

Noble Rats Display Decreased Weight Gain and Visceral Adiposity via Lifelong Exposure to an Isoflavone-Rich Diet

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Abstract

Obesity and cancer represent two of the greatest public health concerns. The relationship between obesity and cancer suggests an important role for visceral adipose tissue (VAT) versus generalized body fat. Male and female Noble rats develop a relatively high incidence of spontaneous and hormone-induced carcinomas. The main purpose of this study was to make an initial assessment of whether a high soy-isoflavonoid diet could decrease VAT and reduce overall body weight gain in Noble rats. The male and female rats were exposed solely to either a high soy-isoflavonoid diet (High-Iso) or a low-isoflavonoid diet (Low-Iso) from conception to adulthood. At the end of the experiment, at 145 days of age, overall weight gain and visceral fat deposition were recorded along with quantification of serum isoflavone levels (genistein, daidzein and equol). Serum isoflavone levels in the High-Iso treatment groups were significantly higher (by approximately 15-fold) compared to animals in the Low-Iso diet groups. Male and female Noble rats fed the High-Iso diet displayed significantly lower body weight gain (by approximately 10%) compared to animals fed the Low-Iso diet. Notably, VAT deposition was reduced by 61% in females but 21% in males fed the High-Iso diet versus the Low-Iso diet. In summary, consumption of a diet high in isoflavones significantly lowered body weight and VAT deposition and a hypothesis may be proposed as to how soy-derived isoflavones may decrease VAT storage areas to potentially improve obesity and cancer outcomes.

Keywords: Isoflavones; Diet; Weight gain; Visceral fat; Noble rat; Cancer outcome

Introduction

Noble Rats Display Decreased Weight Gain and Visceral Adiposity via Lifelong Exposure to an Isoflavone-Rich Diet. The World Health Organization defines obesity as an abnormal or excessive fat accumulation in adipose tissue, to the extent that health is impaired (Obesity-Report WHO consultation, 2000) [1]. Obesity frequency has been steadily increasing over the past decades and in 2007-2008, the prevalence of obesity among US adults was 33.8% and for overweight individuals 68% [2]. Furthermore, obesity is associated with the development and progression of various cancers, including tumors of the colon, esophagus, pancreas, liver, breast, prostate, kidney, endometrium and cardiovascular disease [3-7]. In fact, visceral rather than

generalized body fat appears to play a key role between obesity being link to cancer [6,8]. Many biomarkers, hormones and chemical signals are involved in body weight gain, adipose tissue deposition and cancer outcomes [9,10]. For example, obesity in postmenopausal women has been associated with a higher risk of breast cancer that is linked to estrogen formation within the breast [11,12]. Also, obesity is associated with decreased testosterone levels in males [13]. This suggests a complex interaction between adipokines, inflammatory factors, hormones and other chemical signaling that is just beginning to be revealed.

One approach to investigate the causes of obesity and chronic disease is to examine the evidence for the discordance between modern diets and the evolution of consumption in humankind. In Martin C, et al. [14] summary article such a proposition is reviewed and the authors provide support for the consumption of plant foods as a way to reduce the risk of chronic disease. Such plant food molecules include polyphenolic compounds like the isoflavonoids [14], and there are several studies that suggest isoflavones prevent certain disorders such as breast and prostate cancer and cardiovascular disease [14-17]. However, Western diets generally have a 100-fold lower level of isoflavonoids compared to Asian diets [14].

Notably, several studies from our laboratory have demonstrated that soy diets containing high levels of isoflavonoid compounds have positive influences on obesity, metabolism and prostate health [18-21]. Specifically, body weight and visceral adipose tissue (VAT) deposition parameters were significantly decreased in animals fed an isoflavone-rich diet compared to controls, most likely due to the selective estrogen receptor modulatory (SERM) actions of the isoflavonoid molecules.

However, the Noble rat model has not been studied in this regard as far as body weight and VAT deposition parameters via dietary treatments. The Noble rat model has been used to study spontaneous and hormonal (estrogen- and androgen-induced) carcinogenesis of mammary and prostate/seminal glands [22-25].

The purpose of this study was to determine whether an isoflavonoid-rich diet derived from soy ingredients (with 600

ppm or the High-Iso diet) compared to a low isoflavonoid diet (with 15 ppm or the Low-Iso diet) can influence body weight and VAT deposition when male or female rats were exposed to the treatments from conception to adulthood. In brief, body weight and VAT deposition were significantly lower in the High-Iso vs. the Low-Iso fed animals where female Noble rats displayed the greatest influence of the diet treatments.

Methods

Animals and housing

All procedures involving animals were approved by the Brigham Young University Institutional Animal Care and Use Committee. Fifty to fifty-five day old male and female Noble rats (n=4 by sex) were obtained through the Animal Production Program of the National Cancer Institute (NCI; Frederick, MD, USA). The animals were placed on a 12 hr light/dark schedule (6 am to 6 pm) and allowed ad libitum access to food (see diet treatments) and tap water. The animals stayed on the diet treatments by sex until approximately 90 days old when mating took place as outline below.

All animals were housed in clear plastic cages with wire lids (20 cm x 24 cm x 40 cm standard shoebox with woodchip bedding). The young adult male and female rats were housed individually except during mating. Following delivery, offspring pups were housed with their mothers until age 21 days, at which point they were weaned and placed in separate cages (three to four animals per cage by sex by diet treatment). At 40 days the animals were separated to two animals per cage and finally, at 60 days, placed in individual cages by diet treatment.

Mating

Animals were diet-matched and mated to ensure offspring would be exposed solely to only one of the two diet treatments. During the mating procedure, males were housed within hanging wire cages over cardboard mats. One female was introduced into each male cage, and the cardboard was checked for the appearance of vaginal plugs twice a day (at about 8 a.m. and 5 p.m.) indicating successful mating/insemination. Inseminated females were removed from the male's cage, weighed and replaced with another female. This continued until vaginal plugs were found for all females. All males were fertile and inseminated 4 litters by diet treatment. Pregnancy was confirmed by a 30-40 g weight gain over the first 5-7 days of gestation.

Diet treatments

After arrival at our animal facilities, male and female rats to be mated were immediately placed on either a high soy-isoflavonoid diet (High-Iso) containing isoflavones at a concentration of approximately 600 ppm as glycones and aglycones (Harlan Teklad 8604, Madison, WI, USA) or a low-isoflavonoid diet (Low-Iso) with approximately 15 ppm of soy isoflavones as glycones (Zeigler Bros. Inc., Gardners, PA, USA, Phytoestrogen Reduced Rodent Diet) [26]. The Low-Iso diet contains casein and corn and wheat provided the protein content for this formulation [26]. Offspring were kept on the maternal diet, thus ensuring

strict exposure to only one diet treatment from conception to adulthood. The specific nutritional content for these diets is described elsewhere [26]. The diets were balanced and matched for protein, fat, carbohydrate, amino acids, vitamins and minerals (Table 1).

Table 1: Composition of High Soy-Isoflavonoid (High-Iso) diet or a Low-Isoflavonoid (Low-Iso) diet fed to noble rats.

	High-Iso	Low-Iso
Top 10 ingredients by weight		
1	Dehulled soybean meal	Ground corn
2	Wheat middling	Ground wheat
3	Flaked corn	Fish meal
4	Ground corn	Wheat middling
5	Fish meal	Lactic casein
6	Cane molasses	Dried skin milk
7	Ground wheat	Corn gluten meal
8	Dried whey	Corn oil
9	Soybean oil	Brewers dried yeast
10	Brewers dried yeast	Dry molasses
Proximate composition of the diets		
Protein (g/kg)	243	235
Fat (g/kg)	47	53
Carbohydrate (g/kg)	402	513
Crude fiber (g/kg)	40	25
Neutral detergent fiber (g/kg)	124	67
Ash (g/kg)	74.0	64.5
Genistein + Daidzein (g/kg)	0.62	0.01
Energy density (kJ/kg)	12,560	14,528
Energy from protein (%)	32	27
Energy from fat (%)	14	14
Energy from carbohydrates (%)	54	59
Amino Acids (g/kg)		
Arginine	15.3	11.3
Cystine	3.7	2.7
Histidine	5.8	5.9
Isoleucine	10.0	11.7
Leucine	19.0	21.6
Lysine	14.0	14.2
Methionine	4.2	5.9
Phenylalanine+tyrosine	20.0	20.7
Threonine	9.4	9.4
Tryptophan	2.9	2.5
Valine	11.0	13.6
Minerals (mg/kg)		
Calcium	14,000	12,000
Chloride	5,000	4,300
Cobalt	0.71	0.52
Copper	25	13
Iodine	2	1.84

Iron	300	245
Magnesium	3,000	1,500
Manganese	100	96
Phosphorus	11,000	9,500
Potassium	10,000	5,500
Se	0.34	0.45
Sodium	3,000	3,000
Zinc	80	60
Vitamins (mg/kg)		
Biotin	0.38	0.37
Cholecalciferol	0.060	0.126
Choline	2,530	1,646
Folate	3	3
Menadione	40	31
Niacin	63	76.5
Pantothenic acid	21	31.7
Riboflavin	8	7
Thiamin	27	17.3
Vitamin A	3.78	1.98
Vitamin B-6	13	10
Vitamin B-12	0.050	0.048
Vitamin E	54	22
Linoleic acid (mg/kg)		
Linoleic acid	18.7	22.8

Body weight gain, visceral adipose tissue weight and blood sample collection

Tissue collection occurred at 145 days of age. Animals (n=8-to-10) by diet treatment by sex (representing 4 litters) were weighed (± 0.1 grams). To determine final body weight gained each animal's birth weight was subtracted from their body weight at 145 days of age. Then the animals were anesthetized (ketamine/acepromazine at 75/2.5mg/kg IP) prior to collecting blood samples and VAT. An incision was made from the pelvis to the bottom of the rib cage after which arterial blood was collected from the heart. Subsequently, white adipose tissue (or VAT) was dissected from the caudal surface of the diaphragm to the base of the pelvic cavity and recorded in grams (± 0.01 grams) for each animal.

Determination of isoflavone blood levels

After an arterial blood sample (5-7ml) was collected from each animal, the sample was allowed to clot and then centrifuged to obtain serum, which was then labeled and frozen at -20°C until analysis was performed. Quantification of total isoflavonoid (daidzein, genistein, and equol) serum levels were performed at ESA Biosciences, Inc. Chelmsford, MA, USA, using HPLC-electrochemical detection (ECD) with a lower limit of detection of 5ng/ml for each aglycone. Samples (by diet treatment and sex) were analyzed in duplicate in a single assay along with internal control samples; the intra-assay coefficient of variation was approximately 4%.

Statistical analysis

All data sets were analyzed with Minitab (State College,

PA, USA) software [by ANOVA (unstacked data)], followed by Fisher's pairwise comparisons to determine significance and all data were expressed as the mean \pm standard error of the mean (SEM); $p < 0.05$ was considered significant.

Results and Discussion

Serum isoflavone levels

Serum isoflavone levels in males and females fed the High-Iso diet were significantly higher compared to animals fed the Low-Iso diet (Figure 1). For example, total isoflavone levels in female Noble rats fed the High-Iso diet were approximately 16.8-fold higher compared to the Low-Iso values ($p < 0.001$). Additionally, the total isoflavone levels in male Noble rats fed the High-Iso diet were approximately 13.9-fold higher compared to the Low-Iso values ($p < 0.001$, Figure 1). Equol represented the majority of the total isoflavone values:

- In females, equol represented 77% of the total isoflavone values followed by daidzein (15%), then genistein (8%), and,
- In males, equol represented 72% of the total isoflavone values followed by daidzein (21%) then genistein (7%).

The present data confirm that rodents, in general, produce high circulating levels of the polyphenolic molecule, equol when fed a soy-containing diet [27,28].

The present total isoflavone results by diet treatments were similar to those reported elsewhere for male Long-Evans rats (@ 75 or 100 days old) fed the same diets [20,29]. However, male Noble rat total isoflavone values in the present study fed the

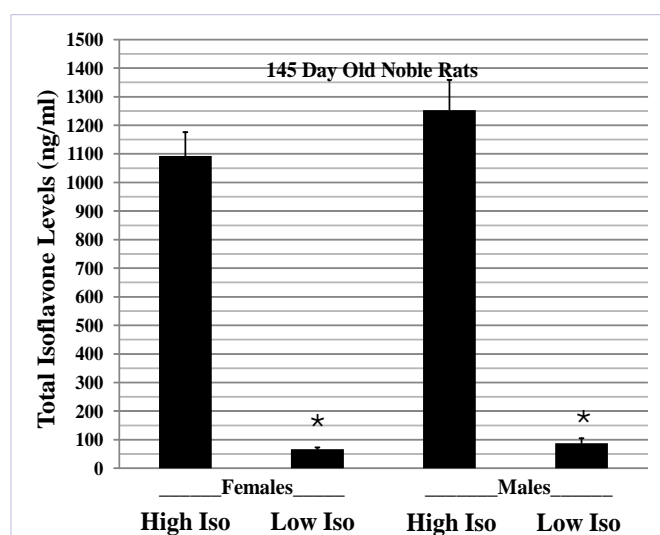


Figure 1: Total circulating serum Isoflavone levels (ng/ml) by sex and diet treatment in noble rats. Isoflavones analysis was performed by HPLC-EC. Symbol (*): significantly lower total Isoflavone levels ($p < 0.001$) in animals fed the Low-Iso diet (containing approximately 15ppm) versus animals fed the High-Iso diet (containing approximately 600 ppm of glycones and aglycones). In general, equol represented 77% of the total Isoflavone values followed by Daidzein (15%) then Genistein (8%) in female and in males equol represented 72% of the total Isoflavone values followed by Daidzein (21%) then Genistein (7%).

High-Iso diet were approximately 25 to 30% lower compared to male Long-Evans rats on the same High-Iso diet [20,29]. This may suggest a difference in isoflavone metabolism and/or clearance between the two rat strains or potential differences in age-related parameters. Also, the equol metabolite represented 73% of the total isoflavones in the present study from male Noble rats fed the High-Iso diet which were similar to those reported for adult male Long-Evans rats on the same diet at 77% for 75 day old or 82% for 100 day old animals [20,29].

Body weight gain and VAT weight

Male and female Nobel rats fed the High-Iso diet displayed significantly lower body weight gain compared to animals fed the Low-Iso diet ($p<0.042$ and $p<0.038$, respectively; Figures 2 & 3). The reduction in body weight gain was greater in the female Noble rats at approximately 13% while in male Noble rats the decrease was approximately 8% in the High-Iso groups compared to animals fed the Low-Iso diet. The present results were similar to previous studies examining young adult and aging (300 day old) Long-Evans rats for decreased body weight gain using the same two diet treatments [20,29].

When VAT weight was examined, VAT deposition was significantly reduced by 61% in females (from approximately 5.4 to 2.1 grams; $p<0.011$) and 21% in males (from approximately 3.9 to 3.1 grams; $p<0.027$) fed the High-Iso diet versus the Low-Iso diet (Figures 4 & 5). The present results were similar to previous findings where feeding Long-Evans rats significantly reduced VAT weights when fed a High-Iso vs. a Low-Iso diet [20,29]. While genistein has been shown to have weight reducing and diabetic protecting properties [30], it is interesting to note that equol represents the major circulating isoflavonoid in the present study that has been shown to have beneficial weight and VAT reducing actions [19,31].

In the present study, the greater VAT deposition in females compared to males, in general, suggests that estrogens play an important role in promoting adipose tissue deposition. For

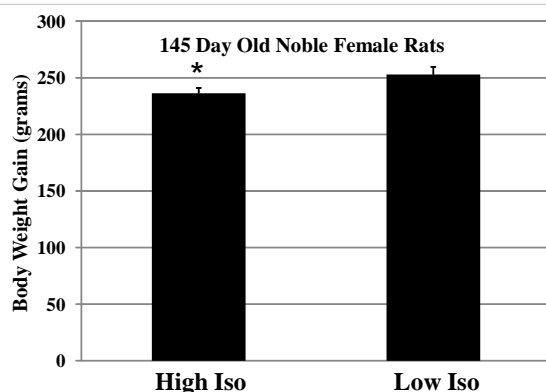


Figure 2: High Isoflavone (High-Iso) versus Low Isoflavone (Low-Iso) lifelong dietary consumption in female noble Rats: influence on body weight gain. Symbol (*): significantly lower body weight gain from birth to adulthood in animals fed the High-Iso diet versus animals fed the Low-Iso diet ($p<0.038$).

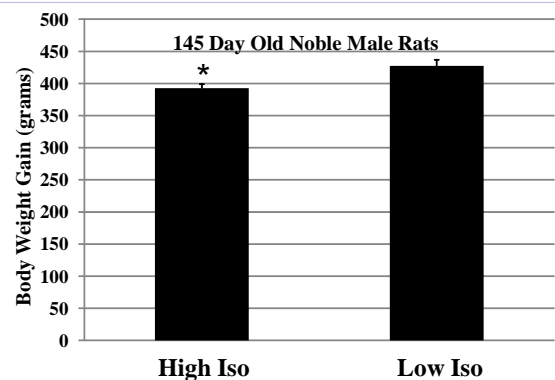


Figure 3: High Isoflavone (High-Iso) versus Low Isoflavone (Low-Iso) lifelong dietary consumption in male noble rats: influence on body weight gain. Symbol (*): significantly lower body weight gain from birth to adulthood in animals fed the High-Iso diet versus animals fed the Low-Iso diet ($p<0.042$).

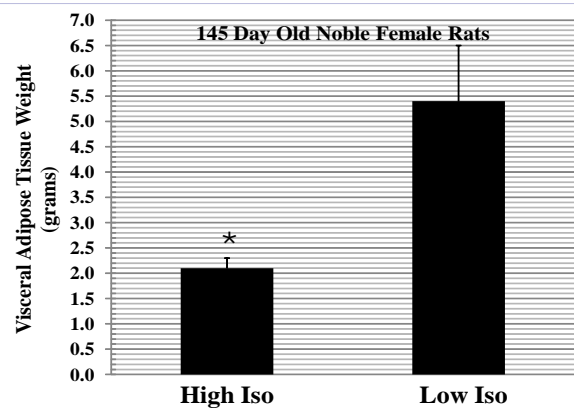


Figure 4: High Isoflavone (High-Iso) versus Low Isoflavone (Low-Iso) lifelong dietary consumption in female noble Rats: influence on visceral adipose deposition. Symbol (*): significantly lower visceral adipose tissue weight ($p<0.011$) in adulthood of animals fed the High-Iso diet versus animals fed the Low-Iso diet.

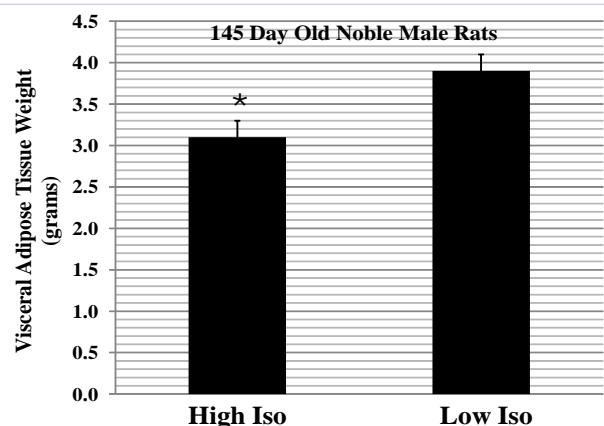


Figure 5: High Isoflavone (High-Iso) versus Low Isoflavone (Low-Iso) lifelong dietary consumption in male noble Rats: influence on visceral adipose deposition. Symbol (*): significantly lower visceral adipose tissue weight ($p<0.027$) in adulthood of animals fed the High-Iso diet versus animals fed the Low-Iso diet.

example, estrogens promote the accumulation of subcutaneous fat [32] while the loss of estrogens is associated with increased visceral fat [33] through its receptors, estrogen receptor alpha (ER α) and estrogen receptor beta (ER β) [34,35]. While ER α plays a major role in body weight regulation [36], ER β functions more as a SERM in the regulation of adiposity [37]. It is known that genistein, daidzein and especially equol are selective ER β agonists [27,28,38] that may play a pivotal role in the significant VAT reduction seen in the present results.

In this manner, the link between visceral fat and chronic disease is well established, especially the connection of VAT deposition in relationship to increased cancer risk [6,8]. By examining the body weight and VAT reducing properties of a diet high in isoflavones may suggest that the Noble rat model might be influenced by consumption of this soy-derived parameter. It is intriguing to consider whether investigators realize how important a laboratory diet may be in altering study outcomes [39,40]. For example, long-term exposure to an isoflavone-rich diet has been shown to enhance testicular and prostate health in Long-Evans rats [41]. Male Noble rats exposed to such an isoflavone-rich diet may alter the outcome of spontaneous and hormonal induced tumors of the reproductive tract, thus influencing cancer parameter outcomes. This hypothesis remains to be tested, but recent evidence suggests that this may be the case since equol has the potential to improve prostate health in animal and human studies [42,43]. Thus, further research is warranted in this important field of study.

In summary, visceral rather than generalized body fat appears to play a key role between obesity being linked to cancer. The present study used the Noble rodent model that examined the influence of lifelong dietary exposure to soy-derived High-Iso compounds on body weight gain and VAT weight in adult female and male animals. Body weight gain and VAT weight were significantly lower in the High-Iso vs. the Low-Iso diet treatment groups, presumably by the isoflavonoid molecules acting as SERMs especially for reductions in VAT deposition. While further research is warranted, the present findings suggest potential impact of isoflavone diets on further Noble rat studies involving spontaneous and hormone-induced tumorigenesis. Finally, the concept that consumption of plant foods as a way to reduce the risk of chronic disease and cancer is supported by the findings of the present study, particularly by isoflavonoid molecules and specifically by the polyphenolic compound, equol.

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