present study provides a molecular basis for the contribution of HT in the Mediterranean diet to the prevention of cardiovascular diseases.