Phytoestrogens limit translation of preclinical results to clinical outcomes

Ask a Nutritionist Series: Impact of phytoestrogens on research, Volume 2

- Isoflavone consumption is higher in rodents fed soybean meal containing lab diets than humans.
- Rodents have higher proportion of circulating unconjugated isoflavones than humans. Unconjugated soy isoflavones have greater activity.
- Rodents are consistent producers of equol, a more physiologically active isoflavone metabolite, while humans are inconsistent equol producers.
- In order to improve translation of rodent results to human outcomes, strong consideration should be given to the avoidance of soybean meal-containing lab animal diets.

Challenge:
Dietary isoflavones limit translation of preclinical results to clinical outcomes.

Solution:
Envigo's minimal isoflavone Teklad Global Rodent Diets lead to reliable, repeatable research results.

Dietary phytoestrogens exert both estrogen dependent and independent effects and have been shown to have broad effects on research outcomes in rodent models (Table 1). Effects of isoflavones observed in rodents may not translate to clinical outcomes in humans due to differences in total intake and endogenous and microbial metabolism (Figure 1).

Rodents fed soy containing diets consume higher levels of isoflavones than humans

Rodents fed soybean meal containing laboratory diets consume higher levels of isoflavones than human populations. Dietary isoflavone level is dependent on the soybean meal inclusion rate and is influenced by soy genetics and growing conditions. Isoflavone levels in rodent diets containing soybean meal can range from approximately 80 – 790 ppm (genistein + daidzein) resulting in an isoflavone intake range of 8 – 118 mg/kg body weight per day (**Table 2**). This intake range is more than 10 times higher than adult Western populations even when scaled for body surface area[1-6] (**Table 2** dark grey columns).

Isoflavone intake levels for infants fed soy formula approach levels consumed by rodents fed diets containing 5 – 10% soybean meal[7], however differences in metabolism and pharmacokinetics of isoflavones likely result in differences in physiological responses to consumed isoflavones.

Research area	Effects described in the literature
Oncology	Modulate tumor growth, latency, multiplicity, metastasis; diminish action of drugs such as tamoxifen and letrozole
Reproductive	Increase uterine weight; accelerate vaginal opening; affect response to exogenous estrogens/xenobiotics
Endocrine	Differences in body composition (weight, adiposity), glucose and insulin homeostatsis, bone density and blood pressure
Neuroscience	Performance differences on tests measuring anxiety behaviors and response to pain stimuli
Immunology	Modulate immune organ development; display anti-inflammatory and antioxidant actions

Table 1. Research areas affected by isoflavone consumption in rodents[25].

Species effects on phase II metabolism results in differences in isoflavone bioactivity

The primary forms of isoflavones consumed by rodents and humans are the glycosides genistin and daidzin. These glycoside forms are poorly absorbed, so once ingested the glycoside moiety is removed via endogenous and microbial β-glucosidases[8-10]. Upon uptake by the enterocyte and liver the majority of free aglycone forms (genistein and daidzein) undergo conjugation via Phase II metabolism for circulation[11]. Glucuronic acid is the primary Phase II conjugate followed by sulfides[12].

Phase II metabolism of isoflavones affects binding and activation of the intracellular estrogen receptor. Conjugated isoflavones are relatively hydrophilic and it is unclear if these compounds can readily pass through the cell membrane to access the intracellular estrogen receptor[11, 13]. An *in vitro* study found binding of murine uterine cytosol estrogen receptors was weaker with conjugated isoflavones compared to unconjugated forms[11]. Once activated, the ER receptor translocates into the nucleus, binding DNA to regulate gene expression. Conjugation of isoflavones limits the downstream binding of nuclear receptors *in vitro* reducing relative potency for the β-ER by ~15 fold for genistein and ~4400 fold for daidzein[13]. Conjugated genistein and daidzein have limited effects on growth of the human MCF-7 cell line compared to aglycone forms[14]. In a transfected cell line with ERα/ERβ ratios mimicking healthy breast cells conjugated isoflavones were found to not be estrogenic[13].

The ability to activate isoflavones via deconjugation at the tissue level differs between species, with rat breast tissue having a ~30 fold higher deconjugation capacity compared to human breast tissue[15]. Isoflavone conjugation influences physiological effects and conjugation ratios differ between rodent strains and sexes as well as between rodents and humans.

In general, the proportion of circulating unconjugated isoflavones is ranked mice > rats > humans[16-18]. Compared to C57BL/6 mice and Sprague-Dawley rats, nude mice and the transgenic mouse model Angptl4b6 have higher proportions of unconjugated circulating isoflavones[16]. While sex does not appear to affect conjugation patterns in humans, sex and hormonal changes do affect conjugation patterns in rodent models[17]. Hormone status in Sprague-Dawley rats affected both oral bioavailability of genistein and circulating conjugated isoflavones[19]. The ability to deconjugate isoflavones at the tissue level has also been shown to differ between rodents and human models[15].

Differences in conjugation patterns between humans and rodents influence isoflavone activity and physiological effects.

Equol production differs in humans and rodents

Equol is a metabolite of daidzein that is exclusively produced by bacteria[20-22]. This metabolite has a greater affinity for the estrogen receptor (ER) and higher biological activity than the parent compound daidzein[20, 23]. Microbial production of equol can be affected by a number of variables in rodents and humans. Feeding a soy containing diet to conventionally raised rodent results in the production of equol but not in germ-free rats[22]. While conventional rodents are consistent equol producers when fed soybean meal, equol production varies in humans. Only 25 – 30% of the adult Western population is considered "equol producers" while 50 – 70% of adults in Japan, China and Korea produce equol[23, 24]. Differences in equol production may be related to differences in nutrient intake and lactase phlorizin hydrolase activity, the enzyme responsible for both the hydrolysis of lactase and deglycosylation of isoflavones[20, 24]. Differences between rodents and humans in circulating levels of equol can affect the translation of preclinical results to clinical outcomes.

Taken together rodents have higher circulating concentrations of isoflavone metabolites with greater affinity for the β-estrogen receptor, thus leading to more pronounced effects than seen in humans. Feeding lab diets containing soybean meal should be avoided in preclinical research studies in order to improve translation of rodent results to human outcomes.

Translating Isoflavone Intakes from Human to Rodents				Typical Rodent Isoflavone Intakes from Soybean Meal in Laboratory Rodent Diets		
Population	**Isoflavone intake, mg/day**	**Human isoflavone intake, mg/kg/day**	**Equivalent rodent intake, mg/kg/day[a]**	**Dietary soybean meal level**	**Typical dietary isoflavone range[b], ppm**	**Rodent isoflavone intake[c], mg/kg/day**
Western adults	1 - 3[(1)]	0.01 - 0.05	0.1 - 0.6	Low	80 - 175	8 - 26
East Asian adults	15 - 50[(4-6)]	0.2 - 0.8	1.4 - 10.2	Medium	150 - 340	15 - 51
Infants fed soy milk	20 - 45[(7)]	6 - 11	20 - 55	High	350 - 790	35 - 118

Table 2. Comparison of consumption of isoflavones in humans and rodents.

a Equivalent Rodent Intake = Human Isoflavone Intake/((animal weight in kg/human weight in kg)$^{0.33}$)[(25)]. Unless provided by the original reference, adult human body weight is assumed to be 60 kg, mouse weight 20 g and feed intake 3 g, rat weigh 150 g and feed intake 15 g.

b Envigo diets containing low (~5%), medium (~10%) and high (~25%) amounts of soybean meal during the same time period[(25)].

c Assuming mice weigh 20 g and consume 3 g diet/day and rats weigh 150 g and consume 15 g diet/day.

References

1. **Boker LK, Van der Schouw YT, De Kleijn MJ, Jacques PF, Grobbee DE, Peeters PH. 2002.** J Nutr 132: 1319-28. https://www.ncbi.nlm.nih.gov/pubmed/12042453
2. **de Kleijn MJ, van der Schouw YT, Wilson PW, Adlercreutz H, Mazur W, et al. 2001.** J Nutr 131: 1826-32. https://www.ncbi.nlm.nih.gov/pubmed/11385074
3. **Horn-Ross PL, Lee M, John EM, Koo J. 2000.** Cancer Causes Control 11: 299-302. https://www.ncbi.nlm.nih.gov/pubmed/10843441
4. **Wakai K, Egami I, Kato K, Kawamura T, Tamakoshi A, et al. 1999.** Nutr Cancer 33: 139-45. https://www.ncbi.nlm.nih.gov/pubmed/10368808
5. **Kim J, Kwon C. 2001. Nutr Res 21: 947-53.** http://www.ncbi.nlm.nih.gov/pubmed/11446978
6. **Yamamoto S, Sobue T, Sasaki S, Kobayashi M, Arai Y, et al. 2001.** J Nutr 131: 2741-7. https://www.ncbi.nlm.nih.gov/pubmed/11584098
7. **Setchell KD, Zimmer-Nechemias L, Cai J, Heubi JE. 1998.** Am J Clin Nutr 68: 1453S-61S. https://www.ncbi.nlm.nih.gov/pubmed/9848516
8. **Day AJ, DuPont MS, Ridley S, Rhodes M, Rhodes MJ, et al. 1998.** FEBS Lett 436: 71-5. https://www.ncbi.nlm.nih.gov/pubmed/9771896
9. **Day AJ, Canada FJ, Diaz JC, Kroon PA, McLauchlan R, et al. 2000.** FEBS Lett 468: 166-70. https://www.ncbi.nlm.nih.gov/pubmed/10692580
10. **Chandrasekharan S, Aglin A. 2013.** J Steroids Hormon Sci S12:004. https://pdfs.semanticscholar.org/6345/545757b1056dfd423fdac9b73eec89fe00f4.pdf
11. **Zhang Y, Song TT, Cunnick JE, Murphy PA, Hendrich S. 1999.** J Nutr 129: 399-405. https://www.ncbi.nlm.nih.gov/pubmed/10024618
12. **Yang Z, Kulkarni K, Zhu W, Hu M. 2012.** Anticancer Agents Med Chem 12: 1264-80. https://www.ncbi.nlm.nih.gov/pubmed/22583407
13. **Beekmann K, de Haan LH, Actis-Goretta L, Houtman R, van Bladeren PJ, Rietjens IM. 2015.** J Steroid Biochem Mol Biol 154: 245-53. https://www.ncbi.nlm.nih.gov/pubmed/26361015
14. **Kinjo J, Tsuchihashi R, Morito K, Hirose T, Aomori T, et al. 2004.** Biol Pharm Bull 27: 185-8. https://www.ncbi.nlm.nih.gov/pubmed/14758030
15. **Islam MA, Bekele R, Vanden Berg JH, Kuswanti Y, Thapa O, et al. 2015.** Toxicol In Vitro 29: 706-15. https://www.ncbi.nlm.nih.gov/pubmed/25661160
16. **Setchell KD, Brown NM, Zhao X, Lindley SL, Heubi JE, et al. 2011.** Am J Clin Nutr 94: 1284-94. https://www.ncbi.nlm.nih.gov/pubmed/21955647
17. **Soukup ST, Helppi J, Muller DR, Zierau O, Watzl B, et al. 2016.** Arch Toxicol 90: 1335-47. https://www.ncbi.nlm.nih.gov/pubmed/26838042
18. **Gu L, House SE, Prior RL, Fang N, Ronis MJ, et al. 2006.** J Nutr 136: 1215-21. https://www.ncbi.nlm.nih.gov/pubmed/16614407
19. **Kulkarni KH, Yang Z, Niu T, Hu M. 2012.** J Agric Food Chem 60: 7949-56. https://www.ncbi.nlm.nih.gov/pubmed/22757747
20. **Larkin T, Price WE, Astheimer L. 2008.** Crit Rev Food Sci Nutr 48: 538-52. https://www.ncbi.nlm.nih.gov/pubmed/18568859
21. **Setchell KD, Brown NM, Zimmer-Nechemias L, Brashear WT, Wolfe BE, et al. 2002.** Am J Clin Nutr 76: 447-53. https://www.ncbi.nlm.nih.gov/pubmed/12145021
22. **Bowey E, Adlercreutz H, Rowland I. 2003.** Food Chem Toxicol 41: 631-6. https://www.ncbi.nlm.nih.gov/pubmed/12659715
23. **Rafii F. 2015.** Metabolites 5: 56-73. https://www.ncbi.nlm.nih.gov/pubmed/25594250
24. **Setchell KD, Brown NM, Summer S, King EC, Heubi JE, et al. 2013.** J Nutr 143: 1950-8. https://www.ncbi.nlm.nih.gov/pubmed/24089421
25. **United States Food and Drug Administration C. 2005.** FDA, ed. http://www.fda.gov/downloads/drugs/guidances/ucm078932.pdf

Contact us

North America 800.793.7287 EU and Asia envigo.com/contactus teklad@envigo.com

Envigo RMS Division, 8520 Allison Pointe Blvd., Suite 400, Indianapolis, IN 46250, United States

envigo.com

RMS-0218-US-01-DS-241