MINIREVIEW

Gut Bacterial Metabolism of the Soy Isoflavone Daidzein: Exploring the Relevance to Human Health

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The indigenous intestinal microflora are involved in a variety of processes within the human body, and are important for maintaining host health. As such, interindividual differences in the ability to harbor certain intestinal bacteria might be associated with interindividual differences in health and/or disease susceptibility. In the last decade there has been considerable interest in phytoestrogen intakes in relation to human health. Daidzein, an isoflavone phytoestrogen found in soy, is metabolized to equol and *O*-desmethylangolensin (*O*-DMA) by intestinal bacteria. The specific bacterium/bacteria responsible for equol and *O*-DMA production in humans have yet to be identified definitively, but *in vitro* and animal studies have suggested that equol and *O*-DMA are more biologically active than their precursor daidzein. Interestingly, substantial interindividual differences in daidzein metabolism exist; following soy or daidzein consumption, approximately 30%–50% of the human population produce equol, and approximately 80%–90% produce *O*-DMA. Observational and intervention studies in humans have suggested that the ability to produce equol and *O*-DMA may be associated with reduced risk of certain diseases including breast and prostate cancers. However, relatively few studies have been conducted to date. In this review, we discuss the available evidence for a relationship between daidzein-metabolizing phenotypes and human health, and suggest potential mechanisms for some of the reported relationships.
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Introduction

The indigenous intestinal microflora are involved in a variety of processes within the human body, and as such, are important in maintaining host health. The primary roles of the intestinal microflora include metabolic (e.g., fermentation of nondigestible dietary components and metabolism of endogenous mucus and dietary compounds), trophic (e.g., control of epithelial cell proliferation and differentiation), and protective (e.g., acting as a barrier to protect against pathogens) (reviewed in Ref. 1). Intestinal bacterial metabolism of dietary components such as the flavonoids and isoflavonoids can result in the production of metabolites that are more biologically active than their precursor, which could ultimately influence their effect on host health (2). On a broader level, comparisons of germ-free and conventional animals have provided evidence for the importance of the intestinal microflora on host health; many biochemical, physiological, and immunological differences have been observed, including a lower basal metabolic rate, smaller lymph nodes, and lower organ weights in germ-free animals compared with conventional animals (3, 4).

The actual number of bacteria in the human colon is unknown, but it has been estimated that there are more than 400 species (3, 5, 6). In some instances, as an alternative to physically isolating and identifying the bacteria, host phenotypes that result from the metabolic functions of certain bacteria can be used to indicate their presence in the intestines. For example, breath levels of methane indicate the presence of methanogenic bacteria (7). Similarly, breath levels of [^{13}C]labeled carbon dioxide (produced by bacterial

breakdown of administered [^{13}C]labeled urea) indicate the presence of *Helicobacter pylori* (8). Thus, the use of such phenotypes can provide information on gut bacterial populations without the need for laborious methods of bacterial identification, such as culture-based methods.

Although stable communities of intestinal bacteria exist within individuals (9), substantial interindividual differences have been observed (9, 10). These differences may ultimately contribute to interindividual variation in health and/or disease susceptibility. In the last decade, there has been growing interest in soy and isoflavone intakes in relation to human health (11), and in this review, we discuss the potential for interindividual differences in intestinal bacterial metabolism of the soy isoflavone daidzein to influence host health.

Daidzein: A Soy Isoflavone

Daidzein belongs to the isoflavone class of the flavonoids. Soy foods are the predominant food sources of the most intensely studied isoflavones (i.e., daidzein and genistein) (12). These are traditionally consumed in relatively high amounts by some Asian populations, such as Chinese and Japanese (13, 14), and in low amounts by Western populations, such as North American and European (15, 16). Isoflavones are structurally similar to the mammalian estrogens (17) and, for this reason, interest has focused primarily on their effects on hormone-dependent conditions. Epidemiologic studies have provided evidence for a potentially protective role for isoflavones in breast cancer, prostate cancer, cardiovascular disease, osteoporosis, and menopausal symptoms (reviewed in Ref. 11). However, such findings have not always been consistent across studies. Potential reasons for these inconsistencies could include the timing of exposure to isoflavones; evidence exists to suggest that exposure in adolescence might be beneficial, at least in terms of breast cancer risk (18–20). Alternatively, or additionally, interindividual differences in isoflavone metabolism could be a contributing factor. The metabolism of genistein in humans is not well characterized, but recent interest has focused on two daidzein metabolites, namely equol and *O*-desmethylangolensin (*O*-DMA) (21, 22). These metabolites have been detected in a variety of body fluids, including blood, urine, feces, prostatic fluid, and breast tissue (23–27). Substantial interindividual variation in their production exists in humans; studies have shown that approximately 30%–50% of individuals in the populations studied are capable of producing equol from daidzein (28–32) and approximately 80%–90% are capable of producing *O*-DMA from daidzein (29, 32, 33). It is possible that the variation in daidzein metabolism may be unique to humans; the majority of studies in animals, including mice, rats, hamsters, cows, pigs, sheep, dogs, monkeys, and chimpanzees (34–42), suggest that all have the capacity to produce equol. Fewer studies have assessed *O*-DMA production in animals; it has been measured in plasma, feces, and urine from monkeys, rats, and chimpanzees (35, 38, 43), but appears to be produced in smaller amounts than equol (38).

Intestinal Bacteria and Daidzein Metabolism

Intestinal bacteria play an essential role in daidzein metabolism. Germ-free animals and young infants with underdeveloped gut microflora do not produce equol or *O*-DMA (44, 45), and *in vitro* incubation of daidzein with human feces results in the production of equol and *O*-DMA (46). In cynomolgus monkeys (38), treatment with certain antibiotics causes marked reductions in plasma levels of equol, and some antibiotics inhibit the *in vitro* production of equol and *O*-DMA by human fecal bacteria (46). Equol and *O*-DMA are likely produced by different bacteria, and the bacteria involved also may differ between individuals; *in vitro*, fecal bacteria from some equol nonproducers can convert daidzein to *O*-DMA (but not equol) (46), and observational studies show that not all equol producers are *O*-DMA producers, and vice versa (33, 47, 48). Furthermore, some antibiotics inhibit equol/*O*-DMA production by fecal bacteria from some individuals, but not others (46).

Several candidate bacteria for daidzein metabolism have been suggested; for example, a *Clostridium* sp (49) and *Eubacterium ramulus* (50, 51) metabolized daidzein to *O*-DMA *in vitro*, and equol has been found in soymilk fermented with some strains of *Bifidobacterium* (52). It has been suggested that other bacteria, including *Escherichia coli*, *Bacteroides ovatus*, *Ruminococcus productus*, and *Streptococcus intermedius* (53, 54), could be involved in daidzein metabolism. However, the human intestinal bacteria responsible for daidzein metabolism have yet to be identified definitively.

Despite the absence of definitive data on the particular bacteria involved in daidzein metabolism, host phenotypes (i.e., urinary excretion of equol and *O*-DMA), can be used to indicate the presence of equol- and *O*-DMA–producing bacteria in the intestines. A convenient way to phenotype large numbers of individuals in populations with low soy consumption patterns is by using a 3-day soy challenge (33, 55). At least 3 days of soy consumption is considered optimal based on pharmacokinetic data (56, 57). For the 3-day soy challenge, individuals supplement their habitual diets with soy protein containing daidzein for 3 consecutive days, and collect a first-void urine sample on the fourth day. Because isoflavones are stable in urine kept at room temperature for at least 14 days (48), the urine sample can be mailed to the laboratory for isoflavone analysis. In studies that have used 24-hr urine collections for phenotype determination, distinct cut-off points for equol-producer/-nonproducer status have been observed (30, 47); however, cut-off points for equol and *O*-DMA producers and nonproducers using first-void urine samples are less pronounced. Detectable levels of urinary equol/*O*-DMA in first-void urine samples have been used to assign daidzein-metabolizing phenotypes (33, 55), but,

depending on the sensitivity of the assay used for measuring isoflavonoids, this could lead to misclassification of the phenotypes. Furthermore, equol has been measured in foods such as cow's milk (58, 59), which, if ingested and subsequently excreted in detectable levels, may lead to misclassification of an individual as an equol producer. Nonetheless, despite the potential for misclassification, the proportions of equol producers in studies using the 3-day soy challenge protocol with first morning urine sample on Day 4 (33, 48, 55) are similar to those reported in studies using a 24-hr urine collection (30, 60, 61). Blood levels of equol and *O*-DMA have been measured in some studies, but the concentrations are considerably lower than in urine (62), thereby increasing the complexity of assigning daidzein-metabolizing phenotypes based on serum or plasma levels, especially in low soy-consuming populations.

Factors Associated with Daidzein-Metabolizing Phenotypes

Reasons for interindividual differences in the ability to harbor the equol- and *O*-DMA–producing bacteria in humans remain unknown. Diet has been suggested to contribute to the ability to produce equol, but results from association studies have been conflicting. For example, Adlercreutz *et al.* (63) reported a positive association between urinary equol concentration and intake of fat and meat in a Japanese population, whereas in a Western population, Rowland *et al.* (47) reported that equol producers consumed significantly less energy as fat and significantly more energy as carbohydrate than equol nonproducers. In another cross-sectional study, equol-producing women had, on average, a higher intake of dietary fiber compared with nonproducers (30). However, in a feeding study, it was not possible to induce equol production by supplementing the diets of nonproducers with high-fiber wheat bran cereal or soy protein (64).

No studies have been designed specifically to assess the stability of the daidzein-metabolizing phenotypes, but several controlled soy intervention studies and longitudinal observational studies indicate that the capacity to produce equol and *O*-DMA likely remains stable in an individual over time (31, 65–67). Two soy intervention studies (68, 69) reported an increase in the number of equol producers with time, but reasons for this are not clear. The apparent stability of the equol- and *O*-DMA–producer phenotypes raises the possibility they might be under some degree of genetic control. Host genetics may influence normal intestinal bacterial populations (70, 71), and one study (48) has investigated the potential role of host genetics on the manifestation of the daidzein-metabolizing phenotypes. Familial correlation and segregation analyses suggested that the daidzein-metabolizing phenotypes might be under some degree of genetic control, but other nongenetic factors also are likely involved.

Equol-Producer and *O*-DMA–Producer Phenotypes: Relevance to Human Health

***In vitro* Studies.** Intestinal bacterial metabolism of bioactive dietary compounds can alter their biological activities (2), which in turn could alter their potential to influence host health. *In vitro* studies suggest that equol and *O*-DMA are more biologically active than their precursor daidzein, and many investigators have focused on the estrogenic potencies of equol and *O*-DMA. In the early 1990s, it was demonstrated that in human endometrial adenocarcinoma cells, equol was more estrogenic than daidzein (72). In several subsequent studies, equol and *O*-DMA have been shown to bind to human estrogen receptors α and β (ERα and ERβ) with a greater affinity than daidzein (73–76). The binding of ERα and ERβ to the estrogen response element (ERE) is an important step in the induction of gene activation. In an *in vitro* assay in which ERE was immobilized on the surface of a sensor chip (77), the concentrations of equol required to increase the binding response of ERα and ERβ to ERE by 50%, as compared with unliganded ER, were 3.5 and 0.4 μ*M*, respectively, whereas the concentrations of daidzein required were greater than 300 and 0.35 μ*M*, respectively. Because daidzein preferentially activated the binding of ERβ to the ERE and equol activated the binding of both ERα and ERβ to the ERE to a similar extent, it was suggested that when daidzein is metabolized to equol it is converted from a more specific activator of ERβ to an activator of both ERα and ERβ (77). The observation that similar concentrations of daidzein and equol increased the binding response of ERβ to ERE (77) is somewhat in contrast with binding affinity data showing that equol binds to ERβ with a greater affinity than daidzein (73, 75), although ER binding does not necessarily predict estrogen agonist activity.

In studies that have assessed estrogen receptor–dependent transcription of β-galactosidase in transfected yeast assays, equol induced transcription to a greater extent than daidzein in yeast carrying human ERα or ERβ (75). *O*-DMA induced transcription to a greater extent than daidzein with ERβ, but did not induce transcription with ERα (74). In MCF-7 cells (an estrogen-responsive breast cancer cell line), equol and *O*-DMA were more potent than daidzein in stimulating cell growth (74, 75), and equol was approximately 100-fold more potent than daidzein in stimulating pS2 (an estrogen-responsive protein) messenger RNA expression in these cells (76). Interestingly, simultaneous exposure of MCF-7 cells to equol and 17β-estradiol reduced the level of pS2 messenger RNA expression seen with 17β-estradiol alone, also suggesting an antiestrogenic role for equol (76). Interpretation of the results of these studies is complicated by the fact that equol can exist as the enantiomers *R*-equol and *S*-equol, and chemically synthesized equol will exist in a racemic mixture unless an enantioselective synthesis is conducted or the enantiomers are separated. The two forms have been shown to differ in

their binding affinities and preferences for ERα and ERβ: *S*-equol has a high binding affinity for, and preferentially binds to, ERβ, whereas *R*-equol binds preferentially to ERα (73). In humans, the metabolism of daidzein to equol results only in the production of *S*-equol (73, 78).

Other *in vitro* studies have shown that equol is a more potent antioxidant than daidzein (79–82), and has a higher effective free fraction in human serum than both genistein and 17β-estradiol (83). In isolated guinea pig ventricular monocytes, equol exerted greater cardioprotective effects than daidzein (84), and in prostatic cell lines, equol was considerably more potent than daidzein in terms of exerting inhibitory effects on proliferation of both benign and malignant cell lines (85). At the same time, the genotoxic potential of equol and *O*-DMA also has been evaluated: in estrogen receptor–negative Chinese hamster lung fibroblasts, equol exhibited greater genotoxic potential than daidzein, but not genistein, at concentrations up to 25 μ*M* (86). In another study (87), equol and *O*-DMA caused a concentration-dependent increase in micronucleus frequency in mouse lymphoma cells (equol, 1–100 μ*M*; *O*-DMA, 0.1–10 μ*M*), whereas daidzein (25–100 μ*M*) did not.

Few studies have compared directly the activities of equol with those of *O*-DMA, but in one study, equol exhibited higher binding affinity than *O*-DMA to ERα (88). To our knowledge, no studies have compared the binding affinities of equol and *O*-DMA with ERβ. In a transfected yeast assay, equol induced transcription of estrogen receptor–dependent β-galactosidase with ERα (75) but *O*-DMA did not (74), whereas both equol and *O*-DMA induced transcription with ERβ (74, 75). In MCF-7 cells, equol and *O*-DMA both stimulated cell proliferation at concentrations ranging from 10 n*M* to 1 μ*M*, but at the highest concentration tested (10 μ*M*), equol stimulated and *O*-DMA inhibited cell proliferation (88). *O*-DMA also was shown to be a more potent inducer of micronucleus formation than equol in mouse lymphoma cells (87). Thus, limited evidence suggests that differences in biological activity may exist between equol and *O*-DMA.

Animal Studies. A large number of animal studies have focused on the biological effects of genistein, primarily because in the late 1980s, it was reported that genistein inhibited tyrosine-specific protein kinases (89), providing evidence for a potential chemopreventive role for genistein (90). Nonetheless, the biological effects of intact soy protein (i.e., containing isoflavones) and purified daidzein also have been investigated in animal models, but few studies have compared the effects of daidzein and genistein. In one study comparing daidzein and genistein, Picherit *et al.* (91) reported that daidzein was more efficient than genistein in preventing ovariectomy-induced bone loss in rats. Given that most, if not all, animals produce equol from daidzein (34–42), it is possible that this finding, and other biological effects that have been associated with daidzein consumption in animals, may be due to the biological properties of equol. In a study in which equol was administered to intact male rats (92), there was a reduction in ventral prostate and epididymal weight, and an increase in circulating levels of luteinizing hormone (LH). *In vitro*, equol specifically bound 5α-dihydrotestosterone (DHT) and, in castrated male rats, equol blocked the trophic effects of DHT on growth of the ventral prostate gland and inhibitory feedback effects on plasma LH levels, without changes in circulating DHT (92). In another study in which racemic equol was administered to female mice either orally or by injection (78), there were modest increases in uterine weight and vaginal cell proliferation, with greater effects seen with injected equol compared with dietary equol.

Human Studies. In humans, it has been suggested that two subpopulations—equol producers and equol nonproducers—respond differentially to soy or isoflavone interventions (93). Differential responses according to equol-producer phenotype are hypothesized to result from direct biological actions of equol itself, given that *in vitro* studies suggest that equol is more biologically active than daidzein (Fig. 1; Refs. 74–76, 79–81, 83–85). As such, responses to soy or isoflavone interventions among equol producers would be driven by daidzein exposure. However, evidence also exists to suggest that a key factor may be the ability to produce equol, irrespective of the amount of soy or daidzein consumed (61). Because intestinal bacteria are responsible for daidzein metabolism, equol production might be a marker of an intestinal bacterial profile associated with human health via unique metabolic actions of either the equol-producing bacteria, or other bacteria that are associated with their presence in the intestines (Fig. 1). Alternatively, because the equol-producer phenotype appears to be stable within an individual (31, 65–67), and because of the possible genetic component to the equol-producer phenotype (48), genetic factors that are either responsible for, or associated with, the equol-producer phenotype could be responsible for health outcomes irrespective of soy or daidzein consumption (Fig. 1). In addition, all of these factors may be involved concurrently. Little work has focused on *O*-DMA producers and nonproducers as two subpopulations, but some evidence exists to suggest that, similar to the equol-producer phenotype, there might be differences between *O*-DMA producers and nonproducers in selected health outcomes (33, 55).

The interpretation of studies in humans assessing health effects associated with equol or *O*-DMA production must take into account (i) whether the study was in a population that regularly consumes soy, or (ii) whether a soy challenge was used to determine daidzein-metabolizing phenotypes. In populations that regularly consume soy, most equol/*O*-DMA producers would be expected to have consistently measurable circulating concentrations of equol/*O*-DMA as a result of regular soy exposure, whereas nonproducers would have low or undetectable levels of equol/*O*-DMA. In contrast, in populations that do not regularly consume soy, it would be expected that all individuals would have low

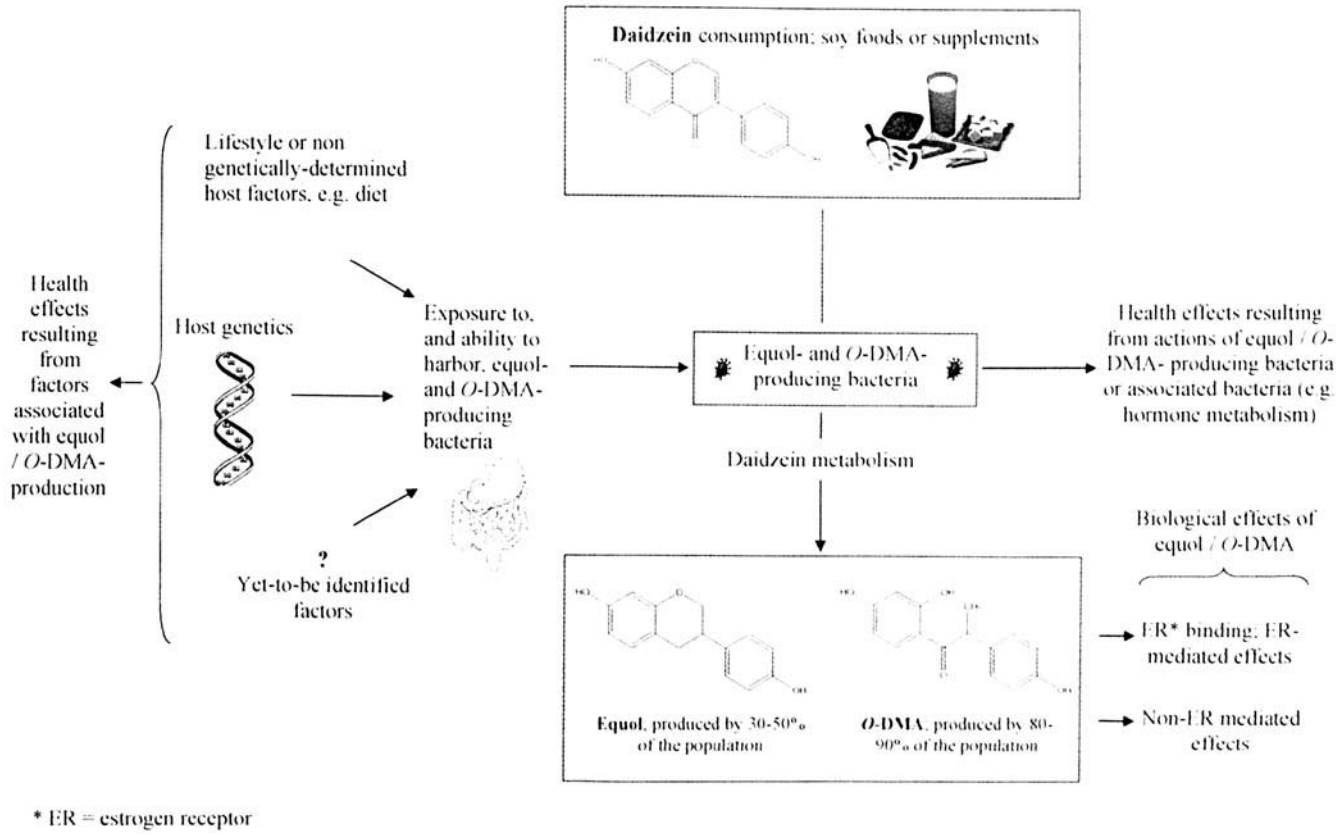

Figure 1. Hypothesized associations between daidzein metabolism and host health.

circulating levels of equol/*O*-DMA, regardless of equol- or *O*-DMA–producer phenotype. Mechanisms for potential associations between equol/*O*-DMA excretion and health may differ in high and low soy-consuming populations; for example, in high soy-consuming populations, associations may be due to the biological effects of equol/*O*-DMA, whereas in low soy-consuming populations, associations may be due to factors associated with the phenotype (e.g., intestinal bacteria or host genetics). However, individuals in low soy-consuming populations who excrete measurable (albeit low) levels of isoflavones and their metabolites are most likely those who have been exposed to dietary soy or other isoflavone-containing foods, resulting in potential confounding of the association between equol/*O*-DMA and health by soy intake.

Daidzein-Metabolizing Phenotypes and Health in High Soy-Consuming Populations. Numerous studies have investigated total isoflavone excretion in relation to health in high soy-consuming populations, but few have assessed the individual contributions of equol and *O*-DMA. We summarize below, and in Table 1, some of the studies that have examined equol and *O*-DMA in relation to host health in high soy-consuming populations.

In a population-based, case-control study among Chinese women (94), breast cancer cases had a nonstatistically significant lower mean urinary *O*-DMA concentration than controls, and there was a nonsignificant trend for a lower risk for breast cancer across increasing tertiles of *O*-DMA excretion. In another study in which data from a smaller sample of these women were reported (95), mean urinary excretion of both equol and *O*-DMA were lower in breast cancer cases than controls, but again, the findings were not statistically significant and no differences were apparent when median excretion was considered. In a study of Asian American women (96), who had been selected for the study based on quartiles of isoflavone intake among controls, fewer cases than controls (39% vs. 49%, respectively) had measurable levels of plasma equol, despite similar isoflavone intakes. These studies suggest that there may be a protective effect of equol and *O*-DMA excretion on breast cancer risk, but the studies may have been insufficiently powered to detect statistically significant effects.

Case-control studies among men residing in countries with high levels of soy consumption have suggested that serum equol levels are associated with a reduced risk of prostate cancer. In a hospital-based study of Japanese prostate cancer cases and controls (28), the proportion of men with detectable serum equol was lower among cases than controls. Equol producers also tended to have a more favorable disease stage and grade at diagnosis. In subsequent studies in which more patients had been accrued (97, 98), the proportion of equol producers was consistently lower in cases than controls among Japanese men. This

Table 1. Summary of Studies on Equol and *O*-Desmethylangolensin (*O*-DMA) Production in Relation to Human Health in High Soy-Consuming Populations[a]

Study population	Cases	Controls	Findings	Reference
Cancer				
Chinese	60 breast	60	Mean urinary equol and *O*-DMA NS lower in cases than controls ($P > 0.05$); median excretion zero among cases and controls	95
Chinese	250 breast	250	Mean and median urinary *O*-DMA NS lower in cases than controls ($P > 0.05$), and NS trend for lower risk of breast cancer with increasing tertiles of *O*-DMA excretion ($P = 0.15$)	94
Asian American[b]	97 breast	97	39% of cases and 49% of controls had plasma equol > ~1 n*M* ($P > 0.05$)	96
Japanese	141 prostate	112	Cases and controls with serum equol ≥ 0.5 ng/ml: all men 40% and 50% ($P = 0.10$), inpatients 30% and 50% ($P = 0.01$), outpatients 48% and 56% ($P = 0.67$)	28
Japanese	133 prostate	162	29% of cases and 46% of controls had serum equol ≥ 0.5 ng/ml ($P = 0.004$)	98
Japanese	52 prostate	151	Similar proportion of EP in cases and controls (67% vs. 75%; $P = 0.38$), but serum equol lower in cases than controls among EP ($P = 0.04$); compared with men with undetectable equol, OR for men with low and high serum equol concentrations were 0.70 ($P = 0.38$) and 0.39 ($P = 0.05$), respectively; P trend = 0.05	99
Korean	61 prostate	61	30% of cases and 59% of controls had serum equol ≥ 0.5 ng/ml ($P = 0.001$)	98
Other				
Korean	15 BPH	10	Equol in plasma and prostate tissue NS higher in men with BPH than controls ($P = 0.08$ and $P = 0.35$, respectively)	100
Korean[c]	21 osteoporosis 29 osteopenia	25	No difference in urinary equol between controls and women with osteopenia or osteoporosis ($P > 0.05$); no correlation between urinary equol and menopausal symptoms or lipid profiles ($P > 0.05$)	101

[a] NS, nonsignificant; EP, equol producer; OR, odds ratio; BPH, benign prostatic hyperplasia.
[b] Study subjects selected according to quartiles of isoflavone intakes among controls; approximately 40% of study subjects were in the two extreme quartiles of soy intake, but isoflavone intakes were similar among cases and controls.
[c] Controls T score > –1, osteopenia T score ≤ –1, but > –2.5, and osteoporosis T score ≤ –2.5.

relationship also was observed in Korean, but not U.S., men (98). In a nested case-control study, also in Japanese men (99), the proportion of men with detectable serum equol was similar among cases and controls. However, there was a reduction in prostate cancer risk with increasing serum equol concentration; compared with men with undetectable equol, the odds ratios for men with low and high serum equol concentrations were 0.70 ($P = 0.38$) and 0.39 ($P = 0.05$), respectively (P trend = 0.05). Similar findings were seen when analyses were conducted on subsets of cases and controls based on serum total testosterone and/or prostate-specific antigen levels, although findings were not statistically significant ($P > 0.05$). In contrast, in Korean men with and without benign prostatic hyperplasia (BPH) (100), levels of equol in plasma and prostate tissue were nonsignificantly higher among those with BPH than among controls. Nonetheless, in general, the studies in Japanese and Korean men suggest a protective effect of equol excretion on prostate cancer risk.

In postmenopausal Korean women (101), there were no significant differences in urinary excretion of equol between women who had normal bone density and women classified as osteopenic or osteoporotic. Menopausal symptoms and lipid profiles also were assessed in that study, but there was no effect of urinary equol concentration on these measures.

If a relationship does indeed exist between equol/*O*-DMA production and host health, it is difficult to evaluate potential mechanisms for such a relationship in high soy-consuming populations because it is not possible to separate the biological effects of equol or *O*-DMA from host factors associated with their production. Furthermore, it is not possible to assess the relative contributions of both biological activities and host factors if acting in combination with one another. The studies to date suggest a potentially protective effect of equol or *O*-DMA excretion on breast and prostate cancer risk, but relatively few studies have been conducted.

It has been suggested that because bioactive compounds may be removed from soy during processing, there may be differences in potential health effects between processed soy foods consumed by Western populations and traditional soy foods consumed by Asian populations (102).

Table 2. Summary of Studies on Equol and *O*-Desmethylangolensin (*O*-DMA) Production in Relation to Human Health in Low Soy-Consuming Populations[a]

Study population	Cases	Controls	Findings	Reference
Cancer				
Australia	144 breast	144	Significant trend toward lower risk of breast cancer across increasing quartiles of equol excretion ($P = 0.009$)	104
United Kingdom	114 breast[b]	219	Urine and serum equol associated with significant increase ($P = 0.01$ and $P = 0.02$, respectively) and urine and serum *O*-DMA associated with nonsignificant increase ($P = 0.20$ and $P = 0.20$, respectively), in risk of breast cancer	62
United States	24 prostate	21	17% cases and 14% controls had serum equol ≥ 0.5 ng/ml ($P = 0.83$)	98
Other				
Netherlands[c]	35 high rate of bone loss	32	Equol excretion weakly positively associated with rate of cortical bone loss at the radius in 5 but not 9 years after menopause	111
United States	0	123	Positive correlation between equol excretion and ratio of 2-OH E_1 to 16α-OH E_1 in women with detectable urinary equol ($n = 55$; $r = 0.38$; $P = 0.005$)	110

[a] 2-OH E_1, 2-hydroxyestrone; 16α-OH E_1, 16α-hydroxyestrone.
[b] Serum isoflavone data available for 97 cases and 187 controls.
[c] Cases represent women who had an annual rate of cortical bone loss at the radius of 2.5% or more; controls represent women with a rate of cortical bone loss at the radius of 0.5% per year or less, during the first 5 years of the study.

The overall impact of processed or traditional soy foods on the intestinal microflora profile is unknown, but studies in humans that have used processed soy foods (33, 48) or chemically synthesized daidzein (56), and studies in Asian populations (28, 99), all have reported equol production in some study participants. This indicates that the source of soy or daidzein consumed does not appear to limit an individual's ability to convert daidzein to equol, although quantitative differences in equol excretion resulting from consumption of processed versus traditional soy foods have not been evaluated.

Daidzein-Metabolizing Phenotypes and Health in Low Soy-Consuming Populations. In general, individuals in Western populations have low soy-consumption patterns (16, 62). As a result, circulating levels and urinary excretion of isoflavones are low, and depending on the sensitivity of the assay used, could hamper the accurate measurement of urine and blood concentrations of isoflavones and their metabolites. Thus, relatively few studies have assessed urinary excretion of equol and *O*-DMA in relation to health in low soy-consuming populations, and caution is needed in the interpretations of those that have (103). The studies described below also are summarized in Table 2.

In a case-controlled study among Australian women (104), although criticized for methodological issues (105), there was a significant trend toward a lower risk of breast cancer across increasing quartiles of equol excretion, after adjustment for known and possible breast cancer risk factors (age at menarche, parity, alcohol intake, and total fat intake). Nonetheless, overall isoflavone excretion in this population was low, and individuals who excreted measurable levels of equol were most likely those who were exposed to dietary soy, resulting in potential confounding by soy intake. In contrast, in a prospective study of urine and serum isoflavones in relation to breast cancer risk among women residing in the UK (62), all isoflavones measured, including daidzein, genistein, equol, and *O*-DMA, were associated with an increased risk of breast cancer, and the findings were significant for urine and serum levels of equol. However, similar to the Australian study (104) and European populations in general (16), dietary intakes of isoflavones were low among these women and resulting equol and *O*-DMA concentrations were low.

Several retrospective and prospective studies have reported an increased risk of breast cancer associated with low urinary excretion of 2-hydroxyestrone (2-OH E_1) relative to 16α-hydroxyestrone (16α-OH E_1) in pre- and postmenopausal women (106–109). Among young to middle-aged women in the United States who were not, on average, regular soy consumers (110), correlations between total isoflavone excretion (sum of genistein, daidzein, *O*-DMA, and equol) and the estrogen metabolites were nonsignificant ($P > 0.05$). Among women with detectable levels of equol, urinary excretion of equol (adjusted for daidzein, genistein, and *O*-DMA excretion) was significantly positively correlated with the ratio of 2-OH E_1 to 16α-OH E_1, suggesting that equol excretion may be associated with estrogen metabolism and that the relationship may be independent of total isoflavones.

In a study of postmenopausal women in the Netherlands (111), equol excretion was weakly positively associated with rate of cortical bone loss at the radius in the 5 years after the menopause. However, this association

Table 3. Summary of Studies on Equol and *O*-Desmethylangolensin (*O*-DMA) Production in Relation to Human Health in Soy/Isoflavone Interventions and Soy Challenge Studies[a]

Study subjects	No.[b]	Intervention	Duration
Premenopausal women	14	SP isolate providing approx. 4, 24, and 47 mg daidzein	Each dose for three menstrual cycles + 9 days
Premenopausal women	30	Isoflavone tablets providing 39 mg daidzein, or placebo	1 year
Premenopausal women	6	60 g SP providing 25 mg daidzein	1 month
Postmenopausal women	28	25 g milk protein, ethanol-washed SP, or intact SP providing 0, 0.5, or 47 mg daidzein, respectively	Each diet for 6 weeks
Postmenopausal women	175	25.6 g SP providing 41 mg daidzein, or milk protein	1 year
Menopausal women with hot flashes	246	Two formulations of red clover isoflavone tablets (57 or 82 mg total isoflavones) or placebo	12 weeks
Postmenopausal women with mild to moderate hypercholesterolemia	75	1 or 2 red clover isoflavone tablets (16 mg formononetin and 0.5 mg daidzein/tablet) or placebo	Each isoflavone dose for 5 weeks, or placebo for entire study
Mildly hypercholesterolemic and/or hypertensive men and postmenopausal women	23	4 servings/day of soymilk or yogurt (80 mg total isoflavones) or dairy-based milk or yogurt	Each diet for 5 weeks
Hypercholesterolemic postmenopausal women	73	40 g isolated SP providing 14 or 26 mg daidzein, or nonfat dry milk	6 months
Premenopausal women	36[c]	Soy challenge	4 days
Postmenopausal women	89	Soy challenge	3 days
Postmenopausal women	55	Soy challenge	3 days

[a] SP, soy protein; EP, equol producer; ENP, equol nonproducer; E1, estrone; E1-S, estrone sulfate; SHBG, sex hormone binding globulin; E2, estradiol; CVD, cardiovascular disease; LDL, low-density lipoprotein; HDL, high-density lipoprotein; 2-OH E_1, 2-hydroxyestrone; 16α-OH E_1, 16α-hydroxyestrone; FSH, follicle-stimulating hormone; IGF-1, insulin-like growth factor–1; IGFBP-3, insulin-like growth factor–binding protein–3.
[b] No. who completed the study unless otherwise noted.
[c] No. of women with baseline data for comparison of EP and ENP.

was not apparent when considering the entire study period (9 years postmenopause), suggesting that the findings may have been due to chance.

Overall, few studies on the effects of equol and/or *O*-DMA on health in low soy-consuming populations have been conducted. In the absence of a soy challenge, it is not possible to determine whether the daidzein metabolites, host characteristics associated with their production, or a combination of these factors are responsible for the reported associations between daidzein metabolites and markers of disease risk. In the studies that have reported a relationship between the daidzein metabolites and disease risk, it is possible that because individuals with detectable levels of isoflavones have most likely been exposed to dietary soy, there is the potential for confounding by soy intakes. Furthermore, and as suggested by Messina (103), caution is needed in the interpretation of studies conducted in low soy-consuming populations because intakes may simply be too low to enable meaningful conclusions to be drawn.

Soy Intervention and Soy Challenge Studies. Soy or isoflavone intervention studies, or studies in which individuals undergo a soy challenge, could provide information on potential mechanisms underlying some of the reported associations between daidzein-metabolizing phe-

Table 3. (Extended)

Study subjects	Findings	Reference
Premenopausal women	In general, EP, compared with ENP, had lower plasma E1, E1-S, androgens, and prolactin, and higher SHBG and progesterone; similar findings at all three doses	61
Premenopausal women	Mean serum E1 and E2 did not differ at months 6 or 12 by equol-producer status	69
Premenopausal women	Greatest lengthening of follicular phase of menstrual cycle in the two women with highest urinary equol excretion	114
Postmenopausal women	No difference between EP and ENP in CVD risk factors at baseline, and response to intervention among EP was no different to response among all women	117
Postmenopausal women	No effect of equol-producer status on response to intervention in terms of plasma lipids, bone density, or cognitive function	118
Menopausal women with hot flashes	No correlation between change in equol or *O*-DMA excretion and change in number of hot flashes	119
Postmenopausal women with mild to moderate hypercholesterolemia	Negative correlation ($\beta = -0.277$, $P = 0.017$) between plasma triglycerides and *O*-DMA excretion with low (one tablet) but not high (two tablets) dose.	116
Mildly hypercholesterolemic and/or hypertensive men and postmenopausal women	No difference in plasma lipids, blood pressure, or arterial compliance between diets for all participants, but significant decrease in total cholesterol, LDL cholesterol, ratio of LDL to HDL cholesterol, triglycerides, and lipoprotein(a) in EP on soy diet	115
Hypercholesterolemic postmenopausal women	Change in plasma *O*-DMA significantly positively associated with change in HDL cholesterol; positive associations also seen with changes in thyroxine and free thyroxine index, and bone mineral density	120
Premenopausal women	No difference in estrogens, androgens, or menstrual cycle length between EP and ENP; $P > 0.05$.	60
Postmenopausal women	EP had 24% ($P = 0.07$) and 27% ($P = 0.06$) higher urinary 2-OH E_1 and ratio of 2-OH E_1 to 16α-OH E_1 than ENP; *O*-DMA producers had 42% ($P = 0.02$), 9% ($P > 0.10$), and 23% ($P = 0.04$) higher 2-OH E_1, ratio of 2-OH E_1 to 16α-OH E_1, and FSH than nonproducers; no difference between producers and nonproducers of either metabolite in serum estrogens, androgens, insulin, leptin, IGF-I, IGFBP-3, or SHBG	33
Postmenopausal women	Mammographic density 39% lower in EP than ENP ($P = 0.04$), and 69% higher in *O*-DMA producers than nonproducers ($P = 0.05$)	55

notypes and health outcomes. Disease risk markers could be compared among daidzein-metabolizing phenotypes in low soy-consuming individuals who have undergone a soy challenge, which may remove the potential for confounding by soy intake. Alternatively, studies with differing doses of daidzein also could provide information on whether equol/*O*-DMA concentrations, or host factors associated with their production, are key in relationships between equol/*O*-DMA excretion and health. Despite the potential for providing information on possible mechanisms, few intervention studies or soy challenge studies have evaluated outcomes stratified on equol- or *O*-DMA–producer status. The studies described below also are summarized in Table 3.

A soy-protein supplementation study in premenopausal women provides evidence for a potentially protective effect of host factors associated with the equol-producer phenotype, rather than equol concentration, on breast cancer risk factors (61). In a randomized, crossover design, women received three isoflavone doses during three diet periods. Women with the equol-producer phenotype ($n = 5$) had circulating levels of hormones that were more likely to be associated with a reduced risk of breast cancer, and this was irrespective of isoflavone dose. If the reported differences in hormone profiles are consistent over a woman's reproductive lifetime, cumulative exposure or exposure at sensitive periods of life to particular steroid hormone profiles could be substantially different by equol-producer phenotype, which may ultimately have an impact on breast cancer risk. Although the findings were irrespective of isoflavone dose, the low soy dose still provided 10 mg of isoflavones per

day, which is higher than average intakes estimated for Western populations (112, 113). It remains to be established whether low doses of isoflavones can produce biological effects in humans. In contrast with that study, two subsequent studies in premenopausal women compared hormone levels between equol producers and nonproducers (60, 69), and did not show significant differences between phenotypes in circulating levels of estrogens or androgens. In addition, in a study of postmenopausal women who had undergone a soy challenge to determine equol- and *O*-DMA–producer status (33), there were no significant differences between equol producers and nonproducers, or between *O*-DMA producers and nonproducers, in serum estrogens, androgens, metabolic hormones (insulin, leptin, insulin-like growth factor–1, and insulin-like growth factor–binding protein-3), or sex hormone-binding globulin. Taken together, these findings suggest that differences in hormone levels between equol producers and nonproducers might only be apparent when individuals are exposed to dietary soy, including low doses (60), but further work is needed to determine more fully the effects of low soy doses in equol producers and nonproducers.

In relation to menstrual cycle events, Cassidy *et al.* (114) observed that with soy supplementation, the two women who had the highest urinary equol excretion had the greatest lengthening of the follicular phase of their menstrual cycles. In contrast, in another study in which equol-producer status was assessed using a soy challenge (60), there were no differences in baseline menstrual cycle length between equol producers and nonproducers.

Some studies have suggested that equol production may be beneficially associated with risk factors for cardiovascular disease. In an intervention study in which mildly hypercholesterolemic and/or hypertensive subjects received soy- or dairy-based milk or yogurt for 5 weeks (115), there were no overall differences in plasma lipids, blood pressure, or arterial compliance between the soy and dairy diets. In a secondary data analysis, the eight subjects who produced equol showed significant reductions in total cholesterol, low-density lipoprotein (LDL) cholesterol, ratio of LDL to high-density lipoprotein (HDL) cholesterol, plasma triglycerides, and lipoprotein(a) with the soy diet, suggesting that equol producers may respond more favorably than nonproducers in terms of cardiovascular disease outcomes to a soy intervention. In postmenopausal women with mild to moderate hypercholesterolemia who received a red-clover isoflavone supplement (116), there was a negative correlation between plasma triglycerides and urinary excretion of *O*-DMA; however, this was only seen in the group taking one but not two isoflavone tablets per day, suggesting that the findings may have been due to chance. Among healthy postmenopausal women given intact soy protein (i.e., with isoflavones) (117), there was a significant vasodilatory response to the intervention, but no effect on blood lipids. A subgroup analysis of the data including only equol producers (n = 10) did not produce different results from the entire study population. A comparison between equol producers and nonproducers in baseline characteristics (including blood lipids) also was made, but again, there were no significant differences between the two groups. In a soy-protein intervention study in postmenopausal women (118), there was no relationship between equol production and change in plasma lipids or in response to soy consumption in terms of bone density or cognitive function, and no correlation between change in equol or *O*-DMA excretion and change in number of hot flashes in another study (119). Among hypercholesterolemic postmenopausal women given soy protein (120), change in plasma *O*-DMA concentration was significantly positively associated with change in HDL cholesterol. Positive associations also were seen with changes in thyroxine and free thyroxine index, and bone mineral density.

In postmenopausal women in the United States who had undergone a soy challenge (33, 55), relationships between daidzein-metabolizing phenotypes and breast cancer risk factors were assessed. Equol producers, compared with nonproducers, had higher urinary 2-OH E1 and ratio of 2-OH E1 to 16α-OH E1; and *O*-DMA producers, compared with nonproducers, had higher 2-OH E1, ratio of 2-OH E1 to 16α-OH E1, and follicle-stimulating hormone (33). In a subgroup of these women, mammographic density was lower in equol producers compared with nonproducers, and higher in *O*-DMA producers compared with nonproducers (55). Given that studies have shown an increased risk of breast cancer associated with low urinary excretion of 2-OH E_1 relative to 16α-OH E_1 (106–109), and that increased radiological breast density is associated with an increased risk of breast cancer (121, 122), these findings suggest that the daidzein-metabolizing phenotypes are associated with breast cancer risk factors in postmenopausal women. Because the women were not regular soy consumers, it is possible that associations between daidzein-metabolizing phenotypes and the breast cancer risk factors might be mediated through host factors associated with the ability to metabolize daidzein to equol/*O*-DMA, rather than the metabolites themselves. However, the small sample sizes limit the interpretation of these studies.

Although soy interventions and soy challenge studies have the potential to provide insight into the nature of associations between daidzein-metabolizing phenotypes and health, few studies have been conducted to fully evaluate this. Overall, the studies that have been conducted to date do not provide compelling evidence that equol producers and nonproducers differ in terms of their response to soy or isoflavone interventions, but this conclusion is based on a limited number of studies with small sample sizes.

Summary and Future Directions

In 2002, Setchell *et al.* (93) hypothesized that the failure to "bacteriotype" individuals for equol-producer status in previous intervention studies of soy or isoflavone

supplements could explain the variable results seen in such studies, and it was suggested that maximal clinical responses to soy protein diets were seen in equol producers. However, some recent studies have failed to demonstrate an effect of equol/*O*-DMA production on response to soy or isoflavone interventions (118, 119), suggesting that this hypothesis may not hold true. Nonetheless, to date, few studies have been specifically designed to examine the effect of equol/*O*-DMA production on human health, which limits the ability to make any inferences regarding the importance, or otherwise, of these phenotypes. Although associations between equol/*O*-DMA production and host health need to be confirmed in additional studies, we include some suggestions below for potential mechanisms for some of the relationships that have been reported to date. In addition, we suggest some areas for future study.

Equol and *O*-DMA are more biologically active than their precursor daidzein in some *in vitro* assays (74–76, 79–81, 83–85). It remains to be established whether differences between equol/*O*-DMA producers and nonproducers reported in some studies are due to the biological effects of equol/*O*-DMA, to host factors associated with their production, or to a combination of these factors. Furthermore, it has yet to be determined whether exposure to low levels of daidzein metabolites in humans can result in clinically relevant physiologic effects. In low soy-consuming populations, intervention studies with different doses of daidzein, or phenotyping individuals for daidzein-metabolizing phenotypes and assessing markers of disease risk, could potentially answer such questions. If responses to an intervention differ by dose, it would suggest an effect via the biological actions of the metabolites, whereas if differences between producers and nonproducers (assessed using a soy challenge) are evident in the absence of an intervention, host factors may be the key element. Alternatively, it is possible that equol/*O*-DMA producers might have an inherent characteristic that results in an enhanced response to all soy isoflavones or some other component in soy, and not just equol or *O*-DMA.

In vitro studies have shown that isoflavones, including equol and *O*-DMA, can inhibit enzymes involved in steroid hormone metabolism, such as aromatase, 5α-reductase, and 17β-hydroxysteroid dehydrogenase (123–125); therefore, some of the observed associations between equol/*O*-DMA and hormone levels and hormone-related factors might be due to their effect on the expression of enzymes involved in hormone metabolism. However, Harris *et al.* (126) recently reported that, *in vitro*, equol is an inhibitor of estrogen sulfotransferase, which could ultimately lead to elevated levels of active estrogens. *In vitro* work with human intestinal bacteria also has shown that estrogen metabolism is carried out differentially by various species of bacteria (127), and *in vivo*, perturbations in colonic microflora can result in alterations in estrogen metabolism (128–132). Thus, it is possible that the daidzein-metabolizing bacteria, or other bacteria associated with their presence, also could be involved in hormone metabolism, although currently there are no data to support this. Studies of oral microflora in women in relation to the onset of puberty and during pregnancy suggest that changes in sex hormones during these times may result in alterations of the oral microbial environment (133–135). This suggests that endogenous hormone levels could perhaps influence the intestinal bacteria and thus the manifestation of the daidzein-metabolizing phenotypes.

Host genetics might influence the human fecal flora; for example, similarities in intestinal bacteria were greater among monozygotic twins than among unrelated individuals (136) or dizygotic twins (71), and a study has shown that there may be a genetic influence on the acquisition of *H. pylori* infection (137). Furthermore, results from a recent population-based family study (48) suggested that the ability to harbor the daidzein-metabolizing bacteria might be under some degree of genetic control. Thus, because there may be a genetic component to the human fecal flora and/or the capacity to harbor the daidzein-metabolizing bacteria, potential differences in disease risk between daidzein-metabolizing phenotypes may result from host genetics associated with these phenotypes (Fig. 1). Alternatively, non–genetically determined host factors associated with the daidzein-metabolizing phenotypes (e.g., lifestyle factors) also might be responsible for associations between daidzein-metabolizing phenotypes and disease risk, although the determinants of the daidzein-metabolizing phenotypes are essentially unknown.

In populations with low soy consumption patterns, the use of a soy challenge is a convenient way to establish daidzein-metabolizing phenotypes (48). As discussed earlier, 24-hr urine collections may be less likely than first-void urine collections to result in misclassification of the phenotypes, but they are not suitable for large-scale epidemiologic studies. Furthermore, although data from small studies have suggested that the daidzein-metabolizing phenotypes are stable within an individual over time (31, 65–67), no studies have been designed specifically to assess this over a long period of time. It is important that, if daidzein-metabolizing phenotypes are to be considered as biomarkers of risk for disease and/or to predict response to a soy or isoflavone intervention, the criteria on which to designate an individual as an equol/*O*-DMA producer and the stability of these phenotypes within individuals over time need to be established.

Identification of the bacterium/bacteria involved in daidzein metabolism is another important goal, and may help to clarify whether or not they play a direct role in modulating factors such as hormone levels. However, data from *in vitro* studies suggest the equol-/*O*-DMA–producing bacterium/bacteria in one individual may not be the same as in another individual (46), which could ultimately lead to subgroups within the daidzein-metabolizing phenotypes in terms of their relevance to human health. This could perhaps explain some of the variability in the reported associations

between daidzein-metabolizing phenotypes and health in human studies. The use of molecular techniques (e.g., polymerase chain reaction–denaturing gradient gel electrophoresis and terminal restriction fragment polymorphism; Ref. 138) to study differences in fecal bacterial profiles between individuals with and without the ability to produce equol and/or *O*-DMA could significantly advance the pursuit to identify the bacteria involved in daidzein metabolism in humans.

It has been established that intestinal bacterial metabolism of dietary compounds including flavonoids and isoflavonoids can alter their biological activities (2), which, in turn, could alter their potential to influence host health. The metabolism of daidzein to equol and *O*-DMA in humans is of particular interest given that (i) substantial interindividual variation in equol and *O*-DMA production exists, and (ii) equol and *O*-DMA may be more biologically active than their precursor daidzein. To date, relatively few studies have been specifically designed to assess daidzein-metabolizing phenotypes in relation to human health. Therefore, little evidence is currently available regarding the potential health effects associated with the ability to produce equol/*O*-DMA, and additional studies specifically designed to address these hypotheses and with larger sample sizes clearly are needed to confirm or refute relationships between daidzein-metabolizing phenotypes and disease risk.

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