The Isoflavonoid - Equol Isomer’s Neuro-physiological Effects After Short-term, Low-dose Administration in Male Rats

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Abstract

Polyphenolic molecules abundant in plants, includes one sub-category the phytoestrogens that have estrogenic and anti-androgenic biological actions. Many studies have investigated the phytoestrogens (i.e., daidzein, genistein) for their hormone actions/health benefits.

Aim: No comparative reports have examined the effects of the isoflavonoid molecule, equol that can exist as enantiomers which has been found in plant and food products. The purpose of this study: to determine the influence of a low dose of R-equol, Racemic equol, or S-equol on neuro-physiological parameters.

Methods: The low dose (0.25 mg/kg/day) of R-equol, Racemic equol or S-equol was administered for one week to Sprague-Dawley male rats from 63 to 70 days of age. At the end of the treatment interval anxiety-related behaviors (via the elevated plus maze), body weight gain, total body weight and ventral prostate weight among the equol treatments. Notably, body weight gain was decreased by 23 %, total body weight declined by 4 %, prostate weight decreased by approximately 18 % and the prostate weight/body weight ratio was the lowest among the equol treatments.

Results: In general, R-equol and Racemic equol displayed significantly decreased anxiety parameters compared to controls in the elevated plus maze for time in the open arms and open arm entries. The 0.25 mg/kg/day short-term (one week) administration of R-equol was the only experimental group that significantly decreased body weight gain, total body weight and ventral prostate weight among the equol treatments. Notably, body weight gain was decreased by 23 %, total body weight declined by 4 %, prostate weight decreased by approximately 18 % and the prostate weight/body weight ratio was the lowest among the equol treatments.

Conclusion: This is the first study to compare the attributes of equol and its enantiomers at low doses for a short-term where R-equol and Racemic equol appear to have the most potent behavioral influences versus that of S-equol, suggesting that R-equol is the contributing factor in these endpoints. These results elucidate that R-equol influences anti-anxiety-like effects and confirm previous reports on equol for this parameter as well as for changes in body weight gain and prostate weight.

Keywords: Polyphenol; Equol; Isomers; Isoflavonoids; Anxiety; Prostate; Body Weight; Rats;

Introduction

Polyphenolic molecules abundant in plants, includes one sub-category the phytoestrogens of which equol is a key metabolite of daidzein, one of the main isoflavonoids present in soy foods [1, 2]. Equol is naturally produced in the intestines of animals and humans, and some individuals, who are capable of producing higher levels of equol than others, commonly are referred to as “equol producers” with reported health benefits [1, 2]. However, equol, unlike its precursor diadzien (or genistein) is unique in having a chiral carbon atom at position C 3 of the furan ring, and therefore, can occur as two distinct isomers as S-equol or R-equol [1, 2]. In brief, both equol isomers specifically bind free 5α-dihydrotestosterone (5α-DHT) to decrease androgen hormone action, but only S-equol has high affinity for estrogen receptor beta (ER β) that has positive effects in brain, skin and prostate tissue sites [2-5]. Apparently, S-equol is the exclusive intestinal metabolite of daidzein in all animals including humans [1, 2, 6]. Additionally, S-equol has been identified in many food products such as fermented soy, tofu, other plants like beans, cabbage, lettuces and animal products such as eggs and milk [7-15]. Remarkably, the metabolism of R- and S-equol in humans appears to be similar [6, 16]. Finally, the biosynthesized form of equol namely racemic equol is very stable where the ratio of the two isomers remains constant. Also, in biologically systems, in general, the isomeric forms of equol are also stable [2, 3].

The equol hypothesis proposed in the late 1990s suggested that the generation of equol and/or consumption of equol containing foods at threshold levels imparted health benefits for various disorders and many age-related diseases [1, 2]. This resulted in a dramatic increase in equol research over the last decade. Overall, the augmented research effort on equol continues to the present day to determine its beneficial health properties [1-3].
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For example, equol is a superior antioxidant, having greater antioxidant capacity than vitamin C or vitamin E and higher antioxidant activity such as preventing oxidative damage [17]. In this regard, equol is known to increase nuclear-factor-erythroid 2-related factor 2 (Nrf2) that plays a key role in the cellular defense against oxidative and xenobiotic stressors by its capacity to induce the expression of genes, which encode detoxifying enzymes and antioxidant proteins [18]. However, in previous research reports, the equol tested was not always clearly identified. In many cases the biosynthesized racemic equol (50 % R-equol and 50 % S-equol) was the compound reported. In dietary intervention studies, where soy was used, the implication of S-equol as the active or key metabolite of daidzein has been examined.

Notably, it is known that R-equol, Racemic equol and S-equol all have the same anti-androgenic properties, where they selectively bind free (5α-DHT) with high affinity, and thereby prevent 5α-DHT from binding the androgen receptor and thus decreasing androgen hormone action in various tissue sites [3-5,19]. Also, equol is known to activate estrogen receptor related gamma (ERRγ), which has important implications for anti-aging mechanisms including enhanced prostate health [20-22]. In more recent investigations that examined human skin gene expression, R-equol, Racemic equol and S-equol were studied as single compounds, which displayed different results dependent upon the equol isomer or racemic mixture tested [20].

It is also known that the equol isomers act like natural Selective Estrogen Receptor Modulators (SERMs) at various tissue sites throughout the body, but particularly in the brain where S-equol and Racemic equol via ER β-selective activation potentiated neural mitochondrial function [1-3, 23]. Remarkably, S-equol has a high affinity for estrogen receptor beta (ER β), whereas, R-equol has some affinity for ER α [1-3, 6]. It is established that ER β activation in the brain mediates anxiety-related behaviors in rodents [24]. In this regard, we have previously reported that rats consuming a soy-rich diet displayed decreased anxiety parameters and decreased body weight, where R-equol and Racemic equol appear to have the most potent behavioral/biological influences.

**Methods**

**Animals and housing**

All procedures involving animals were approved by the Colorado State University Institutional Animal Care and Use Committee. Thirty-two, 50 day-old male Sprague-Dawley rats (experiment 2) were purchased from Charles River Laboratories (Wilmington, MA, USA). All animals were housed in clear plastic cages with wire lids (30 cm L x 24 cm W x 15 cm H, standard shoebox cages with woodchip bedding). The male rats were housed individually during testing. Animals were placed on a 12-hour light/dark schedule (6 am lights on) and allowed ad libitum access to food (low soy diet; Phytoestrogen Reduced Rodent Diet I, Zeigler Bros., Inc, Gardner, PA, USA) and tap water. The standard low-soy containing diet did not contain casein, but corn and wheat provided the protein content for this formulation, the details for this diet are published elsewhere [28]. After approximately 2 weeks, allowing time for the animals to adapt to their new environment and diets, the rats were treated starting on 63 days of age and then tested at approximately 70 days of age in the elevated-plus maze as outlined below.

**Equol Treatments, Chemical Reagents and Supplies**

**Racemic equol, R-equol and S-equol (@ 0.25 mg/kg/day)**

In preliminary pilot studies, the lowest effective dose of R-equol was determined to be 0.25 mg/kg/day for behavioral and biological endpoints. Also, it should be pointed out that high purity R-equol and S-equol crystalline material is extremely expensive. Therefore, rather than formulating a diet containing these isomers the equol treatments were administered via daily subcutaneous injections in DiMethyl Sulfoxide (DMSO) to decrease the amount of equol material used and increase the ease of administration for a short interval of one week in order to provide proof of concept.

Young adult male Sprague-Dawley rats were age and body-weight matched and assigned to four treatment groups: 1) Control- DMSO vehicle, 2) Racemic equol (0.25 mg/kg/day), 3) R-equol (0.25 mg/kg/day) and 4) S-equol (0.25 mg/kg/day), n = 8 animals per group. All equol treatments were dissolved in the DMSO vehicle. According to treatment groups all animals received daily subcutaneous injections (for 7 consecutive days, from 63 to 70 days of age, at approximately 9 am) administered at the nape of the neck and the total volume for each injection was 0.1 cc, which was non-toxic.

**Chemical Reagents and Supplies**

Racemic equol (50% R-equol and 50% S-equol) of high purity (> 98 %) was purchased from LC Laboratories (Woburn, MA, USA). A portion of the racemic equol was separated chemically on a chiral column by commercial standards to yield high purity (> 98.5 %) R-equol or S-equol by Chiral Technologies Inc. (West
Elevated Plus Maze

At the end of the seven consecutive days of treatment the animals were tested in the elevated plus maze (at approximately 3 pm) in order to quantify anxiety-related behaviors [25, 29, 30]. The elevated plus maze consisted of two open arms (10 x 100 cm) and two enclosed arms of the same size, with 50 cm high walls and an open roof similar to that reported previously by our laboratory [25]. At the middle of the intersecting arms an open area was defined by a 10 cm region. The apparatus was elevated 60 cm above the floor and a video camera was suspended above the maze (at approximately 7.5 feet) to record each trial for analysis. Each rat was placed in the same orientation of the center of open area of the maze between the open and closed arms to initiate the trial [30]. The animals were tested individually and only once for 5 minutes. One animal from each treatment group was tested sequentially until all the animals from all treatment groups were tested. The maze was cleaned following each trial (with 70 % ETOH in distilled water) to remove any residue or odors of each animal [30]. The following measures were quantified and analyzed as previously described: 1) percent of total time spent in the open arms and 2) the number of entries into the open arms (defined as both front and hind paws being in an open arm). The personnel conducting and evaluating the video elevated plus maze trials were blind to the treatment groups.

Body Weight Gain and Prostate Weight Measurements

To determine how the equol treatments influenced additional physiological endpoints, all animals were weighed before the treatments started, and then weighed at the end of the treatments to quantify body weight gain in grams using a Mettler balance (+ 0.1 g). Average body weight by treatment groups are shown in Table 1. Also, at the end of the experiments after the elevated plus maze behavioral tests, the rats were sacrificed and the ventral prostate from each rat was removed and weighed (+ 1 milligram, mg).

Statistical Analysis

All data sets were analyzed with Minitab (State College, PA, USA) software [by ANOVA (unstacked data), followed by the Newman-Keuls multiple test to determine significance (where appropriate)] and all data were expressed as the mean ± standard error of the mean; p < 0.05 was considered significant.

Results

Elevated Plus Maze Analysis

At the end of the 7 consecutive days of treatment the animals were tested in the elevated plus maze to quantify anxiety-related behaviors. Anxiety reduction in the elevated plus-maze is indicated by an increase in the time spent in the open arms, and an increase in the number of entries into the open arms among treatment groups.

As shown in Figure 1 treatment with 0.25 mg/kg/day of Racemic equol and R-equol significantly reduced prostate weight compared to control values (see Figure 1). S-equol was not significantly different vs. control values.

When the number of entries into the open arms was analyzed, all equol treatment groups displayed significantly higher numbers of open arm entries compared to control values (see Figure 2).

Finally, while not shown graphically, the Racemic equol and R-equol treated animals displayed significantly longer times (in seconds) in the center area of the elevated plus maze (37.1 ± 6.3 and 36.4 ± 7.6, respectively) compared to control values (17.5 ± 4.1). S-equol values (27.5 ± 4.1) were not significantly different compared to control values. In general, these data show that a low dose of Racemic equol and R-equol is more effective compared to S-equol for significantly reducing anxiety-related behaviors in rodents.

Body Weight Gain and Prostate Weight by Treatments

There were no significant differences in body weight at the start of the experiment (see Table 1). After seven days of treatment with 0.25 mg/kg/day of R-equol significantly decreased body weight gain or total body weights versus control values (see Table 1). There were no significant differences in Racemic equol or S-equol treatment vs. controls. Only R-equol displayed a significant influence on body weight gain.

When ventral prostate weights were examined after seven days of treatment with 0.25 mg/kg/day of R-equol, this equol isomer significantly decreased prostate weight compared to control values (see Table 1). Similar to the body weight gain
parameter, there were no significant differences in Racemic equol or S-equol vs. control values suggesting that R-equol is the most potent isomer for these two dependent variables.

To determine if body weight may induce/influence the differences in prostate weight among the treatments, the prostate weight (mg) was divided by the body weight (per 100 grams) in each animal. As shown in Table 1, prostate weights standardized to body weight did not alter the results, where the R-equol treated animals displayed the lowest prostate to body weight ratio compared to control values. Again, there were no significant alterations in Racemic equol or S-equol vs. control levels for this parameter. Finally, whether body weight gain or final body weights by treatments were examined, the resulting outcomes of these parameters remained the same (see Table 1).

**Discussion**

Several investigations of natural food molecules/products and nutritional components or elements have been shown to reduce anxiety level in rodents, including polyphenolic compounds [31-33].

Previous studies have examined equol’s impact on elevated plus maze either through metabolism via soy dietary intake or by administering racemic equol. In the case of dietary intake of soy-containing diets and subsequent metabolism, it is known that 70-90 % of the total circulating isoflavonoids is represented by the S-equol metabolite [34, 35]. In this regard, we previously showed that dietary soy-containing diet 600 ppm produced anxiolytic effects in the elevated plus maze in young adult male rats at 75 day-old male and female Long-Evans rats similar to the results obtained in the present study. In this regard, we previously showed that dietary soy-containing diet 600 ppm produced anxiolytic effects in the elevated plus maze in young adult male and female Long-Evans rats similar to the results obtained in the present study. Also, in mid-aged (300 day-old) Long-Evans male and female rats consuming the same soy-containing diet long-term (from birth) displayed decreased anxiety parameters in the elevated plus maze. Other studies using a soy-containing diet, soya supplemented diets or the administration of 10 mg/kg/day of racemic equol showed divergent results where decreased or increased anxiety behavioral measurements were obtained [36-39] that may be explained by the influence of the basal diet used in these investigations [40, 41].

**Figure 1:** Open Arm Time: Expressed as a percent of total time in the open arm. Treatment of Sprague-Dawley male rats (63-70 days of age) for 7 consecutive days with 0.25 mg/kg/day of R-equol, Racemic equol, or S-equol. * = Significant increased open arm time (indicating significantly reduced levels of anxiety) in animals treated with 0.25 mg/kg/day of Racemic equol or 0.25 mg/kg/day of R-equol (but not S-equol) versus control levels. n = 8 animals per treatment group, p < 0.05. All values expressed as the mean + SEM.

In examining body weight and prostate parameters, there are several publications demonstrating that soy-containing diets or equol treatment significantly decrease body and prostate weights [3-5, 19, 34, 35, 42, 43]. The mechanisms of how equol decreased body weight include metabolic factors such as thyroid hormone levels, fat metabolism, leptin, adiponectin and insulin levels as well as activation of ERβ both in the central nervous system as well as in peripheral tissue sites in both animals and humans [3-6, 19, 24, 25, 35, 42-49]. Notably, only R-equol at the low dose of 0.25 mg/kg/day for seven consecutive days in young adult male rats significantly reduced body weight. This suggests that this equol isomer has potent and important metabolic actions via mechanisms that are currently unknown but may be explained by previous reports covering metabolic and central nervous system actions of equol (see Figure 3 for the postulated mechanisms of these influences).
Finally, the actions of either Racemic equol or the low dose of R-equol on significantly reducing prostate weight (even when standardized by body weight) demonstrate the important impact of the R-isomer of equol on this parameter. The present results confirm previous reports on this androgen-dependent parameter [4, 4, 19, 34, 42] and the mechanisms of how Racemic equol interact with ERβ, estrogen-related receptor gamma (ERR-γ) and block the potent hormonal actions of 5α-dihydrotestosterone (DHT) to improve prostate human health have been reported and reviewed [3, 5, 19, 22, 25, 34, 35]. Finally, it has been established in 70-80 kg men a daily dose of 12 mg per day is sufficient to significantly decrease symptoms of benign prostatic hyperplasia, representing a dose of 0.16 mg/kg which is close to 0.25 mg/kg/day dose used in the present study [3].

To our knowledge, this is the first report that examined the equol isomers at a low dose and short-term exposure to determine their influence on behavioral, body weight (gain) and prostate parameters. There are no previous published reports on equol isomers in reference to anxiety-related behaviors. In confirmation of the present results, a recent investigation examined human skin gene expression testing R-equol, Racemic equol and S-equol which displayed different results dependent upon the equol isomer or racemic mixture studied [20]. In fact, the R-equol and Racemic equol treatments were, in general, significantly more effective in improving human skin gene expression compared to S-equol [reviewed in 50], findings that support the present results, where Racemic equol or R-equol displayed significant anti-anxiety behaviors using the elevated plus maze.

Conclusions

In summary, since the proposal of the equol hypothesis in the late 1990s, research on this isoflavonoid has increased dramatically [1]. Hundreds of published reports have examined equol and its beneficial effects on hormone and age-related disorders [1-3, 19-24, 27, 43-47] and with the discovery of equol in plants and animal products [2, 7-15] this has stimulated more interest in researching this topic due to its presence in nature.

In this report utilizing the elevated plus-maze, in general, Racemic equol and R-equol displayed significantly decreased anxiety-related parameters compared to S-equol and controls. The 0.25 mg dose of R-equol was the only treatment to significantly decrease body weight gain and prostate weight compared to control values (even when prostate weights were standardized to body weights). This is the first study to compare the attributes of equol and their enantiomers at a low dose on anxiety-related parameters, weight gain and prostate weight where R-equol and Racemic equol appear to have the most potent behavioral/biological influences.

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References

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