

# Endogenous and Exogenous Equol Are Antiestrogenic in Reproductive Tissues of Apolipoprotein E-Null Mice<sup>1-3</sup>

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## Abstract

Equol is an isoflavone (IF) metabolite produced by intestinal microbiota in a subset of people consuming dietary soy. Equol producers may show different responses to soy foods and phenotypes related to cancer risk. Here, we assessed the effects of soy IF, endogenous microbial equol production, and dietary racemic equol in a 3 × 2 × 2 factorial experiment using gnotobiotic apoE-null mice ( $n = 9-11/\text{group/sex}$ ). At age 3-6 wk, equol-producing microbiota were introduced to one-half of the colony ( $n = 122$ ). At age 6 wk, mice were randomized to receive a diet that contained 1 of 3 protein sources: casein and lactalbumin, alcohol-washed soy protein (low IF), and intact soy protein (high IF), with total IF amounts of 0, 42, and 566 mg/kg diet, respectively. One-half of each diet group also received racemic equol (291 mg/kg diet). After 16 wk of dietary treatment, serum isoflavonoid profiles varied with sex, soy IF amount, and intestinal microbiota status. There were no treatment effects on tissues of male mice. In females, reproductive tissue phenotypes differed by equol-producing ability (i.e., microbiota status) but not dietary equol or IF content. Equol producers had lower uterine weight, vaginal epithelial thickness, total uterine area, endometrial area, and endometrial luminal epithelial height compared with nonproducers ( $P < 0.05$  for all), with an association between microbiota status and estrous cycle ( $P > \text{chi-square} = 0.03$ ). Exogenous equol reduced expression of progesterone receptor (PGR) and the proliferation marker Ki67 ( $P < 0.0001$ ) in vaginal epithelium and endometrium; for endogenous equol, only PGR was reduced ( $P < 0.0005$ ). Our findings indicate that equol diminishes estrogen-dependent tissue responses in apoE-null mice. J. Nutr. doi: 10.3945/jn.112.161711.

## Introduction

Epidemiological evidence has shown that the incidence of chronic diseases such as cancer and cardiovascular disease is lower in Asia than in Western countries. Lifestyle factors, including diet, have been identified as potential determinants (1,2). Soy-based foods rich in phytoestrogen isoflavones (IF)<sup>7</sup> are a primary component of many Asian but not Western diets (3). The intake of soy and the primary soy IF, genistein and daidzein, has been widely studied in association with cancer and other chronic

diseases, but the results have been mixed and inconclusive (4). The lack of consensus regarding the health effects of soy IF interventions is due to a variety of potential factors, including variation in IF formulations across studies and interindividual differences in metabolism and response to soy diet (5).

Equol is a metabolite of daidzein produced by intestinal bacteria in ~30% of adult non-Asian and nonvegetarian populations consuming dietary soy protein (6,7). Approximately 10-30% are intermittent equol producers (8,9). Inter-individual variation in the ability to produce equol is a consequence of difference in gut microbial community. Several bacterial strains that can produce equol have been identified in vitro (10); however, the nature of the bacteria and how a person harbors them are incompletely understood. Factors such as dietary habit may modulate the composition and activity of gut microbes, hence affecting equol production (7). Recent evidence suggests that the ability to metabolize daidzein into equol may influence the health-related responses to soy exposure (11). However, it is unclear whether such effects are driven by equol directly or if equol production simply serves as a marker for other intestinal microbiota-mediated effects. Equol has distinct biological activity compared with daidzein and genistein. It binds to estrogen

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<sup>3</sup> Supplemental Methods, Supplemental Figures 1 and 2, and Supplemental Table 1 are available from the "Online Supporting Material" link in the online posting of the article and from the same link in the online table of contents at <http://jn.nutrition.org>.

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<sup>7</sup> Abbreviations used: ASF, Altered Schaedler Flora; CL, casein and lactalbumin; ER, estrogen receptor; HIF, high isoflavone; IF, isoflavone; LIF, low isoflavone; PGR, progesterone receptor.