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Metabolomics profiles of premenopausal women are different based on *O*-desmethylangolensin metabotype

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Abstract

Urinary *O*-desmethylangolensin (ODMA) concentrations provide a functional gut microbiome marker of dietary isoflavone daidzein metabolism to ODMA. Individuals who do not have gut microbial environments that produce ODMA have less favourable cardiometabolic and cancer risk profiles. Urinary metabolomics profiles were evaluated in relation to ODMA metabotypes within and between individuals over time. Secondary analysis of data was conducted from the BEAN2 trial, which was a cross-over study of premenopausal women consuming 6 months on a high and a low soya diet, each separated by a 1-month washout period. In all of the 672 samples in the study, sixty-six of the eighty-four women had the same ODMA metabotype at seven or all eight time points. Two or four urine samples per woman were selected based on temporal metabotypes in order to compare within and across individuals. Metabolomics assays for primary metabolism and biogenic amines were conducted in sixty urine samples from twenty women. Partial least-squares discriminant analysis was used to compare metabolomics profiles. For the same ODMA metabotype across different time points, no profile differences were detected. For changes in metabotype within individuals and across individuals with different metabotypes, distinct metabolomes emerged. Influential metabolites (variables importance in projection score > 2) included several phenolic compounds, carnitine and derivatives, fatty acid and amino acid metabolites and some medications. Based on the distinct metabolomes of producers *v.* non-producers, the ODMA metabotype may be a marker of gut microbiome functionality broadly involved in nutrient and bioactive metabolism and should be evaluated for relevance to precision nutrition initiatives.

Key words: **Equol: Isoflavones: Metabolomics: Microbiome: *O*-desmethylangolensin**

The relationship between gut microbiota and host physiology is complex, and it is well recognised that gut microbiota influence overall health of the human host[1,2], but the mechanisms are not fully elucidated. One possible pathway is through the metabolism of dietary and other orally consumed compounds, such as environmental contaminants and medications. This makes the gut microbiome a potential target for precision nutrition initiatives[3]. Gut microbial species utilise nutrients consumed by the human host as a source of fuel, and the gut microbiome has a critical role in energy harvest[4], glucose and lipid metabolism[5], systematic inflammation[6] and circulating cardiovascular-related proteins[7], which are physiological factors that are associated risk of various chronic diseases, including CVD and cancer. An important feature of the complex gut microbiome is the considerable interindividual variation in species that are present. However, there is notable functional redundancy that is not necessarily captured by comparing individuals' microbiota composition or diversity.

Functional markers based on microbially derived secondary metabolites and metabolomics help address this challenge, and they may serve as important targets for precision nutrition initiatives. There are several known compounds that can be metabolised by microorganisms that reside in the gut and serve as markers of gut microbiome functionality. Metabotype is a term is used to describe metabolic phenotyping of individuals[8], and identifying useful metabotypes has been identified as a means by which to tailor nutrition or pharmaceutical interventions[3,8–10]. One such metabolite with a corresponding producer/non-producer metabotype is *O*-desmethylangolensin (ODMA), which is microbially derived from the isoflavone daidzein[11–13]. Approximately 10–40 % of individuals do not excrete detectable urinary ODMA concentrations after consuming the parent compound daidzein[11,14–21]. Daidzein is an isoflavone, and isoflavones are found in high amounts in soya foods. After soya consumption, approximately 10–40 % of individuals do not excrete detectable urinary ODMA concentrations[11,16–18,20,21].

Abbreviations: PLS-DA, partial least squares discriminant analysis; ODMA, *O*-desmethylangolensin; VIP, variables importance in projection.

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Observational studies suggest that ODMA non-producers have a less favourable breast cancer risk profile[22,23], lower bone density[24] and higher prevalence of obesity[17,19,20]. While ODMA has low physiological activity in the human host because the majority of ODMA in circulation is glucuronidated (bound with a sugar moiety) and has lower binding affinity to hormone receptors than other compounds[25], blood or urine ODMA concentrations provide a functional marker of a gut microbial community capable of metabolising daidzein to ODMA. The production of ODMA is an interesting metabolite among gut microbially derived metabolites because it involves an aromatic ring cleavage[26,27], which is a metabolically costly biotransformation that suggests this metabolism serves an important purpose to detoxify polyphenolic compounds.

An underlying hypothesised mechanism for why ODMA non-producers may be at a higher risk for chronic diseases is that they lack gut microbial functionality to break down potentially harmful compounds. However, there is little know about effects to other compounds associated with having ODMA-producing or not having ODMA-producing bacteria. While there is evidence to support that the ODMA producer *v.* non-producer metabotype may be a functional marker of a gut microbial consortium capable of C-ring cleavage of compounds more broadly than just daidzein (i.e. bacteria involved in ODMA production are also involved in metabolism of other phenolic compounds), there is little known about what compounds may be involved. In particular, one of the bacteria identified to metabolise daidzein to ODMA, *E. ramulus*[28], is well studied and is also involved in the degradation, through ring cleavage, of other phenolic compounds, including quercetin[29], xanthohumol[30], 8-prenylnarigenin[30] and other flavonoids[31,32]. In order to provide some foundational evidence for broader impacts of the ODMA metabotype, metabolomics profiling was used to provide an efficient means to evaluate the differences in small molecule exposure across groups of individuals. As a foundation for future targeted research, the objective of this analysis was to evaluate metabolomics profiles in relation to ODMA metabotype among a group of premenopausal women who participated in soya intervention trial.

Subjects and methods

Study design

Urine biospecimens were collected during a cross-over trial conducted among premenopausal women from 2007 to 2010, and details about the study design and population are published elsewhere[33–35]. Briefly, the BEAN2 trial was a cross-over study with 6 months on a high and a low soya diet, each separated by a 1-month washout period. The objective of the BEAN2 study was to evaluate soya intake and nipple aspirate fluid, a possible indicator of breast cancer risk. Eligibility criteria for the parent study included a normal mammogram, no oral contraceptives, not pregnant, no previous cancer diagnosis or breast surgery, regular menstrual periods, low soya intake and the ability to produce nipple aspirate fluid. Participants had high compliance with the study regimen based on subjective and objective measures[33]. There were eighty-four women from the original trial who had eight stored urine samples over the cross-over intervention, from which sixty samples were selected for metabolomics analysis. Given that the gut microbiome is a dynamic ecosystem[36–38], it is expected that a metabotype can shift over time, but the magnitude of shift is not well documented. In all of the 672 samples over the 13-month study, sixty-six of the eighty-four women had the same ODMA metabotype at seven or all eight time points. Thus, the majority of individuals (79 %) express a relatively consistent metabotype over time, which supports the utility of the metabotype as a biomarker. In order to address the objective of the study, sixty urine samples from twenty women were selected for metabolomics analysis to compare: (1) metabolomic profiles across samples within unchanging ODMA-producer metabotype; (2) metabolomic profiles across samples within changing ODMA-producer metabotypes and (3) metabolomics profiles across ODMA-producer and ODMA non-producer samples.

Identification of Daidzein-Metabolising Metabotypes and Biospecimen Selection

As part of the parent study, daidzein, equol and ODMA concentrations were analysed by liquid chromatography tandem MS (LC-MS/MS) as detailed elsewhere[39]. These previously measured concentrations were used to classify metabotypes. Each urine sample was identified as being ODMA producer/non-producer and equol producer/non-producer based on a cut-off of equol/ODMA:daidzein ratio of 0·018[40]. All samples, except two samples, which were excluded from selection, had daidzein concentrations > 2 nmol/mg creatinine, indicating sufficient presence of the precursor metabolite for metabolite detection.

Figure 1 illustrates the selection of biospecimens for metabolomics analysis and inclusion of samples for the three comparisons. In the eighty-four women from the parent study, forty women (47·6 %) maintained a consistent ODMA-producer (*n* 39) or ODMA non-producer (*n* 1) metabotype across all eight urine samples in a 2-year period. Two samples were randomly selected from the one ODMA non-producer and from nine of the ODMA producers. A random number generator was used to select the nine ODMA producers and to choose the samples from ten individuals with consistent metabotype from samples that also had the same equol producer metabotype in the two samples. For individuals with discrepant metabotype during the 2-year time period, twenty-six women had the same metabotypes at seven time points (31 %) and eighteen women had a different metabotype for two to six of the time points (21 %). Of the eighteen women with at least two instances of producer and non-producer metabotype expression, ten women were selected who had at least two samples each with ODMA producer and ODMA non-producer status and also a consistent equol metabotype in these samples. In total, sixty urine samples from twenty premenopausal women were analysed for untargeted primary metabolism and biogenic amine metabolomic profiles.

Urinary metabolomic analysis

Urine samples were analysed by the National Institutes of Health West Coast Metabolomics Center in the Fiehn laboratory, using

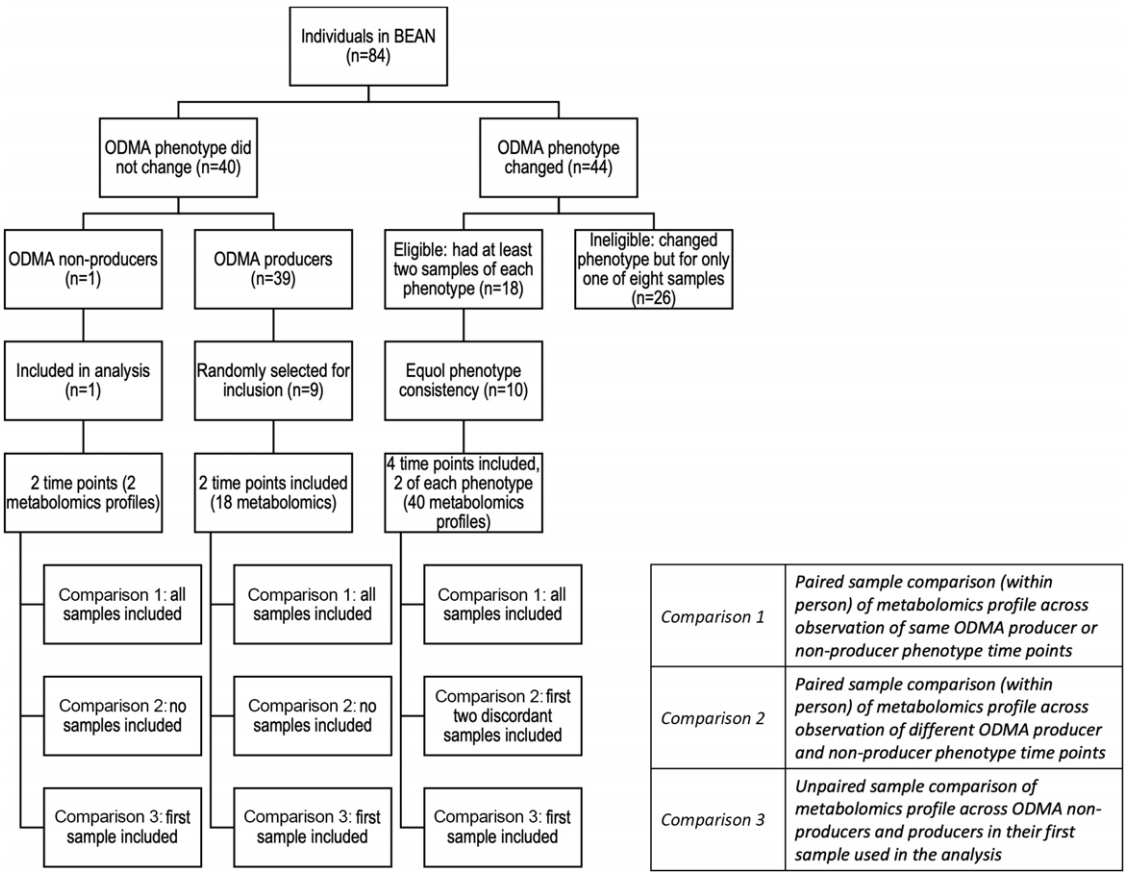

Fig. 1. Urine sample selection among participants in a 2-year soya intervention study. Two or four urine samples were selected from twenty women based on *O*-desmethylangolensin (ODMA) phenotype expression in eight urine samples over time.

established protocols and more detail on these protocols are available elsewhere[41]. To consider broad aspects of metabolism relevant to diet, primary metabolism and biogenic amines platforms were evaluated. In brief, primary metabolism metabolites were assayed using automated liner exchange-cold injection system, gas chromatography-time of flight mass spectrometry (ALEX-CIS GCTOF). Primary metabolism platform covers carbohydrates and sugar phosphates, amino acids, hydroxyl acids, free fatty acids, purines, pyrimidines, aromatics and exposome-derived chemicals. The analytical GC column is protected by a 10 m long empty guard column which is cut by 20 cm intervals whenever the reference mixture QC samples indicate problems caused by column contaminations. At this sequence of column cuts, no detrimental effects are detected with respect to peak shapes, absolute or relative metabolite retention times or reproducibility of quantifications. This chromatography method yields excellent retention and separation of primary metabolite classes (amino acids, hydroxyl acids, carbohydrates, sugar acids, sterols, aromatics, nucleosides, amines and miscellaneous compounds) with narrow peak widths of 2–3 s and very good within-series retention time reproducibility of better than 0·2 s absolute deviation of retention times. Automatic liner exchanges were used after each set of ten injections, which reduces sample carryover for highly lipophilic compounds such as free fatty acids. Mass spectrometry parameters are used as follows: a Leco Pegasus IV mass spectrometer is used with unit mass resolution at 17 spectra s^{-1} from 80 to 500 Da at −70 eV ionisation energy and 1800 V detector voltage with a 230°C transfer line and a 250°C ion source. For data processing, raw data are processed in an untargeted manner by free mzMine 2·0 software to identify peaks up to 300 chromatograms. Alternatively, selected peaks were collated and constrained into Agilent MassHunter quantification method on the accurate mass precursor ion level, using the MS/MS information and the NIST14/Metlin/MassBank libraries to identify metabolites, with manual confirmation of adduct ions and spectral scoring accuracy.

Biogenic amines were assayed using hydrophilic interaction chromatography-electrospray, quadruple time of flight MS, tandem MS (HILIC-ESI QTOF MS/MS). Biogenic amines platform covers acylcarnitines, trimethylamine N-oxide (TMAO), cholines, betaines, *S*-adenosylmethionine, *S*-adenosylhomocysteine, nucleotides and nucleosides, methylated and acetylated amines and di- and oligopeptides. The analytical UHPLC column is protected by a short guard column which is replaced after 400 injections, while the UHPLC column is replaced after 1200 extract injections. This method was validated that at this sequence of column replacements, and no detrimental effects are detected with respect to peak shapes, absolute or relative polar compound retention times or reproducibility of quantifications. This chromatography method yields excellent retention and separation of metabolite classes (biogenic amines, cationic compounds) with narrow peak widths of 5–20 s and very good

within-series retention time reproducibility of better than 6 s absolute deviation of retention times. Internal standards included were: L-arginine, 1-cyclohyxyl-dodeanoic acid urea, 1-methyl-nicotinamide, acetic anhydride, creatine, creatinine, alanine, aspartic acid, glutamic acid, N-methyl-histamine, L-carnitine, L-glutamine, betaine, butyrobetaine, choline, crotonobetaine, trimethylamine N-oxide and valine-tyrosine-valine.

Raw data files are preprocessed without smoothing, three second peak width, baseline subtraction just above the noise level and automatic mass spectral deconvolution and peak detection at signal/noise levels of 5:1 throughout the chromatogram. Apex masses are reported for use in BinBase algorithm. Quantification was reported as peak heights and reported for all annotated metabolites and for database entries that are positively detected in more than 10 % of the samples for unannotated metabolites. Samples were normalised based on creatinine concentrations. Raw data files are available for download from Metabolomics Workbench (http://dx.doi.org/10·21228/M88H76; http://dev.metabolomicsworkbench.org:22222/data/DRCCMetadata.php?Mode=Study&StudyID = ST001928&Access=UjvO7329; http://dev.metabolomicsworkbench.org:22222/data/DRCCMetadata.php?Mode=Study&StudyID = ST001929&Access=BluB5019).

Ethics

Procedures were approved by the University of Hawaii IRB for the parent study and sample collection, and de-identified samples were sent to the West Coast Metabolomics Center and de-identified data was sent to George Mason University (GMU). Due to the de-identification, this specific sub-study was reviewed and classified as not human subjects research by GMU.

Statistical analyses

Stata (version 15) was used for random number generation to identify samples from the parent study. MetaboAnalyst was applied for metabolomics analysis of samples across the three main comparisons (Fig. 1). No data filtering was conducted because of the relatively small number of features (< 5000). Prior to analysis, data were log-transformed and scaled using Pareto scaling (mean-centering and division by the square root of the SD for each variable). Normalisation was visually inspected. For each of the comparisons, the following analyses were performed: univariate *t* tests (false discovery rate cut off for $P < 0{\cdot}1$ was applied) and partial least squares discriminant analysis (PLS-DA). Variables importance in projection (VIP) for PLS-DA were identified from PLS-DA. These analyses were performed on data sets with unannotated and annotated metabolites (complete set) and restricted to annotated only metabolites (annotated subset).

Results

For women compared across two time points with the same ODMA metabotype, no significant difference in the overall primary metabolism or biogenic amine profiles in PLS-DA analysis was detected (Fig. 2(a) and (b)). The first five components of the PLS-DA analysis explained 36·5 % of variation for primary metabolism and 34·1 % of variation for biogenic amines. When individuals changed metabotype and also across producers and non-producers, there was distinct separation of the overall profiles (Fig. 2(c)–(f)). When individuals changed metabotype, the first five components of PLS-DA analysis explained 46·9 % of variation for primary metabolism and 40·1 % for biogenic amines. When comparing interindividual differences across ODMA metabotype, the first five components of PLS-DA analysis explained 45·8 % of variation for primary metabolism and 44·8 % for biogenic amines. The ODMA non-producer metabotype exhibited tighter clustering than the ODMA producer metabotype (Fig. 2(e) and (f)).

Across time points when individuals kept the same metabotype, no primary metabolism or biogenic amine metabolites had a greater than 1·5-fold change between the paired time points and no statistically significant difference was seen in univariate analysis (data not shown). Unannotated metabolites were common among the VIP > 2 for PLS-DA. For each of the comparisons, the annotated metabolites that had a VIP > 2 are presented in Table 1. For comparisons across individuals with different metabotypes (Comparison 3), thirty-three annotated metabolites differed in raw *t* tests ($P < 0{\cdot}01$; eight primary metabolism and twenty-five biogenic amines), but did not remain significant with application of false discovery rate (Table 1). A complete listing of results comparing annotated metabotypes across ODMA metabotype (interindividual and intraindividual differences) is available in Supplemental Table 1. There were some metabolites with $P < 0{\cdot}01$ in the comparisons that did not have a VIP > 2. For comparison 1 (intraindividual, same metabotype across time points), this included malic acid. For comparison 3 (interindividual, different OMDA metabotypes), this included 2-hydroxy-4-methoxybenzaldehyde and 4-aminomethylcyclohexanecarboxylic acid.

For the within-individual comparison of two samples, the compounds that had a VIP > 2 and higher magnitude (not statistically significant) at the non-producer time point included several phenolic compounds (liquiritigenin, phlorobenzophenone, oxybenzone, piperine and lofexidine), ergothioneine and sucrose (Table 1). Carnitine and some its derivatives were lower at non-producer time point in the within-person comparison, but higher in ODMA non-producers for the between-individual comparison. Other differences included fatty acid metabolites, amino acids and metabolites, alkaloids and several medications.

Discussion

Given the lack of information about the importance of the ODMA metabotype on host metabolism of other compounds but the known associations of ODMA metabotype with health outcomes[17–20,22–24], this study was undertaken to explore mechanisms by which the ODMA metabotype may serve as a marker of microbial metabolism beyond daidzein. This type of work is important for informing the utility of the ODMA metabotype as a potential target for precision nutrition. Overall, the results

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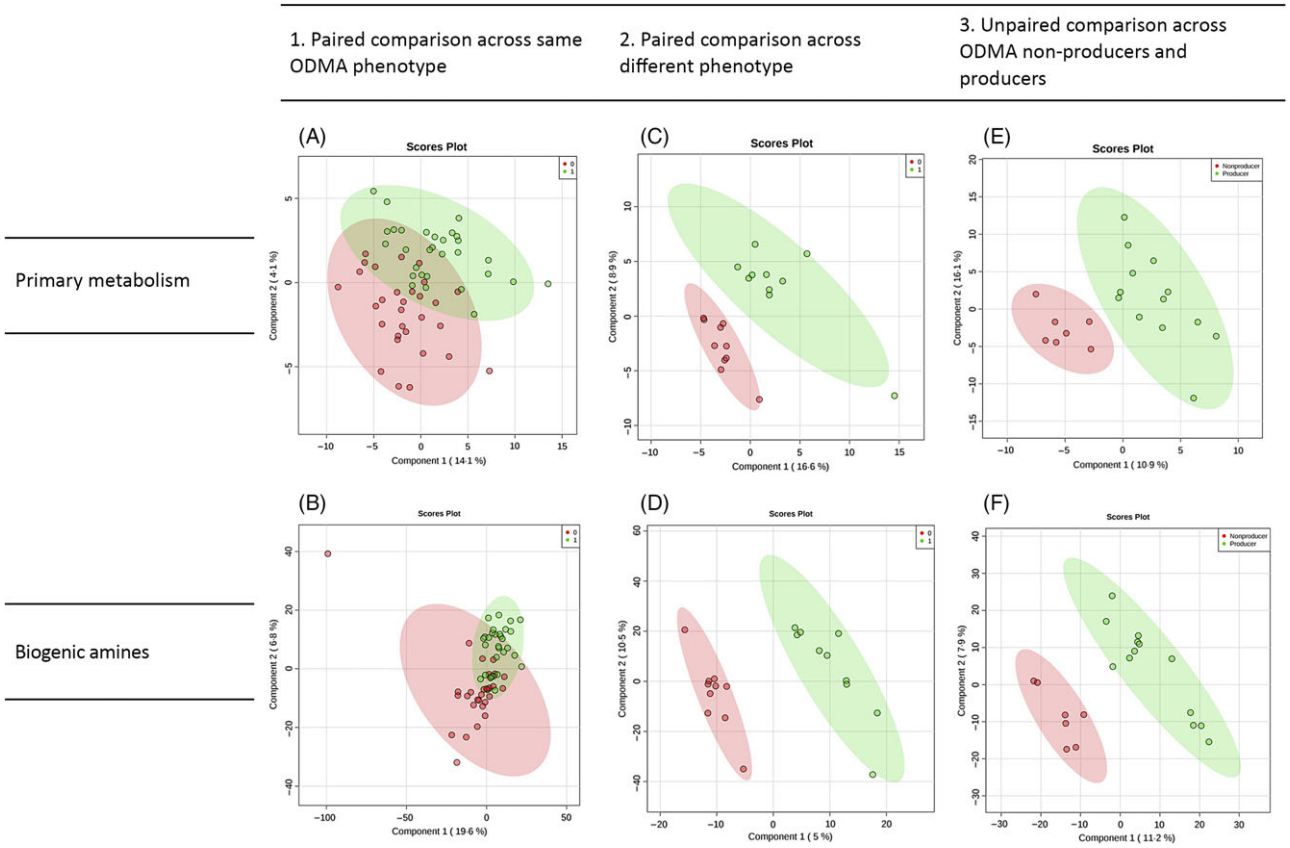

Fig. 2. First two components from partial least squares discriminant analysis (PLS-DA) comparing primary metabolism and biogenic amines untargeted metabolomics profiles in paired (within individual) comparisons across same *O*-desmethylangolensin (ODMA) phenotype (1); paired comparisons across different phenotypes (2); and unpaired (across individuals) comparisons of ODMA non-producer and ODMA producer phenotypes.

suggest that urinary metabolomics profiles are associated with ODMA metabotypes and suggest some avenues for future work.

No difference in metabolomics profiles for primary metabolism or biogenic amines were seen among individuals expressing the same ODMA metabotype at different time points, but metabolomics profiles differed distinctly when individuals changed metabotype across time and between women expressing different ODMA metabotypes. This supports a larger impact on metabolism that is associated with gut microbial environments capable or not capable of metabolising daidzein to ODMA. There is one other study published to-date that has considered metabolomics in relation to the ODMA metabotype. In Reverri *et al.*[42], men and women with higher cardiometabolic risk were divided into groups based on combined equol and ODMA metabotypes: non-producers of equol and ODMA, producers of equol and ODMA and producers of ODMA but not equol. The researchers did not observe any individuals who were producers of equol but not ODMA in their study sample. In their PLS-DA analysis, they observed some separation across the groups for serum metabolomics, but it was not statistically significant. In contrast to the analysis here, they did not see differences across ODMA producers and non-producers in urine metabolomics when comparing ODMA producers and non-producers in equol non-producers. These observations may differ for a variety of reasons, including differences in metabolomics assays and study participants (men and women *v.* women only, and cardiometabolic risk *v.* healthy). Our study was not designed to evaluate the combined impacts of equol and ODMA metabotypes, and future studies could consider the metabotype combination of equol producer/non-producer and ODMA producer/non-producer.

The ODMA metabotype provides a functional marker of gut microbial metabolism of daidzein to ODMA. Some bacteria have been identified *in vitro* to convert daidzein to ODMA, including Aeroto_AUH-JLC108 (derived from *Clostridium sp* AUH-JLC108[43], *Eubacterium ramulus*[28], *Clostridium sp* HGH 136[44] and *Clostrium* rRNA cluster XIVa)[45]. While it possible that other bacteria can metabolise daidzein to ODMA, they have not yet been identified, *Eubacterium ramulus* has been well studied and is informative towards the hypothesis that ODMA producers and non-producers may be a marker of metabolism of other compounds beyond daidzein. *Eubacterium ramulus* is also involved in the degradation or metabolism of other polyphenolic compounds, including quercetin[29], xanthohumol[30], 8-prenylnarigenin[30] and other flavonoids[31,32]. These observations provide evidence that at least one of the bacteria involved in ODMA production metabolises other polyphenolic compounds. This observation that the directionality of some phenolic compounds was higher when individuals were ODMA non-producers than producers provides further support

Table 1. Metabolites identified from partial least squares discriminant analysis (PLS-DA) with variable importance in projection>2 for untargeted metabolomics across between and within persons and metabolites with a raw *t* test $P < 0{\cdot}01$ for between person comparison of *O*-desmethylangolensin (ODMA) non-producer *v.* producer metabotype

	Comparison 1			Comparison 2			Comparison 3			Comparison 3	
	Metabolites VIP > 2	Time 1 *v.* Time 2	p-value	Metabolites VIP > 2	ODMA non-producer *v.* producer	*P*-value	Metabolites VIP > 2	ODMA non-producer *v.* producer	*P*-value	Metabolites with *t* test raw $P < 0{\cdot}01$	ODMA non-producer *v.* producer
Primary metabolism	Galactonic acid	higher	0·0231	Creatinine	lower	0·0879	Tartaric acid	lower	0·0557	Succinic acid	lower
	Gluconic acid	higher	0·0729	Glycerol-3-galactoside	higher	0·5422	Dehydroascorbic acid	higher	0·0675	N-carbamylglutamate	higher
	Galactose	higher	0·1460	Catechol	lower	0·9219				Citric acid	lower
	Quinic acid	lower	0·0405	N-acetyl-d-hexosamine	higher	0·4316				Threonine	higher
	Lyxitol	higher	0·0405	Hydroxyproline	higher	0·4202				Xylose	lower
	Glucose	higher	0·2801	Glycolic acid	lower	0·3337				Dehydroascorbic acid	higher
										Phenol	higher
										Tartaric acid	lower
Biogenic amines	Scopoletin	lower	0·0332	Liquiritigenin	higher	0·2361	Trigonelline	lower	0·0021	4-Aminobenzoic acid	lower
	Atomexetine	higher	0·1403	Ranitidine	lower	0·1775	Pirimiphos-ethyl	higher	0·0085	Trigonelline	lower
	Dopamine	lower	0·0010	Phlorobenzophenone	higher	0·2807	5-Aminoimidazole-4-carboxamide	higher	0·0024	2-Aminophenol	lower
	Pirimiphos-ethyl	lower	0·0312	Cetraxate	lower	0·2091	Acetylcarnitine	higher	0·6426	3-Carboxypropyltrimethyl-ammonium cation	higher
	Moclobemide	lower	0·0290	8-Oxo-2-deoxyadenosine	lower	0·9332	3-Carboxypropyltrimethyl-ammonium cation	higher	0·4387	3-Hydroxybutyrylcarnitine	higher
	Muramic acid	lower	0·1096	1,3,7-Trimethyluric acid	lower	0·0273	Propionylcarnitine	higher	0·0486	N-Carboxyethyl-gamma-aminobutyric acid	lower
	2-phosphonobutyric acid	higher	0·0440	Oxybenzone	higher	0·2118	Ecgonine	higher	0·4627	Acetylcarnitine	higher
	Agmatine	lower	0·1851	3-Carboxypropyltrimethyl-ammonium	lower	0·1519	Taurine	higher	0·1348	3-Hydroxyanthranilic acid	lower
	8-oxo-2-deoxyadenosine	higher	0·0629	Piperine	higher	0·1056	4-Hydroxyhippuric acid	lower	0·8669	Carnitine	higher
	Indolelactic acid	higher	0·0443	Taurine	lower	0·1602	Carnitine	higher	0·3990	Methionine	higher
				Lofexidine	higher	0·0892	N-Methyltyrosine	lower	0·5603	3-Methylcrotonylglycine	lower
				threo-Dihydrobupropion	lower	0·0736	Methionine	higher	0·0802	Homogentisic acid	higher
				Ergothioneine	higher	0·0488	Gly-val	lower	0·9982	Ecgonine	higher
				Propionylcarnitine	lower	0·1934	3-Hydroxybutyrylcarnitine	higher	0·0220	1_15N2-L-Arginine iSTD	lower
				Anserine	lower	0·4770	5-Methoxy-3-indoleacetic acid	lower	0·4639	Propionylcarnitine	higher
				N-Acetylcytidine	lower	0·3223	N-Benzyloxycarbonylglycine	lower	> 0·999	Pipecolic acid	lower
				Synephrine	lower	0·2324	Oxazepam	lower	0·1348	4-Quinolinecarboxylic acid	lower
				Acetylcarnitine	lower	>0·999				5-Aminoimidazole-4-carboxamide	higher
				Triethylene glycol monobutyl ether	lower	0·2324				2-Acetylpyrazine	higher
				Carnitine	lower	0·1782				3-Dehydrocarnitine	lower
				Pirimiphos-ethyl	lower	0·3853				N-2-Furoylglycine	lower
				Sucrose	higher	0·1602				Methohexital	higher
										Methacholine cation	higher
										Lofexidine	lower
										N-Acetylphenylalanine	lower

for the idea that this metabotype is associated with a lower capability for the metabolism of phenolic compounds more broadly than just ODMA. Future *in vitro* work with other compounds would be informative towards understanding the extent by which ODMA-producing bacteria may be influential.

The biotransformation of daidzein to ODMA involves an aromatic ring cleavage[26,27], which is a metabolically costly reaction and suggests this metabolism serves an important purpose in the breakdown of polyphenolic compounds. Many of these polyphenolic compounds are also found in diet and medications and have potential health-promoting effects through anti-inflammatory or other mechanisms[46–48]. On possible benefit of understanding how the ODMA metabotype functions is better characterisation of interindividual differences based on gut microbiome functionality can aid in identifying foods that may have more of health-promoting benefit in particular individuals. Another possible benefit of understanding how the ODMA metabotype functions is providing information about potential mechanisms by the gut microbiome influences cancer and cardiovascular health. The results here provide support that the ODMA metabotype may be involved in the metabolism of other compounds that are influential in health. For example, metabolism of scopoletin, piperine and trigonelline tracked with ODMA metabotype which are bioactives observed in animal and *in vitro* models to have anti-cancer or anti-inflammatory effects[49–52]. More work is needed to understand what specific aspects of composition or functionality of the ODMA metabotype are involved in the metabolism of health-related compounds in order to fully harness utility in disease prevention or treatment.

Some limitations of this study should be considered in the interpretation of the results and foundation for future work. Women in the parent study were all premenopausal and consumed soya; therefore, these results may not be generalisable to other age groups or men. Other than providing the substrate daidzein, soya consumption is not known to influence the ability to produce ODMA[21,53], and the regular consumption of soya is not likely to influence the overall differences in metabolomics profiles. However, there is the possibility for unmeasured confounding by other dietary factors. Given that there is no evidence for dietary differences between ODMA producers and non-producers in other studies, dietary factors are unlikely to fully explain the differences in metabolomics profiles observed here. A strength and unique feature of this study is the availability of samples collected at multiple time points to evaluate intraindividual differences in metabolomics profiles. This design provides control for confounding of numerous personal characteristics that are consistent over time. However, future studies might consider collecting and controlling for dietary intake. Another limitation of the work is that there was a small number of women and lack generalisability, but these results provide important foundations for future work with larger and more diverse study populations.

Nearly 80 % of women in the parent maintained a consistent ODMA metabotype over 13 months (seven or eight of eight urine samples being producer or non-producer). Understanding factors that drive individuals to change metabotype is another area of future work to fully elucidate the utility of the ODMA metabotype as a target for precision nutrition. These metabolomics analyses observed differences in urinary metabolomics profiles by ODMA metabotypew and these differences tracked with changes in metabotype within individual women. Overall, these observations provide further support for the hypothesis that microbial communities capable of metabolising ODMA may also metabolise other compounds important for health. Larger human studies in a variety of populations are needed to confirm these observations and, in order to identify broader impacts for chronic disease mitigation, future *in vitro* work could be used to evaluate the role of ODMA metabotype in the metabolism of other compounds.

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G. M. and A. A. F. acquired the data and conducted laboratory analyses for isoflavone content for the parent study. C. L. F. designed this study, performed data analysis and drafted the manuscript. G. M. and A. A. F. provided review and edits for the final manuscript. All authors have read and approved the final manuscript.

The authors declare no financial or personal conflicts of interest.

Supplementary material

For supplementary material/s referred to in this article, please visit https://doi.org/10.1017/S0007114521004463

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