Phytochemicals added to the feed of ovariectomized adult rats increase brown adipose activity.

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Abstract

Background: Brown adipose tissue (BAT) uncouples respiration, using lipids as an energy source while dissipating heat. Increases in BAT activity are protective against obesity, thus compounds that increase BAT activation may help prevent weight gain. Resveratrol (R) increases BAT activity by upregulating thermogenic genes. As phytochemicals have synergistic properties, our research strategy has included investigation of the efficacy of relatively low concentrations of phytochemical blends on BAT activation.

Methods: Previously, we showed that R combined with genistein (G) and quercetin (Q) reduced weight gain in aged ovariectomized (OVX) female rats. In the current study, OVX rats were fed diets containing doses of phytochemicals with vitamin D (diet 1: 1000 mg/kg G; diet 2: 500 mg/kg G, 200 mg/kg R, and 1000 mg/kg Q; diet 3: 1000 mg/kg G, 400 mg/kg R, and 2000 mg/kg Q).

Results: After 16 weeks, rats in the high dose group had a significantly smaller scapular BAT depot compared to non-OVX controls (0.74 g v 0.92 g; p<0.05). It was hypothesized that the reduction in BAT mass was due to phytochemical-driven increases in BAT lipid metabolism. After 16-weeks reduced lipid content in BAT of the high dose group was seen compared to non-OVX controls (0.35 ± 0.02 v 0.44 ± 0.04; p=0.01). Two-fold increases in BAT-related genes including Sirt3, Nrf1, and Pparg1c were observed in the high dose group compared to non-OVX controls (p<0.10). Similarly, Acc1 expression was increased by 34-fold (p<0.02) and Lipe expression was increased by 29-fold (p<0.004) in the high dose group.

Conclusions: These findings are consistent with our hypothesis that a dietary phytochemical blend increases energy utilization and respiration in BAT. These data provide further support for the anti-obesity effects of synergistic phytochemical combinations.

Introduction

Ovariectomy results in a rapid increase in weight gain and adiposity as a direct result of the loss of 17β-estradiol, the major circulating sex hormone in females. A plethora of research exists on the many mechanisms behind ovariectomy-induced weight gain, however the impact of the loss of estradiol on BAT function is deficient. It has been previously suggested that females have more active BAT stores than males (Cell Physiol Biochem 2007;19:195-204). Unfortunately the affects of ovariectomy on BAT have not been as equally addressed in the literature. Early studies have not identified that a loss of BAT is a major contributing factor to ovariectomy-induced weight gain (Am J Physiol 1986;250:R245-249). Despite the limited research in BAT, increases in adiposity caused by deficiencies in estradiol result in an increased risk for the development of metabolic diseases (Cardiovas Endorinol 2003; 88:2404). Activation of BAT in menopausal females and rodent models of menopause may prove to be a novel way to prevent shifts in fat distribution and gains in total adiposity when estradiol is lost.

Cold activation of BAT has proven to be extremely effective in promoting fat oxidation and weight loss. Activated BAT can burn up to 250 kcal every 3 hours, increasing metabolic rate by 80% (J Clin Invest 2012;22:545-552). While very potent, problems arise with using cold temperatures as a therapeutic agent. In addition to difficulties in maintaining protocol compliance, exposure to cold results in increased appetite (J Comp Physiol Psychol 1979;93:1024-1034). Furthermore, cold temperatures may also increase the risk of sudden cardiac death (Cell Metab 2013;18:118-129). For these reasons, pharmaceutical agents can provide a simiplier method of activating BAT and with possibly increased safety.

Phytochemicals have been identified in the literature to exert effects on BAT functioning. One such example is the nutraceutical compound resveratrol, which is found readily in grapes and their products. Resveratrol has shown to increase mitochondrial biogenesis through enhancing PGC1α and SIRT1 activity (Cell 2006;127:1109-1122). Both of these proteins have been identified as major drivers in both maintaining and inducing brown fat activity (Am J Clin Nutr 2011; 93:884S-890). Furthermore resveratrol also appears to induce UCP1 gene expression in ob/ob mice (FASEB J;23:1032-1040). While resveratrol has shown promise in cell and animal work in helping prevent or alleviate disease progression, resveratrol is not practical for pharmaceutical use in humans. Based off of some animal studies, doses of upwards of 28 g/d of resveratrol supplementation would be required to exert similar affects in humans (Mol Nutr Food Res 2011;55:1177-1185). Fortunately, recent studies have shown that phytochemicals including resveratrol have synergistic properties. Therefore, the role of synergistic blends of phytochemicals needs to be investigated as a way to manage adiposity and prevent obesity.

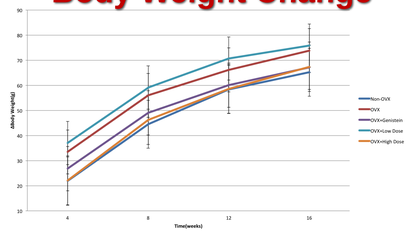
Materials and Methods

-Fischer 344 female rats (retired breeders, n=34) were obtained from the NIA colony from Tacona. They were adapted to a basal maintenance diet (a semipurified phytoestrogen-free casein-based diet, AIN-93M in a meal form with 4% safflower oil) for one week upon arrival. Rats were either ovariectomized or given sham surgery 1 week prior to being fed food containing test compounds for 16 weeks. Food intake was measured on a daily basis and body weights were monitored weekly.
-Upon sacrifice, adipose tissues were dissected and frozen in liquid nitrogen. mRNA was obtained using Trizol Reagent and converted to cDNA using Reverse Transcription kit by Applied Biosystems. Quantitative PCR was performed for adipose-related genes with primers from Applied Biosystems using the 7500 system.
-Total protein was extracted using modified RIPA buffer containing a protease inhibitor cocktail from Sigma-Aldrich. Protein concentrations were measured using the Bradford Assay Reagent (Sigma).
-Lipids were extracted using the Folch method, and the concentration was determined and normalized to protein extraction data.
-Statistics were performed using one-way ANOVA and Fisher LSD post-hoc testing. Significance was set at p<0.05.

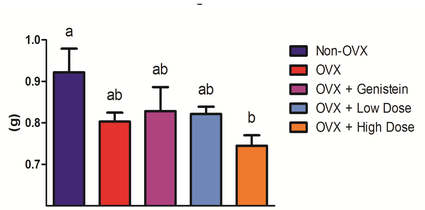
Diet

Non-OVX or OVX	Treatment	VD (IU/Kg diet) 2,5 –OH D3	G (mg /Kg diet) Genistein aglycone	R (mg/Kg diet) Trans - Resveratrol	Q (mg/Kg diet) Quercetin hydrate
Non-OVX	Control	1000	0	0	0
OVX	Control	1000	0	0	0
OVX	VD/G	2400	1000	0	0
OVX	VD/R/Q/G (1)	2400	500	200	1000
OVX	VD/R/Q/G (2)	2400	1000	400	2000

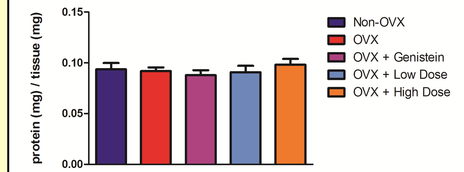
Body Weight Change



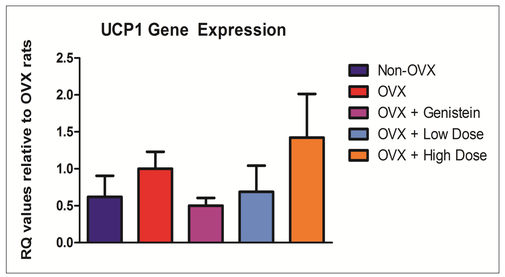
BAT Tissue Weight

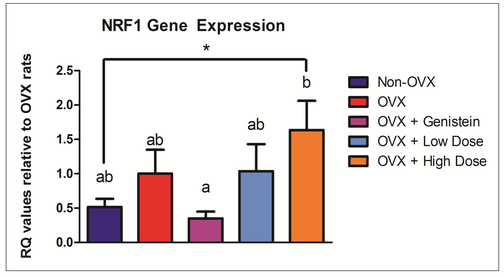


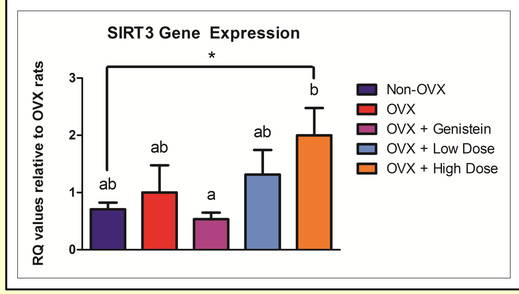
Mitochondrial Protein

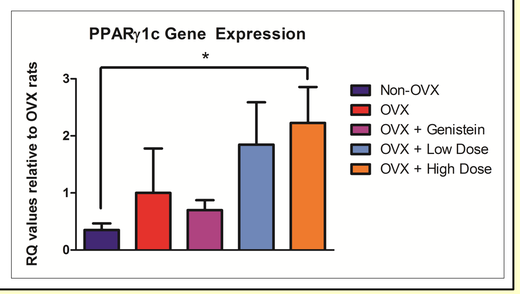


BAT-Related Genes

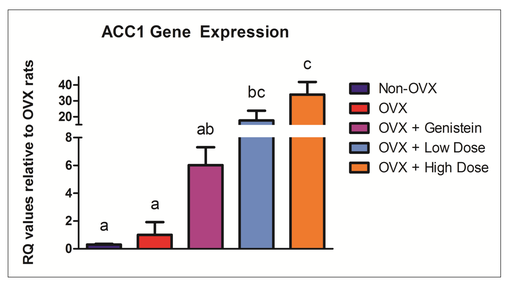


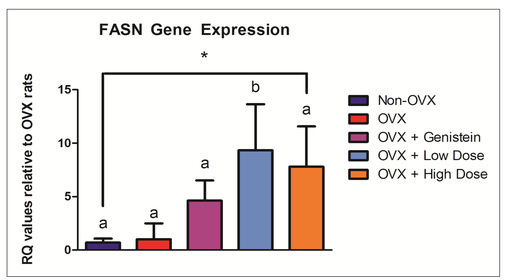




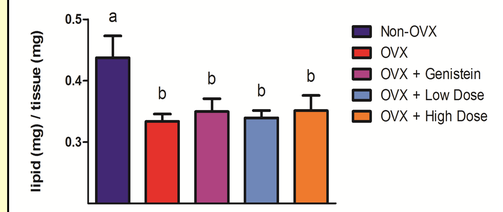


Lipogenic Genes

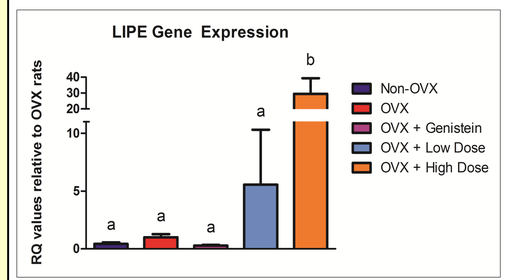


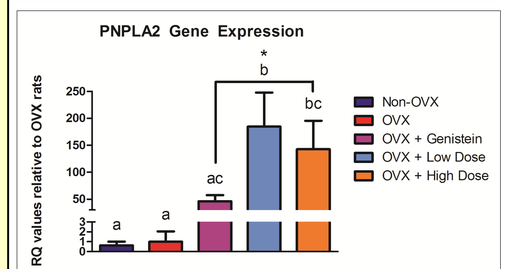


Lipid Concentration



Lipolytic Genes





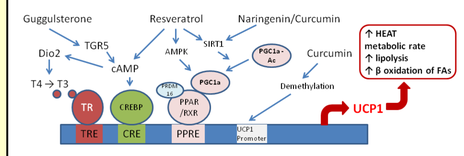
Summary

• Ovariectomy did not significantly reduce scapular BAT weight, however high dose phytochemical treatment resulted in a **loss of BAT weight**. This loss of BAT tissue is presumably due to a reduced lipid concentration as no difference was found in mitochondrial protein content.

• While UCP1 was not significantly upregulated, genes that regulate UCP1 (SIRT3) was significantly increased with treatment. Furthermore genes related to **mitochondrial biogenesis** (NRF1 & PGC1α) was also upregulated.

• Genes related to **lipid synthesis** (ACC1 & FAS) and **mobilization** (HSL & ATGL) was upregulated with phytochemical treatment in a dose-dependent response.

Phytochemical Synergy



Phytochemicals have multiple targets on genes related to thermogenesis. Taking advantage of such synergy reduces the doses required for activating BAT to levels that can be easily ingested in humans. Upregulation of thermogenic genes by phytochemical combinations also is independent of sympathetic nervous system stimulation, and thus is suggested to be safer than pharmaceutical/cold stimulation.

Conclusions

*While our high dose phytochemical blend resulted in a loss of scapular BAT, the reduced lipid concentration along with **upregulated thermogenic, lipogenic, and lipolytic gene expression** indicates a more active tissue.*

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