Published in final edited form as:

Biopharm Drug Dispos. 2019 May ; 40(5-6): 188–194. doi:10.1002/bdd.2182.

Strain-specific Altered Nicotine Metabolism in 3,3'-diindolylmethane (DIM) Exposed Mice

A. Joseph Bloom, PhD, Pramod Upadhyaya, PhD, and Evan D. Kharasch, MD PhD

Department of Genetics, Washington University, St. Louis, MO (AJB), Masonic Cancer Center, University of Minnesota, Minneapolis, MN (PU), and Department of Anesthesiology, Duke University School of Medicine, Durham, NC (EDK)

Abstract

Two indole compounds, indole-3-carbinol (I3C) and its acid condensation product, 3,3'-diindolylmethane (DIM), have been shown to suppress expression of flavin-containing monooxygenases (FMO) and induce some hepatic cytochrome P450s (CYPs) in rats. In liver microsomes prepared from rats fed I3C or DIM, FMO-mediated nicotine *N*-oxygenation was decreased, whereas CYP-mediated nicotine metabolism to nicotine iminium and subsequently to cotinine was unchanged. Therefore, we hypothesized that in mice DIM would also suppress nicotine *N*-oxygenation without affecting CYP-mediated nicotine metabolism. Liver microsomes were produced from male and female C57BL/6J and CD1 mice fed 2500 parts per million (ppm) DIM for 14 days. In liver microsomes from DIM-fed mice, FMO-mediated nicotine *N*-oxygenation did not differ from controls, but CYP-mediated nicotine metabolism was significantly increased, with results varying by sex and strain. To confirm DIM effects *in vivo*, control and DIM-fed CD1 male mice were subcutaneously injected with nicotine, and plasma concentrations of nicotine, cotinine, and nicotine-*N*-oxide were measured over 30 minutes. DIM-fed mice showed greater cotinine concentrations compared to controls 10 minutes following injection. We conclude that DIM's effects on nicotine metabolism *in vitro* and *in vivo* differ between mice and rats and between mouse strains, and that DIM is an effective inducer of CYP-mediated nicotine metabolism in commonly studied mouse strains.

Keywords

nicotine; metabolism; DIM; IC3; mouse

Introduction

Nicotine is metabolized by three pathways: 1) oxidation to nicotine iminium and subsequently to cotinine by cytochrome P450s (primarily CYP2A6 in humans, Cyp2A5 in mice)(Nakajima et al., 1996), 2) oxidation to nicotine-*N*-oxide by FMO1 and FMO3, and 3) glucuronidation by UDP-glucuronosyltransferases. Published evidence indicates that two

Address Correspondence to: Joseph Bloom, Department of Genetics, Washington University in St. Louis, 4566 Scott Ave, Campus Box 8232, St. Louis, MO 63110-1093, United States, Tel.: +1 314 362-5186, Fax: +1 314 362-8571, ajbloom@wustl.edu.

Conflict of Interest: The authors have no conflicts of interest.

indole compounds, indole-3-carbinol (I3C) and its acid condensation product, 3,3'-diindolymethane (DIM), natural derivatives of cruciferous vegetables, suppress FMO activity in both humans and model animals (Cashman et al., 1999; Katchamart et al., 2000). Subjects administered an I3C rich diet for 3 weeks had reduced FMO3 activity as determined by urine trimethylamine to trimethylamine-N-oxide ratio (Cashman et al., 1999). FMO1 protein expression was also significantly decreased in the livers of rats fed I3C or DIM, and liver microsomes prepared from these animals showed a significant decrease in *N*-oxidation of FMO substrates including nicotine. The inhibition was more pronounced for DIM than for I3C, and appeared to be specific to *N*-oxidation, as nicotine *C*-oxidation was unaffected by I3C or DIM treatment in rats (Katchamart et al., 2000).

Heritable differences in nicotine metabolism influence cigarette consumption in smokers. The *CYP2A6* gene is one of the few loci consistently associated with the number of cigarettes smoked per day (Caporaso et al., 2009; Thorgeirsson et al., 2010; Bloom et al., 2013), and a common reduced-function *FMO3* haplotype is associated with altered liability to nicotine dependence (Teitelbaum et al., 2017). Therefore, safe pharmacological means of altering nicotine metabolism have potential as smoking cessation treatments (Sellers et al., 2000; Bagdas et al., 2014). Although I3C and DIM have not been studied related to smoking cessation, they are promising cancer chemoprotective agents (Reed et al., 2006; Bradlow, 2008; Fujioka et al., 2016). In order to determine if DIM also alters nicotine metabolism in mice—a convenient model for assaying nicotine related phenotypes including consumption, dependence and withdrawal—we directly measured nicotine conversion to nicotine-*N*-oxide, iminium, and cotinine, both in liver microsomes and *in vivo* in DIM-fed mice.

Methods

All protocols were approved by the Washington University Institutional Animal Care and Use Committee and comply with the US National Research Council's Guide for the Care and Use of Laboratory Animals, the US Public Health Service's Policy on Humane Care and Use of Laboratory Animals, and Guide for the Care and Use of Laboratory Animals. 8-week old C57BL/6J mice (Jackson Laboratory, Bar Harbor, ME, USA) and CD1 mice (Charles River, Wilmington, MA, USA) were fed Purina 5058 mouse chow or modified LabDiet 5058 containing 0.25% i.e. 2500 ppm DIM (Purina TestDiet, Richmond, IN, USA) for 2 weeks prior to experiments, based on prior published methods (Katchamart et al., 2000).

Microsome incubations

Microsomes were prepared from flash-frozen tissues. Five mice were used for each sex, strain and condition. Tissues were homogenized in ice-cold potassium phosphate buffer pH 7.4, 6x with a glass-teflon homogenizer, and centrifuged 20 min at 9,000 *g*. The S9 fraction was centrifuged 60 min at 100,000 *g* to obtain a microsomal pellet which was resuspended, further homogenized 6x, centrifuged another 60 min at 100,000 *g* and finally resuspended and homogenized 6x in potassium phosphate buffer containing 20% glycerol for storage at -80°C . Prior to freezing, protein concentrations were determined by the Bradford Protein assay. All incubations were carried out in 96-well PCR plates in 50 μl total volume at 37°C , as previously described (Teitelbaum et al., 2017) 10 minutes in tricine buffer containing 1

mM NADPH (Sigma-Aldrich) and 100 μ M nicotine (Toronto Research Chemicals, Toronto Canada). Reactions were quenched by adding 10 μ l 15% zinc sulfate containing 200ng/ml of the internal standard, (d₃)-nicotine-N-oxide (Toronto Research Chemicals, Toronto Canada). Samples were centrifuged and supernatants removed for LC/MS analysis.

DIM disposition in mice

Blood samples were collected by cardiac puncture in isoflurane anesthetized animals, immediately placed on ice, and centrifuged to separate plasma. Other tissues were collected, flash frozen in liquid nitrogen, and stored at -80°C for further processing. Liver and brain tissues were first homogenized with a glass-teflon homogenizer in ice-cold potassium phosphate buffer pH 7.4. 250 μ l sample was added to 250 μ l PBS plus 40 μ l D2-DIM (200 pmol), vortexed 15 min at room temperature, and extracted twice with 1 ml t-butyl methyl ether. The organic extracts were evaporated to dryness and 40 μ l 70:30 10mM ammonium acetate buffer: Acetonitrile added. Samples were further vortexed and centrifuged (2000G) for 2h at room temperature. The supernatant liquid was transferred to GC-Vial and 2 μ l injected on the LC-MS. DIM concentrations in plasma, liver and brain were determined as previously described with slight modifications (Fujioka et al., 2016). Analyses were done on a TSQ Quantum Discovery Max instrument (Thermo Fisher Scientific) in the positive ion mode with N₂ as the nebulizing and drying gas. MS parameters: spray voltage 3.2kV; sheath gas 25; capillary temperature 250C; collision energy 13 V; scan width 0.01 amu; Q2 gas pressure 1.0 mTorr; source CID 9 V; tube lens 45. Mass spectrometer data were acquired and processed by Xcalibur software version 1.4 (Thermo Scientific). Two μ l of the sample were injected from an auto sampler using an Agilent 1100 capillary LC system (Agilent Technologies) equipped with a 5 μ m, 150 $\times$ 0.5mm Zorbax SB-C18 column (Agilent Technologies). The initial conditions were 40% 10 mmol/L NH₄OAc and 60% methanol, percent methanol was then increased to 75% over 15 min and for 1 minute, then increased to 95% over 1 minute and held for 2 minutes, the system was then returned to initial conditions over 1 minute and to equilibrate for 12 minutes before injection for the next sample. Flow rate was held constant at 10 μ l /min throughout the separation. The mass transitions monitored were m/z 247.14 to m/z 130.10 for DIM (typical retention time 17.92 minutes) and m/z 249.14 to m/z 132.10 for [²H₂]-DIM (typical retention time 17.82 minutes). Quantitation was done by comparing the MS peak area ratio of DIM to that [²H₂]-DIM using a calibration curve prepared by plotting the MS peak area ratio of DIM to [²H₂]-DIM against their concentration ratios using standard mixes containing constant levels of [²H₂]-DIM and varying levels of DIM. The detection limit of the assay was 0.4pmol/ml.

Nicotine disposition in mice

Mice were injected subcutaneously in the back with 0.35 mg/kg nicotine (nicotine hydrogen tartrate, Sigma-Aldrich, St. Louis, MO, USA) in sterile saline, anesthetized with isoflurane and blood was drawn by terminal cardiac puncture after 5, 10, 20 or 30 minutes, immediately placed on ice, and centrifuged to separate plasma. Five mice were used for each time-point. Nicotine and nicotine metabolites were analyzed by liquid chromatography tandem mass spectrometry based on previous methods (Teitelbaum et al., 2017) and quantified using the ratio of metabolite to internal standard. Cotinine, nicotine-N-oxide and nicotine iminium standards were purchased from Toronto Research Chemicals (Toronto,

Canada). LC-MS/MS analyses were performed on an API 4000 Q-TRAP triple quadrupole mass spectrometer (Applied Biosystems Sciex, Foster City, CA) equipped with an electrospray source. The HPLC system consisted of two LC 20AC pumps with a CTO-20A column oven, SIL-20A autosampler, DGU-20A3 degasser, FCF-11AL valve, and a CBM 20A controller (Shimadzu, Columbia, MD, USA). The chromatographic separation was performed on an xBridge column (150 × 2.1, 3.5µm, Waters, Milford, MA, USA) with a pre-column inline 0.2 µm filter. The injection volume was 5 µl and the oven temperature was 40 °C. Mobile phase (0.3 ml/min) was (A) 4.5 mM ammonium acetate pH 4.0 and (B) 4.5 mM ammonium acetate in acetonitrile using the following program: 1% B for 4 minutes, linear gradient to 25% B between 4 and 5 minutes, held at 25% B until 6.0 minutes, and then re-equilibrated to initial conditions between 6.5 and 8.5 minutes. The instrument was operated in positive-ion mode at 450 °C with an ion spray voltage of 5500 V, entrance potential of 10 V and exit potential of 22 V. The curtain gas was set at 20, ion source gas 1 at 30, and ion source gas 2 at 40. Transitions monitored for nicotine were m/z 163→130, for nicotine iminium m/z 161→130, for cotinine, m/z 177→98, for nicotine-*N*-oxide m/z 179→132, and for deuterated (d3)-nicotine-*N*-oxide, m/z 182→132.

Data analysis

Differences between groups were tested by analysis of variance (ANOVA). Further reported p-values are from post-hoc two-tailed t-tests. All analyses were performed using the statistical software R (R Core Team).

Results

DIM detection in mouse plasma, liver, and brain

The presence of DIM was first confirmed in plasma, liver and brain tissue from male and female C57BL/6J and CD1 mice fed chow containing 2,500 ppm DIM for two weeks. DIM was detected in all samples measured, with concentrations highest in liver (average 1910; range 1262–3210 pmol/ml), followed by plasma (average 292; range 219–468 pmol/ml) and brain (average 70; range 38–98 pmol/ml). DIM was also detected in tissues from control mice fed regular chow. Relative to the average DIM-fed mouse, <1% as much DIM was detected in control plasma (2.6 pmol/ml), <0.01% DIM in control liver (0.06 pmol/ml) and <3% DIM in control brain (1.0 pmol/ml).

Nicotine metabolism in DIM-fed mouse liver microsomes

To determine the effects of DIM on hepatic nicotine metabolism in mice, liver microsomes were prepared from male and female C57BL/6J and CD1 mice fed standard rodent chow or chow containing 2,500 ppm DIM for two weeks. Two nicotine metabolites were then measured in incubations of microsomes with nicotine substrate: nicotine-*N*-oxide, the product of FMO-mediated *N*-oxidation, and nicotine iminium ion, the primary initial product of CYP-mediated *C*-oxidation. Nicotine *N*-oxidation was not significantly lower in DIM-fed mice of any sex or strain (Figure 1). However, nicotine *C*-oxidation was significantly greater in microsomes from DIM-fed mice (ANOVA $p < 5 \times 10^{-6}$, Figure 2). Consistent with prior reports that Cyp2a5 expression is female-predominant (Kirby et al.,

2011), we also found significantly higher ($p < 0.005$) nicotine C-oxidation in female-derived liver microsomes from both strains.

In vivo nicotine metabolism in DIM-fed mice

To determine if the effects of DIM on nicotine metabolism observed in microsomes corresponded with similar effects *in vivo*, we measured plasma concentrations of nicotine metabolites in CD1 male mice subcutaneously injected with nicotine. Male CD1 mice were chosen because of 19-fold difference in nicotine-C-oxidation activity we observed in liver microsomes from DIM-fed CD1 male mice relative to the more modest differences observed in the other three sex/strain groups (2.4–3.5 fold increases). Cotinine was measured because nicotine iminium ion is rapidly converted to cotinine *in vivo*. Consistent with our microsome incubation results, plasma cotinine concentrations were significantly greater in DIM-fed animals following nicotine injection (ANOVA $p < 0.005$, Figure 3). The mean area under the curve (AUC) of cotinine for DIM-fed mice was significantly greater than for control mice (2424 ± 347 vs. 1671 ± 366 ng*min/ml, $p < 0.02$). Nicotine plasma concentrations at 10 minutes were significantly reduced in DIM-fed mice compared to controls ($p < 0.01$, Figure 4), though the mean AUCs of nicotine for DIM-fed and control mice were not statistically significantly different (2791 ± 408 vs. 3268 ± 460 ng*min/ml). The calculated half-life of nicotine was not significantly greater in DIM-fed mice than control mice (8.0 ± 2.0 vs. 8.2 ± 1.0 minutes). The discrepancy between the changes in AUC between cotinine and nicotine may be explained by altered elimination of either molecule related to DIM exposure, as comparisons of mouse strains have shown the elimination half-lives of nicotine and its metabolite to be independent of one another (Siu and Tyndale, 2007). Consistent with prior reports (Petersen et al., 1984) nicotine-N-oxide plasma concentrations were lower than cotinine concentrations, but we did not see a statistically significant difference between DIM-fed and control animals (55 ± 16 vs. 85 ± 27 ng*min/ml).

Discussion

Cruciferous vegetables such as cabbage, broccoli, and other leafy vegetables are abundant in glucosinolates, a family of organic compounds believed to mediate the observed epidemiological associations between vegetable consumption and reduced cancer risk (Verhoeven et al., 1996; Herr and Buchler, 2010; Liu and Lv, 2013; Wu et al., 2013a; Wu et al., 2013b). One especially abundant glucosinolate, glucobrassicin, is converted through mastication to I3C, and subsequently undergoes acid condensation to DIM in the stomach. Prior studies demonstrated that humans fed an I3C rich diet showed reduced FMO3 activity (Cashman et al., 1999) and dietary exposure to I3C or DIM in rats induced multiple CYP isoforms including CYP1A1, 1A2, 2B $\frac{1}{2}$, and 3A, while simultaneously reducing FMO1 protein content and FMO-mediated catalytic activity (Bjeldanes et al., 1991; Wortelboer et al., 1992a; Wortelboer et al., 1992b; Stresser et al., 1994; Larsen-Su and Williams, 1996; Katchamart et al., 2000). Interestingly, nicotine C-oxidation was not increased in I3C or DIM-exposed rats (Katchamart et al., 2000), presumably because there was no induction of CYP2A3, the rat ortholog of mouse Cyp2a5 and human CYP2A6. However, due to the concurrent reduction of FMO-activity, nicotine N-oxidation in rats fed 2,500 ppm I3C or 1,000 ppm DIM was reduced to below the limits of detection.

We became interested in I3C and DIM as a means to pharmacologically alter the balance of nicotine metabolism by selectively reducing FMO activity. Heritable differences in nicotine metabolism influence cigarette consumption among dependent smokers. CYP2A6, the enzyme responsible for the majority of nicotine metabolism (70–80%) in most people, is encoded by a highly polymorphic gene, and the mechanism of its genetic influence is straightforward: smokers with reduced CYP2A6 function metabolize nicotine more slowly, delaying the onset of the withdrawal symptoms that compel nicotine addicts to reach for the next cigarette. Differences in CYP2A6 activity do not primarily influence an individual's liability to nicotine dependence, but rather the frequency of smoking once the subject has become addicted (Bloom et al., 2012). However, in a recently published study, we demonstrated that a common human *FMO3* haplotype that leads to a two-fold reduction in *in vivo* nicotine *N*-oxidation is also associated with altered liability to nicotine dependence (Teitelbaum et al., 2017). In a large sample European Americans seeking treatment for smoking cessation (the U. Wisconsin Transdisciplinary Tobacco Use Research Center smoking cessation trials (Piper et al., 2007; McCarthy et al., 2008; Piper et al., 2009)), a significant association was found between *FMO3* genotype and scores reported for the Fagerström Test of Nicotine Dependence (FTND), the most widely used instrument for measuring nicotine dependence. Among the questionnaire items from the FTND, 'time to first cigarette' and 'cigarettes per day' are the two strongest predictors of smoking cessation (Baker et al., 2007; Mercincavage et al., 2013; Rohsenow et al., 2013). Importantly, the association between *FMO3* genotype and FTND score was driven entirely by time to first cigarette, and unlike *CYP2A6*, *FMO3* genotype was not significantly associated with overall cigarette consumption among dependent smokers.

Given their numerous advantages, mice are a powerful model system for examining mechanisms and factors that influence nicotine consumption, dependence and withdrawal (Hall et al., 2012; Stoker and Markou, 2013; Jackson et al., 2015). Mice are also a preferred model species for nicotine metabolism studies because they have significantly higher *in vivo* CYP2A activity than rats, and FMO makes a larger contribution to nicotine metabolism in mice than in humans or other species (Raunio et al., 2008). But, contrary to our expectations based on results from rats, we did not see a selective reduction of FMO-mediated nicotine metabolism in DIM-exposed mice, male or female from two commonly-studied strains, but rather an increase of nicotine *C*-oxidation across all mice and varying by strain. Therefore, we concluded that although DIM or I3C may provide a safe, effective means to reduce FMO activity in humans, they are not a selective means to alter such activity in mice. However, manipulating nicotine *C*-oxidation is also being pursued for its smoking cessation potential (Sellers et al., 2000; Bagdas et al., 2014), and we demonstrate here a pharmacological means to increase nicotine *C*-oxidation that has yet to be exploited in mice. Significant to the use of DIM as a reagent in mouse, we also saw large strain- and sex-specific differences in the effect of DIM on nicotine *C*-oxidation. The mechanisms underlying these differences are not addressed by this study, but could include genetic differences in the regulatory regions of the *Cyp2a5* gene or in the transcription factors that mediate DIM's influence on *Cyp2a5* expression. *Cyp2a5* expression and activity have been shown to vary between mouse strains (Poca et al., 2017). Such genetically determined differences in enzyme expression may also explain the differences in FMO activity we detect between strains. Sex differences in FMO

expression were also previously reported (Falls et al., 1995). Alternatively, differences in DIM's effect on nicotine C-oxidation may reflect variation in DIM exposure due to strain and sex differences in DIM absorption or in total feed consumption. These questions will need to be considered in future studies, and point to the potential usefulness of the mouse model for interrogating DIM's regulation of CYP gene expression.

Acknowledgments:

This work was funded by grant K01DA034035 from NIDA.

References

- Bagdas D, Muldoon PP, Zhu AZ, Tyndale RF, and Damaj MI (2014) Effects of methoxsalen, a Cyp2a5/6 inhibitor, on nicotine dependence behaviors in mice. *Neuropharmacology* 85:67–72. [PubMed: 24859605]
- Baker TB, Piper ME, McCarthy DE, Bolt DM, Smith SS, Kim SY, Colby S, Conti D, Giovino GA, Hatsukami D, Hyland A, Krishnan-Sarin S, Niaura R, Perkins KA, and Toll BA (2007) Time to first cigarette in the morning as an index of ability to quit smoking: implications for nicotine dependence. *Nicotine Tob Res* 9 Suppl 4:S555–570. [PubMed: 18067032]
- Bjeldanes LF, Kim JY, Grose KR, Bartholomew JC, and Bradfield CA (1991) Aromatic hydrocarbon responsiveness-receptor agonists generated from indole-3-carbinol in vitro and in vivo: comparisons with 2,3,7,8-tetrachlorodibenzo-p-dioxin. *Proc Natl Acad Sci U S A* 88:9543–9547. [PubMed: 1658785]
- Bloom AJ, Baker TB, Chen LS, Breslau N, Hatsukami D, Bierut LJ, and Goate A (2013) Variants in two adjacent genes, EGLN2 and CYP2A6, influence smoking behavior related to disease risk via different mechanisms. *Hum Mol Genet*.
- Bloom AJ, Harari O, Martinez M, Madden PA, Martin NG, Montgomery GW, Rice JP, Murphy SE, Bierut LJ, and Goate A (2012) Use of a predictive model derived from in vivo endophenotype measurements to demonstrate associations with a complex locus, CYP2A6. *Hum Mol Genet* 21:3050–3062. [PubMed: 22451501]
- Bradlow HL (2008) Review. Indole-3-carbinol as a chemoprotective agent in breast and prostate cancer. *In Vivo* 22:441–445. [PubMed: 18712169]
- Caporaso N, Gu F, Chatterjee N, Sheng-Chih J, Yu K, Yeager M, Chen C, Jacobs K, Wheeler W, Landi MT, Ziegler RG, Hunter DJ, Chanock S, Hankinson S, Kraft P, and Bergen AW (2009) Genome-wide and candidate gene association study of cigarette smoking behaviors. *PloS One* 4:e4653. [PubMed: 19247474]
- Cashman JR, Xiong Y, Lin J, Verhagen H, van Poppel G, van Bladeren PJ, Larsen-Su S, and Williams DE (1999) In vitro and in vivo inhibition of human flavin-containing monooxygenase form 3 (FMO3) in the presence of dietary indoles. *Biochem Pharmacol* 58:1047–1055. [PubMed: 10509757]
- Falls JG, Blake BL, Cao Y, Levi PE, and Hodgson E (1995) Gender differences in hepatic expression of flavin-containing monooxygenase isoforms (FMO1, FMO3, and FMO5) in mice. *J Biochem Toxicol* 10:171–177. [PubMed: 7473608]
- Fujioka N, Ransom BW, Carmella SG, Upadhyaya P, Lindgren BR, Roper-Batker A, Hatsukami DK, Fritz VA, Rohwer C, and Hecht SS (2016) Harnessing the Power of Cruciferous Vegetables: Developing a Biomarker for Brassica Vegetable Consumption Using Urinary 3,3'-Diindolylmethane. *Cancer prevention research* 9:788–793. [PubMed: 27538743]
- Hall FS, Markou A, Levin ED, and Uhl GR (2012) Mouse models for studying genetic influences on factors determining smoking cessation success in humans. *Ann N Y Acad Sci* 1248:39–70. [PubMed: 22304675]
- Herr I and Buchler MW (2010) Dietary constituents of broccoli and other cruciferous vegetables: implications for prevention and therapy of cancer. *Cancer Treat Rev* 36:377–383. [PubMed: 20172656]

- Jackson KJ, Muldoon PP, De Biasi M, and Damaj MI (2015) New mechanisms and perspectives in nicotine withdrawal. *Neuropharmacology* 96:223–234. [PubMed: 25433149]
- Katchamart S, Stresser DM, Dehal SS, Kupfer D, and Williams DE (2000) Concurrent flavin-containing monooxygenase down-regulation and cytochrome P-450 induction by dietary indoles in rat: implications for drug-drug interaction. *Drug Metab Dispos* 28:930–936. [PubMed: 10901703]
- Kirby GM, Nichols KD, and Antenos M (2011) Cyp2a5 induction and hepatocellular stress: an adaptive response to perturbations of heme homeostasis. *Curr Drug Metab* 12:186–197. [PubMed: 21395539]
- Larsen-Su S and Williams DE (1996) Dietary indole-3-carbinol inhibits FMO activity and the expression of flavin-containing monooxygenase form 1 in rat liver and intestine. *Drug Metab Dispos* 24:927–931. [PubMed: 8886600]
- Liu X and Lv K (2013) Cruciferous vegetables intake is inversely associated with risk of breast cancer: a meta-analysis. *Breast* 22:309–313. [PubMed: 22877795]
- McCarthy DE, Piasecki TM, Lawrence DL, Jorenby DE, Shiffman S, Fiore MC, and Baker TB (2008) A randomized controlled clinical trial of bupropion SR and individual smoking cessation counseling. *Nicotine Tob Res* 10:717–729. [PubMed: 18418793]
- Mercincavage M, Branstetter SA, Muscat JE, and Horn KA (2013) Time to first cigarette predicts cessation outcomes in adolescent smokers. *Nicotine Tob Res* 15:1996–2004. [PubMed: 23811009]
- Nakajima M, Yamamoto T, Nunoya K, Yokoi T, Nagashima K, Inoue K, Funae Y, Shimada N, Kamataki T, and Kuroiwa Y (1996) Role of human cytochrome P4502A6 in C-oxidation of nicotine. *Drug Metab Dispos* 24:1212–1217. [PubMed: 8937855]
- Petersen DR, Norris KJ, Thompson JA (1984) A comparative study of the disposition of nicotine and its metabolites in three inbred strains of mice. *Drug Metab Dispos* 12:725–31. [PubMed: 6150822]
- Piper ME, Federman EB, McCarthy DE, Bolt DM, Smith SS, Fiore MC, and Baker TB (2007) Efficacy of bupropion alone and in combination with nicotine gum. *Nicotine Tob Res* 9:947–954. [PubMed: 17763111]
- Piper ME, Smith SS, Schlam TR, Fiore MC, Jorenby DE, Fraser D, and Baker TB (2009) A randomized placebo-controlled clinical trial of 5 smoking cessation pharmacotherapies. *Arch Gen Psychiatry* 66:1253–1262. [PubMed: 19884613]
- Poca KS, Parente TE, Chagas LF, Leal BS, Leal HS, Paumgarten FJ, and De-Oliveira AC (2017) Interstrain differences in the expression and activity of Cyp2A5 in the mouse liver. *BMC research notes* 10:125. [PubMed: 28298240]
- Raunio H, Pokela N, Puhakainen K, Rahnasto M, Mauriala T, Auriola S, and Juvonen RO (2008) Nicotine metabolism and urinary elimination in mouse: in vitro and in vivo. *Xenobiotica* 38:34–47. [PubMed: 18098062]
- Reed GA, Arneson DW, Putnam WC, Smith HJ, Gray JC, Sullivan DK, Mayo MS, Crowell JA, and Hurwitz A (2006) Single-dose and multiple-dose administration of indole-3-carbinol to women: pharmacokinetics based on 3,3'-diindolylmethane. *Cancer Epidemiol Biomarkers Prev* 15:2477–2481. [PubMed: 17164373]
- Rohsenow DJ, Martin RA, Tidey JW, Monti PM, and Colby SM (2013) Comparison of the cigarette dependence scale with four other measures of nicotine involvement: correlations with smoking history and smoking treatment outcome in smokers with substance use disorders. *Addict Behav* 38:2409–2413. [PubMed: 23644751]
- Sellers EM, Kaplan HL, and Tyndale RF (2000) Inhibition of cytochrome P450 2A6 increases nicotine's oral bioavailability and decreases smoking. *Clin Pharmacol Ther* 68:35–43. [PubMed: 10945314]
- Siu EC and Tyndale RF (2007) Characterization and comparison of nicotine and cotinine metabolism in vitro and in vivo in DBA/2 and C57BL/6 mice. *Mol Pharmacol* 71:826–34. [PubMed: 17158199]
- Stoker AK and Markou A (2013) Unraveling the neurobiology of nicotine dependence using genetically engineered mice. *Curr Opin Neurobiol* 23:493–499. [PubMed: 23545467]
- Stresser DM, Bailey GS, and Williams DE (1994) Indole-3-carbinol and beta-naphthoflavone induction of aflatoxin B1 metabolism and cytochromes P-450 associated with bioactivation and detoxication of aflatoxin B1 in the rat. *Drug Metab Dispos* 22:383–391. [PubMed: 8070314]

- Teitelbaum AM, Murphy SE, Akk G, Baker TB, Germann A, von Weymarn LB, Bierut LJ, Goate A, Kharasch ED, and Bloom AJ (2017) Nicotine dependence is associated with functional variation in FMO3, an enzyme that metabolizes nicotine in the brain. *Pharmacogenomics J.*
- Thorgeirsson TE, Gudbjartsson DF, Surakka I, Vink JM, Amin N, Geller F, Sulem P, Rafnar T, Esko T, Walter S, Gieger C, Rawal R, Mangino M, Prokopenko I, Magi R, Keskitalo K, Gudjonsdottir IH, Gretarsdottir S, Stefansson H, Thompson JR, Aulchenko YS, Nelis M, Aben KK, den Heijer M, Dirksen A, Ashraf H, Soranzo N, Valdes AM, Steves C, Uitterlinden AG, Hofman A, Tonjes A, Kovacs P, Hottenga JJ, Willemsen G, Vogelzangs N, Doring A, Dahmen N, Nitz B, Pergadia ML, Saez B, De Diego V, Lezcano V, Garcia-Prats MD, Ripatti S, Perola M, Kettunen J, Hartikainen AL, Pouta A, Laitinen J, Isohanni M, Huei-Yi S, Allen M, Krestyaninova M, Hall AS, Jones GT, van Rij AM, Mueller T, Dieplinger B, Haltmayer M, Jonsson S, Matthiasson SE, Oskarsson H, Tyrfingsson T, Kiemeny LA, Mayordomo JI, Lindholt JS, Pedersen JH, Franklin WA, Wolf H, Montgomery GW, Heath AC, Martin NG, Madden PA, Giegling I, Rujescu D, Jarvelin MR, Salomaa V, Stumvoll M, Spector TD, Wichmann HE, Metspalu A, Samani NJ, Penninx BW, Oostra BA, Boomsma DI, Tiemeier H, van Duijn CM, Kaprio J, Gulcher JR, McCarthy MI, Peltonen L, Thorsteinsdottir U, and Stefansson K (2010) Sequence variants at CHRNA3-CHRNA6 and CYP2A6 affect smoking behavior. *Nat Genet* 42:448–453. [PubMed: 20418888]
- Verhoeven DT, Goldbohm RA, van Poppel G, Verhagen H, and van den Brandt PA (1996) Epidemiological studies on brassica vegetables and cancer risk. *Cancer Epidemiol Biomarkers Prev* 5:733–748. [PubMed: 8877066]
- Wortelboer HM, de Kruif CA, van Iersel AA, Falke HE, Noordhoek J, and Blaauboer BJ (1992a) Acid reaction products of indole-3-carbinol and their effects on cytochrome P450 and phase II enzymes in rat and monkey hepatocytes. *Biochem Pharmacol* 43:1439–1447. [PubMed: 1567468]
- Wortelboer HM, van der Linden EC, de Kruif CA, Noordhoek J, Blaauboer BJ, van Bladeren PJ, and Falke HE (1992b) Effects of indole-3-carbinol on biotransformation enzymes in the rat: in vivo changes in liver and small intestinal mucosa in comparison with primary hepatocyte cultures. *Food Chem Toxicol* 30:589–599. [PubMed: 1521833]
- Wu QJ, Yang Y, Vogtmann E, Wang J, Han LH, Li HL, and Xiang YB (2013a) Cruciferous vegetables intake and the risk of colorectal cancer: a meta-analysis of observational studies. *Ann Oncol* 24:1079–1087. [PubMed: 23211939]
- Wu QJ, Yang Y, Wang J, Han LH, and Xiang YB (2013b) Cruciferous vegetable consumption and gastric cancer risk: a meta-analysis of epidemiological studies. *Cancer science* 104:1067–1073. [PubMed: 23679348]

Figure 1.

Nicotine-*N*-oxide produced by liver microsomes from DIM-fed and control mice. Each column represents microsomes from five mice. The boxplots provide summaries of the data distributions for each group. A box represents the interquartile range, which includes 50% of values. The line across the box indicates the median. The whisker lines extend to the highest and lowest values that are within 1.5x the interquartile range. Further outliers are marked with circles.

Figure 2.

Nicotine iminium produced by liver microsomes from DIM-fed and control mice. Each column represents microsomes from five mice. The boxplots provide summaries of the data distributions for each group. A box represents the interquartile range, which includes 50% of values. The line across the box indicates the median. The whisker lines extend to the highest and lowest values that are within 1.5x the interquartile range. Further outliers are marked with circles. * $p < 0.01$, ** $p < 0.0001$.

Figure 3. Plasma cotinine concentrations in DIM-fed and control CD1 male mice subcutaneously injected with 0.35 mg/kg nicotine. Each data point represents the mean $\pm$ standard deviation of 5 mice. * $p < 0.03$.

Figure 4. Plasma nicotine concentrations in DIM-fed and control CD1 male mice subcutaneously injected with 0.35 mg/kg nicotine. Each data point represents the mean $\pm$ standard deviation of 5 mice. * $p < 0.01$.