O-Desmethylangolensin: The Importance of Equol’s Lesser Known Cousin to Human Health1,2

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ABSTRACT

The objective for this paper was to review human studies of O-desmethylangolensin (O-DMA) concentrations and of O-DMA producers compared with nonproducers in the context of results from in vitro studies. O-DMA is an intestinal bacterial metabolite of daidzein, an isoflavone compound observed to have phytoestrogenic properties. Not all individuals harbor bacteria capable of metabolizing daidzein to O-DMA, and individuals can be classified as O-DMA producers and nonproducers. O-DMA is less structurally similar to 17β-estradiol than its parent compound, daidzein; thus, it may exhibit different biological actions than daidzein. Evidence from in vitro studies suggests that O-DMA has several cancer-related biological actions. However, results from human metabolic studies and observational studies of disease risk suggest that these actions may not be physiologically relevant in vivo due to the amount and form (primarily glucuronide) of circulating O-DMA. Apart from circulating O-DMA concentrations, the underlying bacteria may have a distinct physiological role. Urinary excretion of O-DMA in humans is a marker of harboring intestinal bacteria capable of C-ring cleavage. Bacterial C-ring cleavage reactions are relevant to other phytochemicals that may exert biological actions in vivo that are stronger than the actions of O-DMA; thus, the role of the phenotype may extend beyond daidzein metabolism. There are a limited number of studies that have evaluated disease risk factors in relation to being an O-DMA producer, with mixed results. Further research evaluating disease risk in relation to the O-DMA-producer phenotype from the perspective of intestinal microbial composition is recommended. Adv. Nutr. 2: 317–324, 2011.

Introduction

O-desmethylangolensin (O-DMA)3 and equol were first identified in human urine in the 1980s (1,2) and were later identified as products of intestinal bacterial metabolism of daidzein, an isoflavone compound found in high amounts in soy (3). O-DMA is structurally similar to 17β-estradiol and has been evaluated in vitro for phytoestrogenic properties. Both O-DMA and equol exhibit producer phenotypes, i.e., some people harbor bacteria capable of producing O-DMA and equol (O-DMA producers and equol producers, respectively), whereas other people do not harbor such bacteria (4–7). Since their discovery, the published research on equol has increased exponentially to >600 papers, whereas published literature in which O-DMA was specifically evaluated is a little more than 100 papers. The objective of this review was to summarize more recent human studies in the context of what was observed in vitro and the seminal human studies. The aggregation of this knowledge in this review provides a foundation for future directions regarding O-DMA and the O-DMA-producer phenotype.

Current status of knowledge

Biochemistry and metabolism

The precursor to O-DMA is daidzein, an isoflavone compound that exhibits phytoestrogenic activities in vitro (8–10). Whereas daidzein and its metabolite equol are diphenolic compounds structurally similar to 17β-estradiol, one of the rings of O-DMA is cleaved (Fig. 1). Thus, O-DMA may arguably be considered more structurally similar to other polyphenol chemicals, such as phloretin, which is found in high amounts in apples (11), than to its parent compound, daidzein, or its “cousin,” equol. O-DMA can exist in several forms: monoglucuronide, diglucuronide, sulfate, and free aglycone.

The production and metabolic fate of O-DMA is illustrated in Figure 2. Daidzein exposure occurs primarily through the diet; dietary daidzin (the glycoside form of daidzein) is found in high amounts in unfermented soy foods and dietary daidzein is found in high amounts in fermented...
soy foods (12). A small percentage of individuals may also be consuming formononetin, a precursor to daidzein, from supplements that contain clover. Daidzin can be hydrolyzed by β-glycosidases in the intestinal tract and evidence supports that this can begin in the small intestine (13). Intestinal bacteria in the large intestine can metabolize daidzein to dihydrodaidzein, which can then undergo ring cleavage by other intestinal bacteria to form O-DMA; formation of O-DMA has not been observed in the ileum and is thought to occur only in the large intestine (13). There is some recent evidence to suggest that O-DMA may be further partially metabolized to resorcinol and 2-(4-hydroxyphenyl) propionic acid by particular bacteria (14). The aglycone O-DMA can be absorbed, where it can be conjugated in the intestinal mucosa or the liver to O-DMA glucuronide or O-DMA sulfate (15). O-DMA can be released from the liver into circulation or into the bile and undergo enterohepatic recirculation. It appears that >95% of circulating O-DMA is glucuronidated (16). The glucuronide form is water soluble and can be excreted via the kidneys. The kidney is the main route of excretion of O-DMA. A small amount is excreted in feces; however, the amount excreted in feces appears to have high interindividual variation (17). It was observed in a sample of 4 women that 97% of urinary O-DMA was glucuronidated and ~1.2% was free O-DMA (18).

Individuals who harbor bacteria capable of producing O-DMA are commonly referred to as O-DMA producers and, similarly, individuals who harbor bacteria capable of producing equol are commonly referred to as equol producers. Evidence from in vitro studies supports that the bacteria that produce O-DMA are distinct from the bacteria that produce equol (19,20). In addition, observational studies suggest that the O-DMA-producer and equol-producer phenotypes are independent of each other, i.e. the capacity to harbor O-DMA-producing bacteria is not influenced by whether the individual has the capacity to harbor equol-producing bacteria (21). However, the presence of both producer phenotypes within the same individual may influence the circulating or urinary concentrations of O-DMA and equol. Small human intervention studies have observed an inverse relationship between the excretion of O-DMA and equol in the presence of soy consumption, observations that suggest that in the presence of both equol-producing and O-DMA-producing bacteria, the preferred metabolic pathway is to the metabolism of daidzein to O-DMA.

Figure 1 Chemical structures of 17β-estradiol, O-desmethylangolensin, and structurally similar phytochemicals.

Figure 2 Production and metabolism of O-desmethylangolensin in humans (adapted from a publically available image from National Institute of Diabetes and Digestive and Kidney Diseases, NIH; available at catalog.niddk.nih.gov/ImageLibrary).
equol (22–24). However, this has not been consistently observed (25,26).

Bacteria confirmed to metabolize daidzein to O-DMA (i.e. cleaving the C-ring) in vitro include \textit{Eubacterium ramulus} (27) and \textit{Clostridium} sp HGH 136 (28). Recently, a genus from the \textit{Clostridium} cluster XIVa, named strain SY8519, was isolated from a human fecal sample and identified to also produce O-DMA (29). Strain SY8519 exhibited structural similarity to bacteria in the genus \textit{Eubacterium} and genus \textit{Rosburia}. \textit{E. ramulus} inhabits the human gastrointestinal tract with wide variation in the mean cell count, ranging in one study of 20 individuals from 0.01 to 0.36% of total bacteria cell counts (30). In cultures of \textit{E. ramulus} isolated from human fecal samples and incubated with 0.5 nmol/L daidzein, O-DMA appeared to be an end product of metabolism (27). Interestingly, \textit{E. ramulus} has been observed to metabolize other polyphenolic compounds to phenolic acids in vitro (examples of dietary sources are included in brackets), including quercetin-3-glucoside (31) and its aglycone quercitin (32–34) [apples, bee pollen, berries, onions (35)], nar-ingenin (34) [citrus, particularly grapefruit (35)], kaempferol (34) [endive, kale (35)], phloretin (34) [apples, tomatoes (11,36)], and luteolin (33,34) [carrots, peppers (37)]. These observations suggest that the presence of O-DMA-producing bacteria may be relevant beyond the ability to transform daidzein to O-DMA, as the bacteria may transform other polyphenolic compounds associated with human disease risk.

**Presence in biological matrices**

O-DMA has been detected in several matrices in addition to serum, plasma, and urine, including prostatic fluid (38,39), breast milk (40), amniotic fluid (41), and cord blood (41). Compared to other isoflavones and their metabolites, O-DMA appears to be a minor metabolite in biological matrices (42). Evidence suggests that the majority of isoflavones and metabolites peak in circulation about 12 h after a daidzin/daidzein intake and little remains in circulation, without sustained daidzin/daidzein consumption, after 72 h (43). Drawing a parallel from work that has looked at dietary equol consumption (44–46), low levels of O-DMA in biological matrices may also reflect consumption of small amounts of O-DMA from foods of animal origin, but this has not been specifically studied. As expected, urine and serum levels are low in populations that are on average low soy consumers. In a large study of individuals in the United States, urine and serum concentrations were 4.34 and 1.0 μg/L, respectively (47,48). In comparison, Kunisue et al. (49) evaluated urine concentrations from several countries and observed average urinary O-DMA concentrations of 110 μg/L in Japanese participants. In both of these populations, urinary excretion ranged from nondetectable to the 95th percentile of 217 μg/L in the U.S. population and a maximum of 1600 μg/L in the Japanese population; detectable concentrations were observed in 79% of U.S. and 85% of Japanese samples (47,49). This observation highlights the wide variability of O-DMA excretion, even within countries where high-soy consumption is common.

**Biological actions**

Several biological actions of O-DMA have been observed in vitro. In the past decade, O-DMA has been evaluated for effects on growth and integrity of cancer cells (9,39,50–54), binding to and transactivation of estrogen, androgen, and progesterone receptors (8–10,55), osteoclast formation (56), modulation of immunological markers (57), binding to and transactivation of PPARγ (58), superoxide radical scavenging activity (59), and leptin secretion inhibitory activity (60).

Relationships with nuclear receptors and effects on cancer cells have been the most studied in vitro activities. Recently, Takeuchi et al. (10) compared the agonistic and antagonistic activities of various compounds, including O-DMA and structurally similar phloretin, against estrogen receptor (ER)-α, ERβ, androgen receptor (AR), glucocorticoid receptor, thyroid hormone receptor (TR)-α1, and TRβ1. Although O-DMA did not exhibit in vitro agonistic or antagonistic activities toward glucocorticoid receptor, TRα1, or TRβ1, in Chinese hamster ovary cells, O-DMA and enterolactone were the only 2 compounds tested to have AR antagonistic activity. However, a study in yeast suggested that O-DMA may bind to but not transactivate AR (55). O-DMA also exhibited agonistic activities against ERα and ERβ, with 20% relative effective concentration of 2.4 × 10⁻⁸ and 1.8 × 10⁻⁸ mmol/L, respectively. For comparison, these relative effective concentrations were similar to their parent compound, daidzein, lower than phloretin, and greater than estradiol (O-DMA had <5% of the percent activity relative to estradiol). These results are similar to other studies that have evaluated ERα, ERβ, and AR. Hwang et al. (8) observed O-DMA to have 6% of the relative binding affinity (RBA) of estradiol to ERα and 37% of the RBA of estradiol to ERβ. For most assays conducted, O-DMA exhibited weaker effects than daidzein. The authors noted that despite the stronger estrogenic potency observed with the RBA to ERβ, the inhibitory effects in transfection assays were weaker.

It is important to note that the free aglycone O-DMA may exhibit different effects than O-DMA glucuronide, which is the predominant form in human circulation. Results from in vitro studies suggest that the glucuronide has some activity, but lower activity than the aglycone. Kinjo et al. (9) observed the binding of O-DMA glucuronide to human ERβ (hERβ) was similar to that of daidzein glucuronides and the binding of the O-DMA aglycone to hERβ was similar to that of estradiol. O-DMA glucuronide also exhibited hERβ-dependent β-galactosidase induction activity similar to that of daidzein and genistein glucuronides, albeit the activity of these compounds appeared relatively weak.

O-DMA effects on cancer cell growth and integrity in vitro have been studied for several cell lines, including MCF-7 (human breast cancer) (9,52), MDA-MB-231 (human breast cancer) (50,52), BG-1 (human ovarian cancer) (52), LNCaP (human prostate) (39,51), LAPC-4 (human prostate) (51), HT29 (human colon cancer) (54), and L5178Y (mouse lymphoma) (53). Results from the studies of breast cancer and ovarian cancer cells suggest that O-DMA exerted stronger
effects on ER-insensitive cells than on ER-positive cells (50,52). Kinjo et al. (9) observed that the glucuronide form inhibited MCF-7 growth, but much weaker than did the aglycone form. Antiproliferative effects of O-DMA were observed in vitro on benign prostatic epithelial cells and LNCaP cells (39). In another study, O-DMA suppressed growth of LAPC-4 cells, but not LNCaP cells; this combination of actions was not observed for genistein, daidzein, and other metabolites, which all suppressed both cell types (51).

**O-DMA concentrations and disease risk**

Several studies have evaluated O-DMA concentrations in participants in the EPIC-Norfolk cohort (61–65), which is part of a multicenter prospective study (66). In the EPIC-Norfolk studies, spot urine samples and serum samples were used to evaluate phytoestrogen exposure. Because the samples reflect participants’ usual diets and this is on average a low-soy-consuming population, the concentrations of O-DMA were low compared to what would be observed in a high-soy-consuming population. Low et al. (63) observed no significant association of urinary or serum O-DMA concentrations with sex hormones in postmenopausal women; however, in a later analysis with a larger sample and higher geometric mean urinary O-DMA concentrations, a positive association was observed between urinary O-DMA and plasma estradiol (no associations with other hormones were observed) (61). In men from the EPIC-Norfolk cohort, urinary and serum O-DMA concentrations were not associated with plasma androstenediol glucuronide, free androgen index, testosterone, or sex hormone binding globulin (62). Urinary and serum O-DMA concentrations were also not significantly associated with breast cancer, colon cancer, or prostate in the EPIC-Norfolk cohort (64,65). It is possible that because this was a low-soy-consuming population, O-DMA values were too low or that there was insufficient variation in the exposure to detect associations. However, other studies in both low-soy-consuming and high-soy-consuming populations support a lack of association between urinary or circulating concentrations of O-DMA with breast cancer (67–70).

Hedlund et al. (39) also noted that in human participants, 98% of men produced O-DMA during the soy metabolism study, but only 4 of 36 men had circulating O-DMA concentrations ≥ 1 μmol/L, which was the dose the authors noted as the minimum at which to produce measurable anti-proliferative effects. These observations underscore that the presence of O-DMA producing bacteria, even in the presence of soy consumption, does not result in high circulating concentrations of O-DMA in all O-DMA-producing individuals.

**O-DMA-producer phenotype**

Classification of individuals as O-DMA producers or non-producers is done based on the presence of O-DMA in urine, blood, or cultured bacteria from feces. Most studies that have evaluated the phenotype have used urinary concentrations of O-DMA. There is no agreed-upon cutoff of urinary O-DMA concentrations and some studies evaluate the absolute excretion or excretion in a ratio to daidzein. Prevalence of O-DMA producers using fecal incubations is challenging, because not all culture media are capable of supporting the growth of O-DMA-producing bacteria (43). Because the daidzein precursor is needed for the production of O-DMA and the half-life of O-DMA in the body is short (43), phenotyping should be done after a soy or isoflavone challenge or in the presence of sustained high-soy intake. Based on larger studies evaluating urinary excretion in soy-consuming populations or after a soy challenge, ~80–95% of individuals are O-DMA producers (4–7).

As reviewed by Atkinson et al. (4), there are several factors that may influence an individual’s ability to produce O-DMA, including dietary and environmental factors, host genetics, or nonmodifiable host factors (e.g. age). In a study of 410 individuals aged 10–95 y, an inverse association was observed for the relationship between age and the prevalence of O-DMA producers (21). In the cross-sectional analysis, predicted prevalence (from statistical modeling) of being an O-DMA producer was highest in younger participants (~95% in 10-y olds) and appeared to reach a plateau in adulthood at ~80%. This is an interesting observation, because it parallels data that supports that almost all children and adolescents harbor Oxalobacter formigenes, bacteria capable of metabolizing oxalate, but there is a lower percentage of adults, ~75–80%, who harbor O. formigenes (71,72). Evidence primarily from cross-sectional human studies suggests that the microbiome may shift during key transitional life periods (73), such as during growth and weaning in infancy and aging in the elderly, and that the microbiome is influenced by both heritable and nonheritable factors (21,73–79). One study has evaluated familial aggregation of the O-DMA-producer phenotype (21). There was some suggestion of modest correlations between closely related family members (e.g. siblings) to be concordant on phenotype, but small pedigree sizes limited the ability to fully evaluate familial aggregation for the O-DMA-producer phenotype. Two studies observed that individuals who self-report as being Asian were less likely to be O-DMA producers (21,80), which is also supported by the observation that Korean American females had a lower prevalence of O-DMA producers than Caucasian American females living in the same geographic area (7). Two studies also observed relationships with weight, but it is difficult to draw conclusions from the relationships, because O-DMA producers were more likely to be taller in one study (80) and greater height was associated with lower prevalence in the other study (21). Factors that were not associated with the phenotype included sex, diet, weight or BMI, and smoking (21,80).

Four key exposure patterns arise when considering the O-DMA-producer phenotype across different populations (Table 1). Evaluation of the O-DMA-producer phenotype in the presence of high, frequent daidzein consumption put the phenotype in the role of being a potential effect modifier of the relationship between soy or isoflavone consumption and health outcomes, i.e. does the effect of soy or isoflavones on health outcomes differ across the phenotypes? Evaluation of the O-DMA-producer phenotype, determined by soy or
isoflavone challenge, in the absence of regular daidzein consumption provides information about the independent role of an O-DMA–producing microbial profile, i.e. do O-DMA–producing bacteria or correlated bacteria have an effect on human health? There is ample evidence to suggest that the indigenous microbiome is a contributor to human host health (81,82); thus, it is possible that the O-DMA–producing bacterial profile alone exerts influence on human health.

**O-DMA–producer phenotype and disease risk**

A few populations have been studied for health outcomes in relation to the O-DMA–producer phenotype. Mostly recently, Guo et al. (83) evaluated 202 community-based participants, males and females aged 20–69 y, from Guangzhou, China. Participants consumed isoflavone supplements (48.12 mg daidzein/d) for 3 d prior to collecting a 24-h urine sample. Fifty-one percent of women and 42% of men were classified as being O-DMA producers (defined as having a urinary O-DMA:daidzein ratio of >0.018). No significant associations were observed for serum lipids, blood pressure, uric acid, BMI, or waist:hip ratio in relation to being an ODMA producer. In 2 separate studies in the Seattle, WA area, the O-DMA–producer phenotype was evaluated in relation to breast cancer risk factors in premenopausal women aged 40–45 y (84,85) and postmenopausal women aged 50–75 y (86–88). Both studies utilized a 3-d soy challenge reaction. Other phytochemicals undergo C-ring cleavage reactions. For example, E. ramiulus metabolizes both daidzein and quercetin. It is hypothesized that the O-DMA–producing bacteria metabolize other phytochemicals that may influence disease risk. The proportion of glucuronide to aglycone is high and the actions of the glucuronide are weaker than the aglycone. Even among O-DMA producers, O-DMA constitutes a minor metabolite of daidzein and concentrations in most individuals are low. Overall, these observations suggest that circulating concentrations of O-DMA may not be physiologically relevant for disease risk. This is supported by the lack of association between urinary and circulating concentrations and breast cancer in human studies.

However, the O-DMA–producer phenotype may be physiologically relevant for disease risk. The method of classifying O-DMA producers and nonproducers is not standardized; however, it is well established that not all individuals excrete O-DMA after daidzin/daidzein consumption. A small number of studies have published results evaluating disease risk factors in relation to the O-DMA–producer phenotype, with mixed results. The O-DMA–producer phenotype acts as a marker of harboring bacteria capable of C-ring cleavage reactions. Other phytochemicals undergo C-ring cleavage reactions. For example, E. ramiulus metabolizes both daidzein and quercetin. It is hypothesized that the O-DMA–producing bacteria metabolize other phytochemicals that may influence disease risk. Further research is recommended to elucidate disease risk in relation to the phenotype as a marker of intestinal bacterial composition.

**Conclusions**

The review of the evidence from in vitro and human studies suggests that while O-DMA may exert several cancer-related biological actions in vitro, including impairment of the growth of ER-insensitive breast cancer cells, circulating concentrations in humans likely have limited, if any, impact on disease risk. The proportion of glucuronide to aglycone is high and the actions of the glucuronide are weaker than the aglycone. Even among O-DMA producers, O-DMA constitutes a minor metabolite of daidzein and concentrations in most individuals are low. Overall, these observations suggest that circulating concentrations of O-DMA may not be physiologically relevant for disease risk. This is supported by the lack of association between urinary and circulating concentrations and breast cancer in human studies.

**Acknowledgments**

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**Literature Cited**


Table 1. O-DMA exposure groups that arise from the combination of the O-DMA–producer phenotype and dietary intake of daidzin/daidzein

<table>
<thead>
<tr>
<th>Phenotype</th>
<th>Dietary intake</th>
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<tbody>
<tr>
<td>O-DMA producer</td>
<td>Exposure to daidzein, O-DMA, and O-DMA–producing microbial profile</td>
</tr>
<tr>
<td>O-DMA nonproducer</td>
<td>Exposure to daidzein and O-DMA–nonproducing microbial profile</td>
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