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Dietary Isoflavone-Dependent and Estradiol Replacement Effects on Body Weight in the Ovariectomized (OVX) Rat

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Abstract

17 β -Estradiol is known to regulate energy metabolism and body weight. Ovariectomy results in body weight gain while estradiol administration results in a reversal of weight gain. Isoflavones, found in rodent chow, can mimic estrogenic effects making it crucial to understand the role of these compounds on metabolic regulation. The goal of this study is to examine the effect of dietary isoflavones on body weight regulation in the ovariectomized rat. This study will examine how dietary isoflavones can interact with estradiol treatment to affect body weight. Consistent with previous findings, animals fed an isoflavone-rich diet had decreased body weight ($p < 0.05$), abdominal fat ($p < 0.05$), and serum leptin levels ($p < 0.05$) compared to animals fed an isoflavone-free diet. Estradiol replacement resulted in decreased body weight ($p < 0.05$), abdominal fat ($p < 0.05$), and serum leptin ($p < 0.05$). Current literature suggests the involvement of cytokines in the inflammatory response of body weight gain. We screened a host of cytokines and chemokines that may be altered by dietary isoflavones or estradiol replacement. Serum cytokine analysis revealed significant ($p < 0.05$) diet-dependent increases in inflammatory cytokines (keratinocyte-derived chemokine). The isoflavone-free diet in OVX rats resulted in the

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Conflict of Interests

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regulation of the following cytokines and chemokines: interleukin-10, interleukin-18, serum regulated on activation, normal T cell expressed and secreted, and monocyte chemoattractant protein-1 ($p < 0.05$). Overall, these results reveal that estradiol treatment can have differential effects on energy metabolism and body weight regulation depending on the presence of isoflavones in rodent chow.

Keywords

energy metabolism; leptin; cytokines

Introduction

The ovariectomized (OVX) rat is a well-established model of post-menopausal weight gain [1, 2]. It has previously been shown that complete ovary removal in the rat leads to increased body weight gain and food consumption [2]. Administration of exogenous 17β -estradiol (E2) to the OVX rat prevents this body weight gain demonstrating that E2 plays a significant role in regulating metabolic pathways [2]. E2 is synthesized and released primarily from the ovaries; however, bone, adrenals, and adipose tissue also contribute to the overall circulating estrogen levels [3]. E2 is the predominant estrogen in adult female rats and acts via the ligand-activated nuclear estrogen receptors (ER): ER α and ER β [4–6]. E2 binds with equal affinity to both isoforms of the ER. These receptors have overlapping expression in peripheral and central tissues [7]. By treating OVX rats with propylpyrazoletriol (PPT), an ER α selective agonist, our laboratory has previously demonstrated that ER α mitigates E2 action on body weight by reducing post-OVX weight gain [8]. Similarly, ER α knockout male and female rats, but not ER β knockout animals, have decreased energy expenditure and become hyperphagic resulting in excess white adipose tissue deposition [9, 10]. Thus, it appears that endogenous E2 is involved in the homeostatic metabolic regulation of an organism via ER α -mitigated actions.

Adipose tissue plays an active role as a secretory endocrine organ involved in homeostatic regulation [11]. Leptin is an adipocyte-secreted hormone that crosses the blood brain barrier to act as a central nervous system adiposity signal. Leptin receptors (Ob-R) are found within hypothalamic regions that control food intake and energy expenditure, resulting in leptin's ability to regulate body weight [12]. Leptin and E2 signaling pathways interact in hypothalamic nuclei that regulate food consumption and body weight [13]. In addition to leptin, other adipokines, such as cytokines and chemokines, are released from adipose tissue and may also play a critical role in regulating body weight gain and energy expenditure [14].

Obesity, characterized as a low-grade inflammatory response, results in the infiltration of macrophages to the adipose tissue, which in turn, increases the release of inflammatory cytokines [15]. Increased body weight results in elevated levels of a host of pro-inflammatory cytokines such as interleukin-6 (IL-6), tumor necrosis factor α (TNF α), and keratinocyte-derived chemokine (GRO/KC) [16–18]. Previous studies show that in genetically obese (ob/ob) mice and diet-induced obese mice, circulating GRO/KC is elevated [18]. Likewise, interleukin-10 (IL-10) is elevated in obese chimpanzees [19]. Elevated

interleukin-18 (IL-18) levels are correlated with impaired insulin sensitivity and metabolic syndrome [20].

Estrogens and cytokines have a bidirectional interaction, regulating the other's production and activity [21, 22]. Estrogen is shown to have anti-inflammatory effects [23, 24]. Estrogens can interact with and regulate cytokine activity, offering another mechanism for estrogen-mediated metabolic regulation [22]. In both in vivo and in vitro estrogen deficient models, estrogen treatment suppresses IL-6 production [25]. Activation of both isoforms of the ER also results in a decrease of IL-6 gene expression [26]. Estrogen administration has also been shown to downregulate central TNF α and IL-10 [22]. Likewise, previous studies show that OVX animals fed either a normal diet or a high-cholesterol diet had increased monocyte chemoattractant protein-1 (MCP-1). Administration of E2 decreases MCP-1 protein and mRNA expression, further alluding to a potential role of E2 in regulating weight-induced inflammatory response [27].

Phytoestrogens, which act as estrogen mimetics, are compounds found in rodent chow. Naturally occurring non-steroidal phytoestrogens can elicit estrogenic and anti-estrogenic effects due to their structural similarity to estrogen and ability to interact with both isoforms of the ERs [28–30]. Isoflavones, found in plants and soy products, are the most abundant class of phytoestrogens found in standard rodent chow. A number of studies have shown that isoflavones present in food may affect reproduction, metabolism, and sexually dimorphic behaviors [31]. Young mice exposed to isoflavones have reduced fertility, evidenced by decreased number of live litters and percent of females able to deliver [32]. Similarly, isoflavones are shown to have a role in modulating metabolic homeostasis. Gonadally intact and OVX female rats exposed to an isoflavone-rich diet have decreased body weight relative to animals exposed to an isoflavone-free diet [33–35]. Therefore, isoflavones can regulate energy expenditure and metabolic pathways likely via ER action.

The current study examined the interaction of dietary isoflavones and E2 replacement on metabolic regulation in the OVX rat. E2 treated OVX rats were fed either standard rodent chow or isoflavone-free chow. The effects of isoflavones and E2 on body weight, adipose tissue, food and water consumption, as well as, serum leptin were determined. Due to the precedence to profile cytokine and chemokine changes in response to dietary components and hormone replacement, we screened serum adipokines that may play a role in the metabolic regulation. Our results suggest that the presence of isoflavones in diet may modulate the metabolic effects of E2 in the OVX rat.

Materials and Methods

Forty adult (2 months of age) female Sprague–Dawley rats, weighing 200–225 gms, were purchased ovariectomized from an approved vendor (Sprague-Dawley Outbred Rat SD, Harlan, Indianapolis, IN, USA). Rats were pair-housed and placed on a reverse light/dark cycle (12:12L:D cycle with lights out at 13:00 h). The housing room was temperature- and humidity-controlled at 22–25 °C and 50 % humidity. Upon arrival, rats were individually handled daily and acclimated to the facility for 7 days. Rats had ad libitum access to either standard feed (2018 Teklad Global 18 % Protein Rodent Diet; Harlan, Madison, WI, USA)

or an isocaloric isoflavone-free (IF) feed (Modified AIN-93G Purified Rodent Diet with Corn Oil Replacing Soybean Oil; Dyets, Inc., Bethlehem, PA, USA) [36] and water. These diets did not differ significantly in macronutrient, mineral and vitamin composition. In brief, the energy density of the AIN-93 IF diet is 3 766 kcal with 19.3 % protein, 64 % carbohydrates and 16.7 % fat [36]. The Teklad Rodent diet has an energy density of 3 100 kcal with 18.6 % protein, 44.2 % carbohydrates and 6.2 % fat [37]. The standard and IF diets were analyzed for isoflavone content using a high performance liquid chromatography (HPLC) based analysis according to the procedure of Franke et al. [36, 38]. Analysis of the standard rodent chow shows an average total of 199.4 µg/g of isoflavone aglycone equivalents (89.3 µg/g genistein + 87.9 µg/g daidzein + 22.2 µg/g glycitein). Analysis of the IF diet reveals 0 µg/g isoflavones (Table 1). All handling and care of animals was conducted in accordance with the National Institutes of Health Guide for Care and Use of Laboratory Animals and approved by the Institutional Animal Care and Use Committee at the Uniformed Services University of the Health Sciences.

Experimental design

This study was designed to examine the interaction of dietary isoflavones and E2 replacement on body weight regulation in the OVX rat. The design of this experiment will allow for examination of the acute effect of diet alterations on body weight regulation. Replacement of E2 will allow for examination of the interaction of diet and hormone on metabolic control. This experiment was conducted as a 2 [vehicle (VEH) or E2 treatment] × 2 (standard food or isoflavone-free (IF) food] factorial design. Animals were randomly assigned to one of 4 treatment groups, each containing 10 animals. Rats were weighed daily throughout the 27-day duration of the experiment (Fig. 1). Briefly, after 7 days of acclimation to the facility, where animals were fed standard chow, rats either remained on standard chow or were switched to an IF diet (day 0). Animals were exposed to the designated food for 14 days, a length of time previously shown to alter body weight and behavior in the rat [39]. Food and water measurements were recorded daily as consumption per pair-housed cage. After 13 days of exposure to the designated feed, rats were assigned to receive either VEH or E2 treatment (administered from day 13 to day 27). The timeframe for E2 administration is based on previous studies in our laboratory where E2, via the same administration technique, significantly reduced body weight gain in the OVX rat [2].

OVX and osmotic pump

E2 was administered via subcutaneous implantation of a 14-day osmotic mini-pump (Alzet Model 2002 0.5 ml, Durect Co., Cupertino, CA, USA) following previously established protocols [2]. Briefly, rats were anesthetized using isoflurane (Isoflurane, USP, Phoenix Pharmaceuticals Inc., St. Joesph, MO, USA). On day 13 (Fig. 1), mini-pumps were placed between the shoulder blades and contained either VEH (27 % hydroxypropyl-β-cyclodextrin in sterile water, Acros Organics, Fair Lawn, NJ, USA) or E2 (Sigma Chemical Co., St. Louis, MO, USA) dissolved in VEH. Mini-pumps gradually released either VEH or E2 at a continuous rate of 0.05 µl/h and administered 0.25 mg/kg body weight/day. The dose used was based on previous studies to mimic circulating E2 levels during the proestrus phase of the rodent estrous cycle [2, 40, 41].

Collection of serum and tissue

Rats were euthanized using carbon dioxide inhalation between 09:00 and 11:00 h on experimental day 27 (Fig. 1). Trunk blood was collected immediately and stored on ice until it was centrifuged at $4\,000 \times g$ for 20 min for serum collection. Serum was stored at $-80\text{ }^{\circ}\text{C}$ until use. Abdominal fat and the uterus were removed, weighed, and stored at $-80\text{ }^{\circ}\text{C}$.

Enzyme-linked immunosorbent assay (ELISA)

Serum leptin levels were determined using a sandwich ELISA kit (SPI Bio, Montigny Le Bretonneus, France) following the manufacturer's protocol. Intra-assay coefficients of variance were 7 %.

Bio-Plex Pro™ Rat Cytokine Assay

Serum cytokine levels were determined using the Bio-Plex Pro™ Rat Cytokine 24-Plex Assay (Catalog No. 171K1001M; Bio-Rad, Hercules, CA, USA) following the manufacturer's protocol. In brief, after thawing, serum was centrifuged again at $10\,000 \times g$ for 10 min at $4\text{ }^{\circ}\text{C}$ to remove precipitates and platelets. Samples were diluted 1:4 in Bio-Rad sample diluent. Plate was read at High RP1 (PMT) setting using the Bio-Plex 100 system (Bio-Rad, Hercules, CA, USA). A complete list of cytokines tested is found in Table 2.

Statistical analysis

The interactions of diet composition and hormone replacement were analyzed using GraphPad Prism 6 (La Jolla, CA, USA). Body weight was analyzed using a 2-way analysis of variance (ANOVA) with repeated measures across time. Food and water consumption at day 13 (prior to E2 treatment) were analyzed using an unpaired t-test. At day 27, food and water consumption were analyzed using a 2-way ANOVA followed by Fisher's least significant difference (LSD) post hoc test. The total change in food and water weight was used for analysis. Abdominal fat weight, uterine weight, serum leptin and serum cytokine levels were analyzed using a 2-way ANOVA followed by Fisher's LSD post hoc test. A value of $p < 0.05$ was considered significant.

Results

Body weight

We examined the effect of dietary isoflavones and E2 replacement on body weight gain in the OVX rat. A repeated measures analysis revealed a significant effect of time [$F(6,72) = 11.48$, $p < 0.05$], treatment [$F(2,72) = 136.4$, $p < 0.05$] and interaction [$F(3,36) = 5.448$, $p < 0.05$]. At day 13 (food exposure only), animals fed standard chow weighed significantly less than animals fed IF chow ($p < 0.05$). On day 27, OVX animals receiving E2 replacement weighed significantly less than VEH treated animals within the respective food conditions ($p < 0.05$). At day 27, standard chow fed animals weighed significantly less than IF animals within the respective hormone group ($p < 0.05$) (Fig. 1).

Food and water consumption

Food consumption was measured daily and values at days 13 and 27 were analyzed using an unpaired t-test and a 2-way ANOVA, respectively. Analysis was completed on food consumed per paircaged food consumed. At day 13, an unpaired t-test revealed a significant difference between standard chow fed animals and IF fed animals. Animals fed IF chow consumed less food than animals fed standard chow ($p < 0.05$) (Fig. 2a). Data is expressed as total grams of food consumed from day 0 to day 13. At day 27, after E2 administration, a 2-way ANOVA revealed a significant effect of food [$F(1,16) = 10.55$, $p < 0.05$], no significant effect of hormone treatment and no significant interaction. E2 treatment significantly decreased food intake in IF diet fed animals ($p < 0.05$). Animals receiving E2 in the standard diet group had increased food consumption compared to E2 treated animals in the IF diet group ($p < 0.05$) (Fig. 2b). Data is expressed as total grams of food consumed between day 13 and day 27.

Water consumption was also measured daily and values at day 13 and day 27 were analyzed using an unpaired t-test and a 2-way ANOVA, respectively. Analysis was completed on water consumed per pair-cage. There were no significant differences in water consumption at day 13 or day 27 (Fig. 2c,d). Day 13 data are expressed as volume of water consumed from day 0. Day 27 data is expressed as volume of water consumed from day 13 to day 27.

Abdominal fat and uterine weights

At day 27, abdominal fat and the uterus were obtained and weighed. A 2-way ANOVA of abdominal fat weight revealed a significant effect of food [$F(1,36) = 41.86$, $p < 0.05$], a significant effect of hormone treatment [$F(6,72) = 11.48$, $p < 0.05$], and no significant interaction. Animals fed a diet rich in isoflavones had significantly decreased abdominal fat weight compared to animals fed an IF diet ($p < 0.05$). Fisher's LSD post hoc test revealed a significant decrease in abdominal fat with E2 in the standard chow diet ($p < 0.05$) (Fig. 3a). The observed changes in abdominal fat weight correlated to the body weight changes.

Uterine weight was measured to determine E2 replacement efficacy. A 2-way ANOVA revealed no significant effect of food, a significant effect of hormone [$F(1,36) = 48.48$, $p < 0.05$], and no significant interaction. E2 replacement significantly increased uterine weight in OVX animals ($p < 0.05$) (Fig. 3b).

Serum adipokines

Circulating leptin levels were measured at day 27. A 2-way ANOVA showed a significant effect of food [$F(1,36) = 11.59$, $p < 0.05$], a significant effect of E2 treatment [$F(1,36) = 11.43$, $p < 0.05$], and no significant interaction. IF diet fed animals had a significant increase in serum leptin levels compared to standard chow fed animals ($p < 0.05$). E2 replacement significantly decreased serum leptin levels in both standard and IF diets compared to the respective VEH condition ($p < 0.05$) (Fig. 4a). Serum cytokine levels were measured at day 27. Of the analytes measured, 5 were altered by either food condition or hormone treatment (Fig. 4b–f, Table 2). Each cytokine was analyzed individually using a 2-way ANOVA. Analysis of GRO/KC revealed a significant effect of food [$F(1,30) = 4.251$, $p < 0.05$], a significant effect of hormone [$F(1,30) = 8.894$, $p < 0.05$] and a significant interaction

[F(1,30) = 6.306, $p < 0.05$]. In VEH treated animals, serum GRO/KC levels significantly increased in animals fed an IF diet ($p < 0.05$). E2 administration significantly decreased GRO/KC expression only in animals fed the IF chow ($p < 0.05$) (Fig. 4b). Analysis of regulated on activation, normal T cell expressed and secreted (RANTES) revealed no significant effect of food, a significant effect of hormone [F(1,30) = 9.232, $p < 0.05$], and no significant interaction. In animals fed an IF diet, E2 treatment significantly decreased serum RANTES levels ($p < 0.05$) (Fig. 4c). A 2-way ANOVA of IL-10 revealed a significant effect of food [F(1,30) = 5.968, $p < 0.05$], no significant effect of hormone and no significant interaction. E2 treated animals fed IF chow had significantly decreased expression of IL-10 compared to E2 treated animals fed standard chow ($p < 0.05$) (Fig. 4d). Analysis of serum IL-18 levels revealed no significant effect of food, no significant effect of hormone and a significant interaction [F(1,30) = 8.285, $p < 0.05$]. E2 replacement significantly decreased serum IL-18 in IF fed animals compared to VEH treatment and also compared to E2 treated, standard chow fed animals ($p < 0.05$) (Fig. 4e). A 2-way ANOVA of MCP-1 revealed no significant effect of food, a significant effect of hormone [F(1,30) = 20.54, $p < 0.05$] and no significant interaction. E2 replacement decreased MCP-1 levels compared to VEH treatment within the respective food condition ($p < 0.05$) (Fig. 4f).

Discussion

In this study, we demonstrate that the removal of isoflavones from standard rat chow influenced the response to E2 treatment in the OVX rat. Consistent with previous findings [33–35, 42], OVX animals fed a diet rich in isoflavones have reduced body weight, adipose tissue and serum leptin levels while having increased food intake. These results suggest a potential dietary isoflavone-dependent metabolic regulation. Consistent with previous findings, we demonstrate that E2 replacement in the OVX rat results in a decrease of body weight, abdominal fat weight and serum leptin [2]. However, we show that this effect of E2 occurs regardless of the presence of dietary isoflavones. Current literature suggests the involvement of inflammatory signals in response to body weight changes [11, 43]. Removal of dietary isoflavones resulted in E2-dependent decreases in serum levels of GRO/KC, RANTES, IL-10, IL-18, and MCP-1. E2 treatment decreased serum MCP-1 in animals fed standard chow. With these results, we begin to evaluate how the interaction of isoflavones and E2 may regulate metabolic processes in regards to acute changes in dietary composition.

To assess the interaction of dietary isoflavones and E2 replacement on metabolic regulation, we measured body weight and adiposity markers in the OVX rat. Dietary isoflavones resulted in a decrease of body weight gain and fat accumulation in the OVX rat. This may be a result of isoflavones effects on adipocytes or their interaction with ERs. In rat adipocytes, isoflavones, specifically genistein, are shown to inhibit lipogenesis and enhance lipolysis, by affecting downstream metabolic cascades [44]. The prolonged presence of isoflavones in diet regulates serum glucose levels, adipokine expression and transcription factors involved in lipid metabolism [45]. In a dose dependent manner, the administration of genistein, both in vivo and in vitro, induces adipocyte apoptosis [46]. Although genistein has a higher affinity for ER β , ER α knockout mice have no metabolic response to injected or dietary isoflavones, demonstrating an ER α -dependent mechanism [30, 47]. Activation of ER α may account for the estrogenic-like reduction in body weight and adipose tissue observed after

isoflavone exposure. However, isoflavones may also effect metabolic processes via non-estrogenic mechanisms, as suggested by our observations in uterine weight [44]. Isoflavones are shown to increase energy expenditure and metabolic regulation [48]. E2 treatment resulted in increased uterine weight; however, isoflavones did not alter uterine weight. Therefore, it is likely that isoflavones may act via other mechanisms. Although a non-estrogenic mechanism of isoflavones is possible, it is important to further investigate the affinity of isoflavones for ER α , as well as, the interaction of isoflavones with ERs in the presence of circulating estrogen.

Serum leptin levels are used as a measure to assess adiposity [2, 50]. We observed increased circulating leptin that correlated with increased body weight. Leptin is synthesized and released from white adipose tissue to elicit central nervous system regulation of feeding behavior and body weight [51]. Mice with mutations in the long form of the leptin receptor (Ob-R) are severely obese, indicating that this is the key signaling form of the receptor [14, 52]. Ob-R mediates leptin's action via the JAK-STAT signaling pathway, resulting in activation of proopiomelanocortin (POMC) transcription [53], a pathway likely involved in the estrogenic effect we observed. POMC activation promotes satiety and increases energy expenditure. After E2 treatment, we noticed a decrease in adiposity and circulating leptin, potentially as a result of the crosstalk of these 2 hormones in the hypothalamus [54]. Isoflavones may regulate Ob-R as another mechanism independent of ER, however, further studies are needed to elucidate this role.

Like estrogen, isoflavones are shown to regulate key components of central and peripheral control of feeding behavior and energy expenditure. In our study we observed decreased body weight gain in animals fed an isoflavone-rich diet. Surprisingly, these animals had increased food consumption compared to animals fed a diet free of isoflavones. The independent effects that E2 and isoflavones have on central and peripheral regulation may explain the observed changes in the OVX model; however, how these compounds interact to further control and regulate feeding, energy and metabolic behaviors still needs to be explored.

Metabolic dysregulations are marked by increased circulating inflammatory markers [11, 62]. Mice with increased body weight and adiposity have increased numbers of mature macrophages, introducing adipose tissue's active role in the inflammatory response [11]. To this end, we screened for circulating levels of various cytokines that were altered in response to food and hormone. We observed, in certain dietary or hormone conditions, that a change in body weight correlated with a change in circulating cytokine/chemokine expression. As shown in Table 2, we noticed no difference in IL-6 and TNF α , 2 pro-inflammatory cytokines highly correlated with adiposity, both experimentally and clinically [11, 43, 63, 64]. We did, however, show changes in circulating levels of GRO/KC. Utilizing the ob/ob (genetically obese mouse) model and the diet-induced obesity model, previous studies also show increased adipose tissue and plasma expression of GRO/KC [18]. The receptor for GRO/KC is expressed in white adipose tissue, increased in obesity and regulated by TNF α [67]. We observed that removal of isoflavones from diet increased body weight and also induced increased expression of serum GRO/KC, making this a potential chronic marker for adiposity.

Current experimental literature examining the effect of isoflavone presence on cytokines is sparse; however, estrogen's anti-inflammatory properties may modulate cytokine activity [68]. At physiological concentrations, E2 inhibits cytokine secretion in whole blood cultures [69]. E2 also inhibits monocyte adhesion in hypercholesterolemic rabbits, by potentially regulating MCP-1. In OVX animals fed a high cholesterol diet, MCP-1 protein and mRNA are elevated. E2 treatment reverses this effect [27]. In our study, the primary effect on cytokine and chemokine expression patterns occurred upon the administration of E2 when isoflavones were removed from the diet. E2 replacement in rats exposed to an acute removal of dietary isoflavones resulted in decreased levels of circulating RANTES, IL-10, and IL-18. Estradiol administration may exert its effects via other cytokines (i. e., TNF α) or nitric oxide [27]). This seems unlikely for our study since we observed no alteration in circulating TNF (data not shown), potentially due to the level of weight gain observed in our animals. Alteration in adipokine expression may not occur in overweight animals, but only in animals classified as obese [19].

In our study, the removal of ovarian estrogens and dietary isoflavones sensitized the cytokine/chemokine response to E2, which may explain why we observe the decreases in cytokine expression primarily in IF chow fed animals. In ER α and ER β knock-out mice, E2 treatment does not produce anti-inflammatory effects in response to lipopolysaccharide injury [77]. Therefore, E2 replacement may have an enhanced cytokine effect in cases where dietary isoflavones do not activate ERs. Conversely, the availability of isoflavones may desensitize the anti-inflammatory effect of E2 due to changes in ER expression [78]. Further research is required to determine the interaction of endogenous E2 levels with isoflavones to potentially alter receptor expression, ratio and/or sensitivity.

In summary, OVX rats treated with E2 had decreased body weight, abdominal fat, and serum leptin levels compared to animals treated with VEH. Isoflavones potentiate the effect of E2 to further decrease body weight gain and abdominal fat weight. Central leptin signaling may mediate this effect. Since isoflavones exhibit similar effects to E2, it is proposed that isoflavones act via estrogenic pathways to regulate body weight. Cytokines and chemokines are involved in the regulation of processes involved in body weight control. Circulating cytokine/chemokine levels are decreased in E2 treated OVX animals fed an isoflavone-free diet. By removing isoflavones from the diet, this potentially sensitizes the receptors to the estrogenic-dependent cytokine effect. These findings suggest that dietary isoflavones are an important factor to consider when determining the neural and metabolic response to estradiol, however, the exact mechanism(s) of this interaction remains to be determined. One limitation of this study is that only one time point was used. Future studies are needed to look at the factors identified in this study at various time points. The results of this study address the interaction of isoflavones and estradiol in metabolic regulation and provide a preliminary profile of cytokine and chemokine expression patterns in the OVX rat.

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Fig. 1.

Effect of isoflavone presence and hormone treatment on body weight in the OVX rat. On day 0, animals were either continued on standard chow diet or introduced to an IF diet. On day 13, animals received either VEH or E2 for the remainder of the study (14 days). On day 27 tissue was collected. IF chow fed animals had increased body weight compared to standard chow fed animals (at day 13 and day 27). E2 treatment prevented post-OVX weight gain in both diet conditions (at day 27). Data points represent average body weight values $\pm$ SEM of $n = 10$ animals per treatment group; * $p < 0.05$.

Fig. 2.

The presence of isoflavone and hormone treatment on food and water consumption at days 13 and 27. **a** Amount of food consumed on day 13 (prior to E2 treatment). Animals fed an IF diet had decreased food consumption compared to animals fed standard chow. **b** Amount of food consumed at day 27 (after E2 treatment). E2 replacement decreased food consumption in IF diet animals. The combination of E2 treatment and IF chow resulted in decreased food consumption compared to E2 treated animals fed standard chow. **c** Amount of water consumed at day 13. There is no difference in water consumption regardless of food condition. **d** Amount of water consumed on day 27. There was no difference regardless of food or hormone treatment. Bars represent mean values $\pm$ SEM. At day 13, $n = 10$ per treatment group. At day 27, $n = 5$ per treatment group; * $p < 0.05$).

Fig. 3. Effect of dietary isoflavones and hormone treatment on abdominal fat weight and uterine weight in the OVX rat. **a** Animals exposed to isoflavones in standard chow had decreased abdominal fat weight compared to animals fed an IF diet. In the standard chow condition, E2 treatment decreased abdominal fat weight. **b** As expected, animals treated with E2 had increased uterine weights compared to animals treated with VEH. All samples were collected at day 27. Bars represent mean values $\pm$ SEM of $n = 10$ animals; * $p < 0.05$).

Fig. 4.

Circulating adipokines as a result of food condition and E2 treatment in the OVX rat. **a** Animals fed standard chow had an increased levels of circulating leptin compared to animals fed an IF diet. E2 replacement decreased the amount of serum leptin within each food condition. **b** IF diet alone (VEH) resulted in increased GRO/KC expression compared to standard chow. E2 treatment resulted in decreased serum GRO/KC in animals fed IF chow. **c** In animals fed an IF diet, E2 replacement decreased serum RANTES expression. **d** After E2 administration, IF chow fed animals had decreased IL-10 expression compared to standard chow fed animals. **e** Serum IL-18 levels were decreased in E2 treated, IF chow fed animals compared to E2 treated, standard chow fed animals. Within the IF chow fed condition, animals receiving E2 replacement had decreased IL-18 compared to animals receiving VEH. **f** E2 treatment decreased serum expression of MCP-1 within each food condition compared to VEH. Samples were collected on day 27. Bars represent mean $\pm$ SEM of $n = 6-10$ animals per treatment group; * $p < 0.05$).

Table 1

Isoflavone content in standard rodent chow and isoflavone-free (IF) rodent chow.

Isoflavone		Standard chow $\mu\text{g/g}$ food	Isoflavone-free (IF) chow
Daidzein 7- <i>O</i> -glucoside	Daidzin	51.1 ± 1.6	0
Glycitein 7- <i>O</i> -glucoside	Glycitin	14.0 ± 1.7	0
Genistein 7- <i>O</i> -glucoside	Genistin	39.9 ± 1.0	0
Daidzein 7- <i>O</i> -glucoside-6''-malonate	Daidzin malonate	26.0 ± 1.5	0
Glycitein 7- <i>O</i> -glucoside-6''-malonate	Glycitin malonate	7.0 ± 1.1	0
Genistein 7- <i>O</i> -glucoside-6''-malonate	Genistin malonate	9.3 ± 0.4	0
Daidzein 7- <i>O</i> -glucoside-6''-acetate	Daidzin acetate	1.2 ± 0.1	0
Glycitein 7- <i>O</i> -glucoside-6''-acetate	Glycitin acetate	25.5 ± 0.1	0
Genistein 7- <i>O</i> -glucoside-6''-acetate	Genistin acetate	1.0 ± 0.1	0
Daidzein aglycone	Daidzein	0	0
Glycitein aglycone	Glycitein	23.1 ± 1.0	0
Genistein aglycone	Genistein	0.8 ± 0.3	0

Standard chow shows an average total of 199.4 $\mu\text{g/g}$ of isoflavone aglycone equivalents. Analysis of the IF diet reveals 0 $\mu\text{g/g}$ isoflavones

Table 2

Full listing of cytokines tested utilizing the Bio-Plex Pro™ Rat Cytokine 24-Plex Assay.

Assayed cytokines – No change regardless of diet or hormone				Altered cytokines
EPO	G-CSF	GM-CSF	IFN- γ	GRO/KC
IL-1 α	IL-1 β	IL-2	IL-4	IL-10
IL-5	IL-6	IL-7	IL-12p70	IL-18
IL-13	IL-17A	M-CSF	MIP-1 α	MCP-1
MIP-3 α	TNF- α	VEGF		RANTES

Of the 24 analytes, 5 were significantly altered either by diet or by hormone. The altered cytokines are listed on the right hand column of the table