

PRECISION ANALYTICALINC.

OBJECTIVE

3,3'-diindolylmethane (DIM) is a non-FDA-regulated supplement that is the derivative and primary *in vivo* product of indole-3-carbinol (I3C), a dietary phytoestrogen found in cruciferous vegetables. DIM has been studied in multiple clinical trials as a potential cancer prevention agent with mixed results. Because DIM is thought to offer protection against breast cancer, some postmenopausal women choose to use it, and, in some cases, some providers recommend it to their patients. Many of these patients may be concurrently receiving menopausal hormone therapy (MHT), and since DIM's mechanism of action involves alteration of estrogen metabolism, it is possible that a drug-drug interaction exists. The objective of this study was to examine the effect of DIM on the estrogen profiles of postmenopausal women receiving MHT treatment in the form of a transdermal estradiol (E2) patch.

DESIGN

This was a retrospective observational cohort study for which data were collected from a database containing 144,561 laboratory accessions from 129,883 patients processed between January 1, 2016 and December 9, 2019 (Clinical Trials ID: NCT04305093). Laboratory measurements included urinary E2 and 9 other urinary estrogen metabolites. From this database, 1458 results were available for postmenopausal women using a transdermal E2 patch, of which 108 indicated that they were concurrently taking a DIM/I3C supplement. For comparison, results were also available for premenopausal women using DIM (n = 909), premenopausal women on no therapy, and postmenopausal women on no therapy. Wilcoxon rank sum tests, Kruskal-Wallis tests, and paired t-tests were used to assess differences between groups.

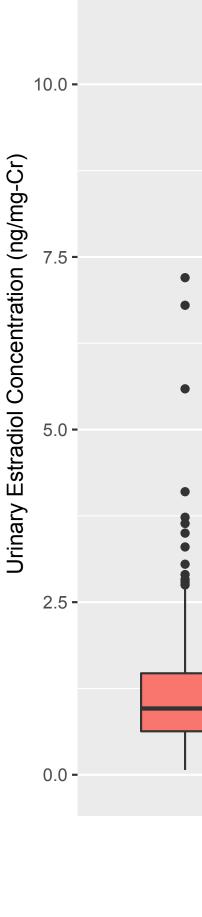
RESULTS

When compared to postmenopausal women using a transdermal E2 patch but not concurrently taking DIM, women using an E2 patch plus DIM had statistically significant alterations in their urinary estrogen profiles. These alterations were similar to those observed in the profiles of premenopausal women using DIM. Multivariate regression analyses to account for age, body mass index (BMI), E2 patch dose, and urinary creatinine confirmed the observed differences in estrone (p=0.004), estriol (p<0.0001), the 2-hydroxymetabolites (2-OHE1: p=0.004; 2-OHE2: p=0.004), 4-hydroxyestradiol (p=0.01), 16-hydroxyestrone (p=0.02); however, E2 (p=0.19) and 4-hydroxyestrone (p=0.1) no longer reached significance for the effect of DIM. Although E2 was no longer significant in the multivariate analysis, the point estimate (beta=0.042) was similar to the findings in premenopausal women with a 9.2% decrease in urinary E2 concentrations at each increased dose of the E2 patch.

The Impact of 3,3'-Diindolylmethane on the Estrogen Profile of Postmenopausal Women Being Treated with a Transdermal Estradiol Patch Mark Newman, MS1*; Doreen Saltiel MD, JD1; Bryan P. Mayfield, PharmD1,2; Frank Z. Stanczyk, PhD, MS3 Precision Analytical. Inc. McMinnville OR: 2 Texas Tech University Health Sciences Center Lerry H. Hodge School of Pharmacy Dallas TX: 3 University of Southern ¹Precision Analytical, Inc, McMinnville, OR; ²Texas Tech University Health Sciences Center Jerry H Hodge School of Pharmacy, Dallas, TX; ³University of Southern

Variabl

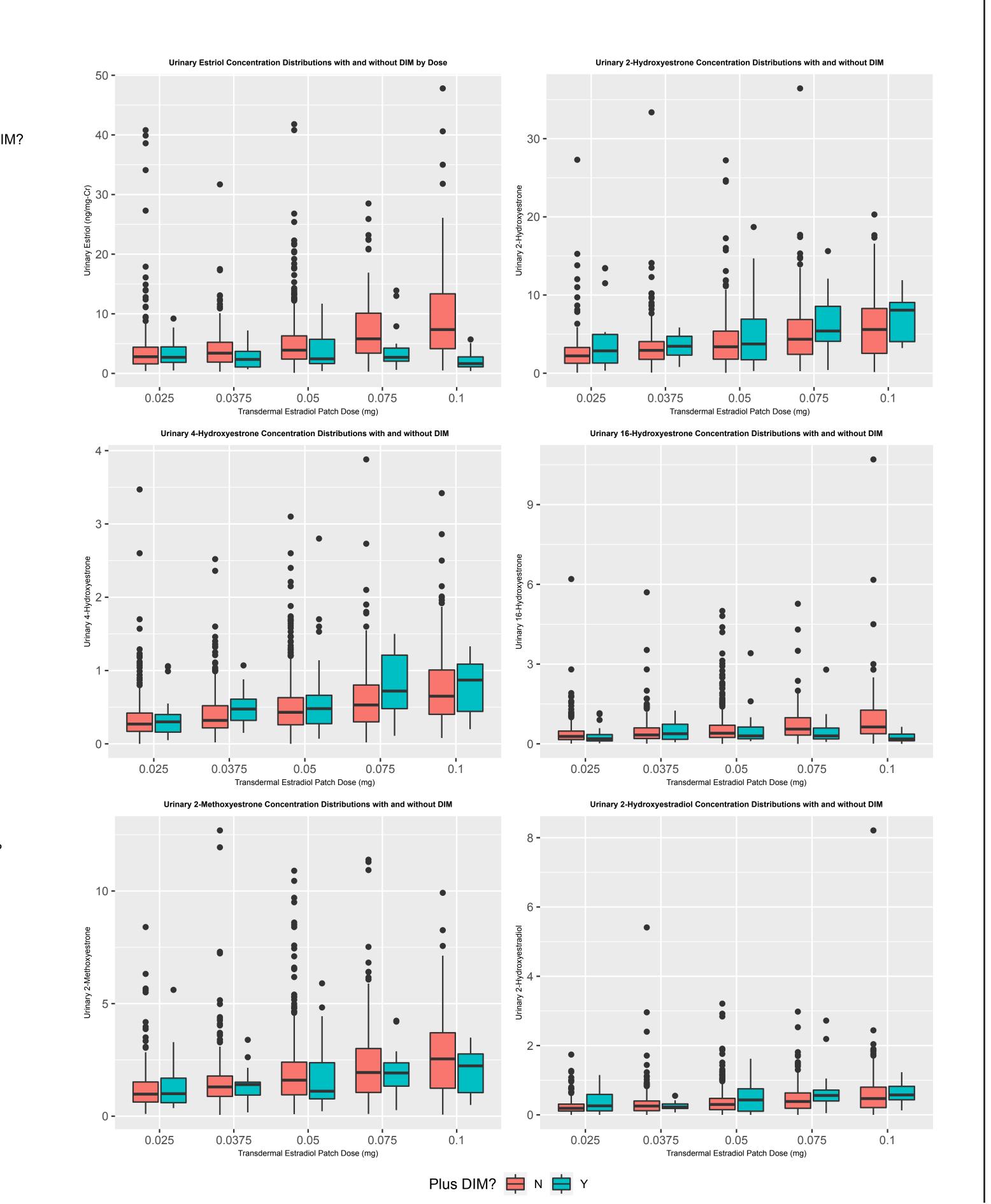
Age at (BMI Estradio 0.025 0.0375 0.05 0.075 Mediar



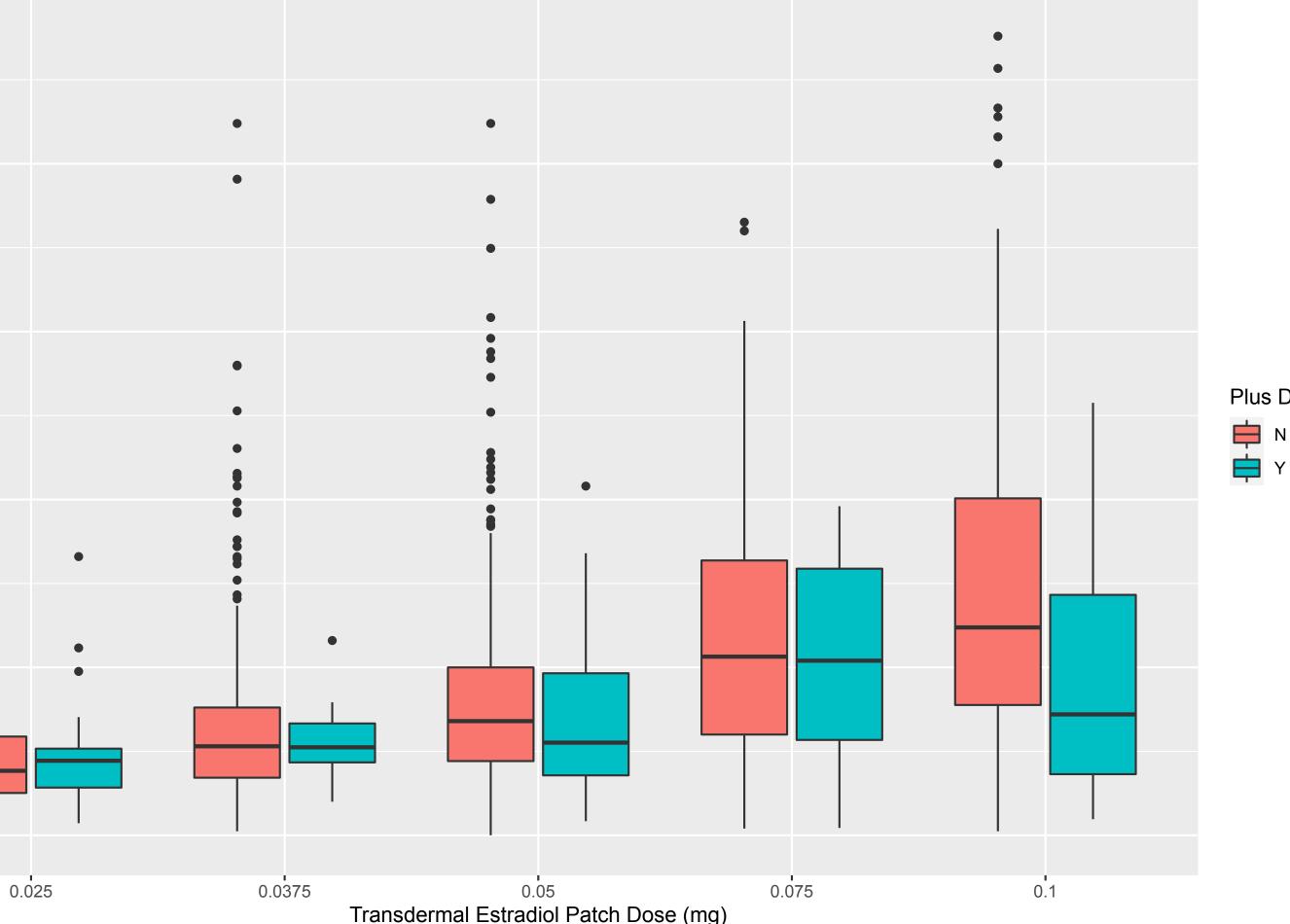
Comparing E2 Patch Only and E2 Patch Plus DIM

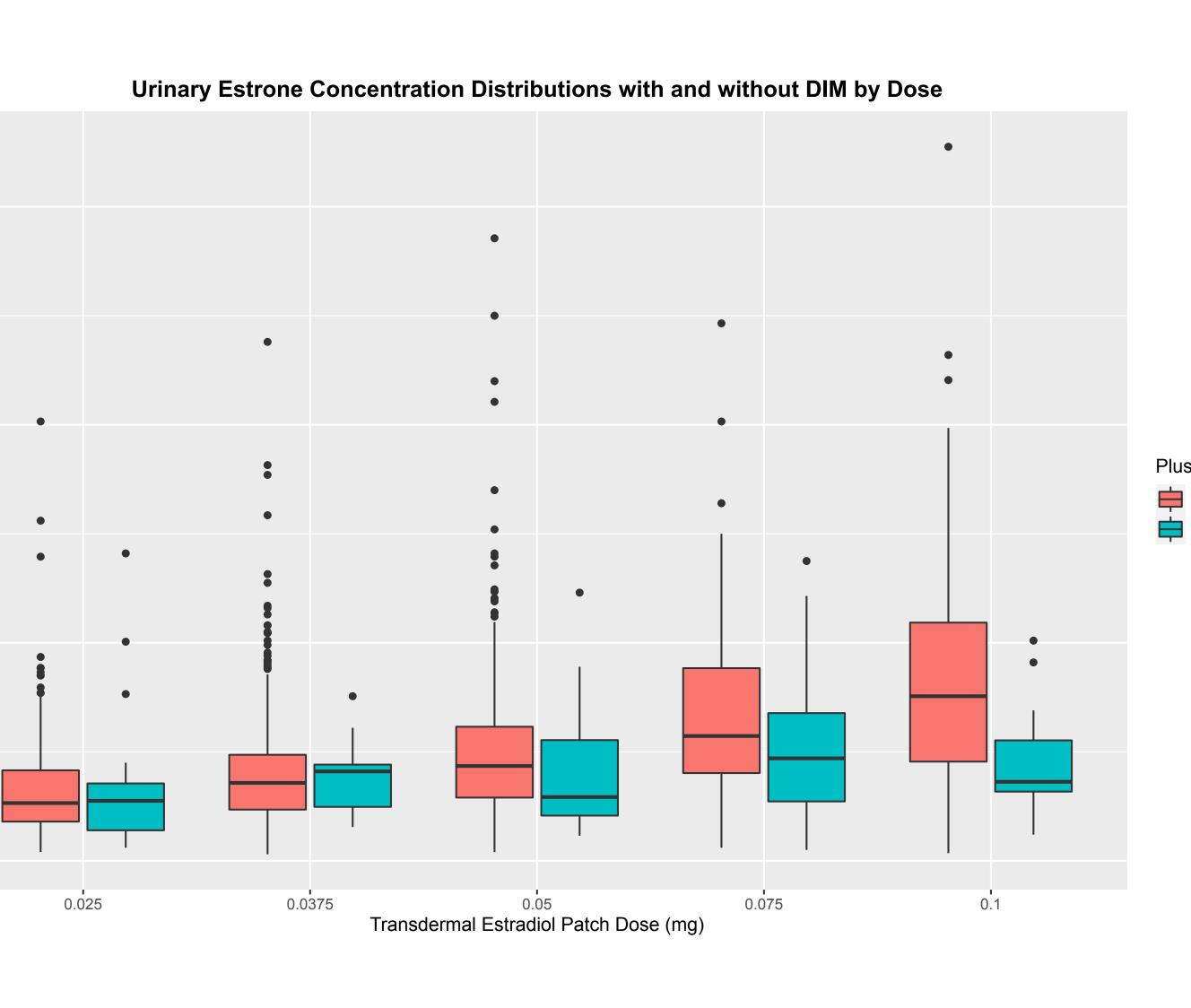
Patient Characteristics					
	Therapy Received				
le	Patch Only , N = 1,350 ⁷	Patch + DIM , N = 108 ⁷			
Collection	57 (53, 61)	58 (54, 64)			
	23.8 (21.5, 26.3)	22.8 (20.6, 25.9)			
iol Patch Dose (mg))				
5	261 (19%)	23 (21%)			
75	300 (22%)	16 (15%)			
	475 (35%)	36 (33%)			
	164 (12%)	19 (18%)			
	150 (11%)	14 (13%)			
n (IQR); n (%)					

Urinary Estrogen Metabolite Concentrations with and without DIM					
	Therapy Received				
Urinary Estrogen Metabolite (ng/mg-Cr)	Patch Only , N = 1,350 ¹	Patch + DIM , N = 108 ⁷			
Estrone	8.30 (5.10, 12.60)	6.40 (4.20, 9.90)			
Estradiol	1.58 (0.91, 2.60)	1.32 (0.87, 2.44)			
Estriol	3.80 (2.20, 6.50)	2.60 (1.48, 4.45)			
2-hydroxyestrone	3.16 (1.73, 5.10)	3.92 (2.31, 7.00)			
4-hydroxyestrone	0.40 (0.23, 0.64)	0.48 (0.29, 0.85)			
16-hydroxyestrone	0.40 (0.21, 0.70)	0.28 (0.16, 0.58)			
2-methoxyestrone	1.45 (0.89, 2.30)	1.39 (0.80, 2.29)			
2-hydroxyestradiol	0.28 (0.14, 0.48)	0.40 (0.17, 0.68)			
4-hydroxyestradiol	0.10 (0.10, 0.20)	0.10 (0.10, 0.20)			
Missing Values	386	28			
2-methoxyestradiol	0.30 (0.20, 0.50)	0.30 (0.10, 0.40)			
Missing Values	382	26			
Total Estrogens	20.90 (14.00, 31.30)	17.95 (13.50, 27.40)			
¹ Median (IQR)					



Urinary Estradiol Concentration Distributions with and without DIM by Dose



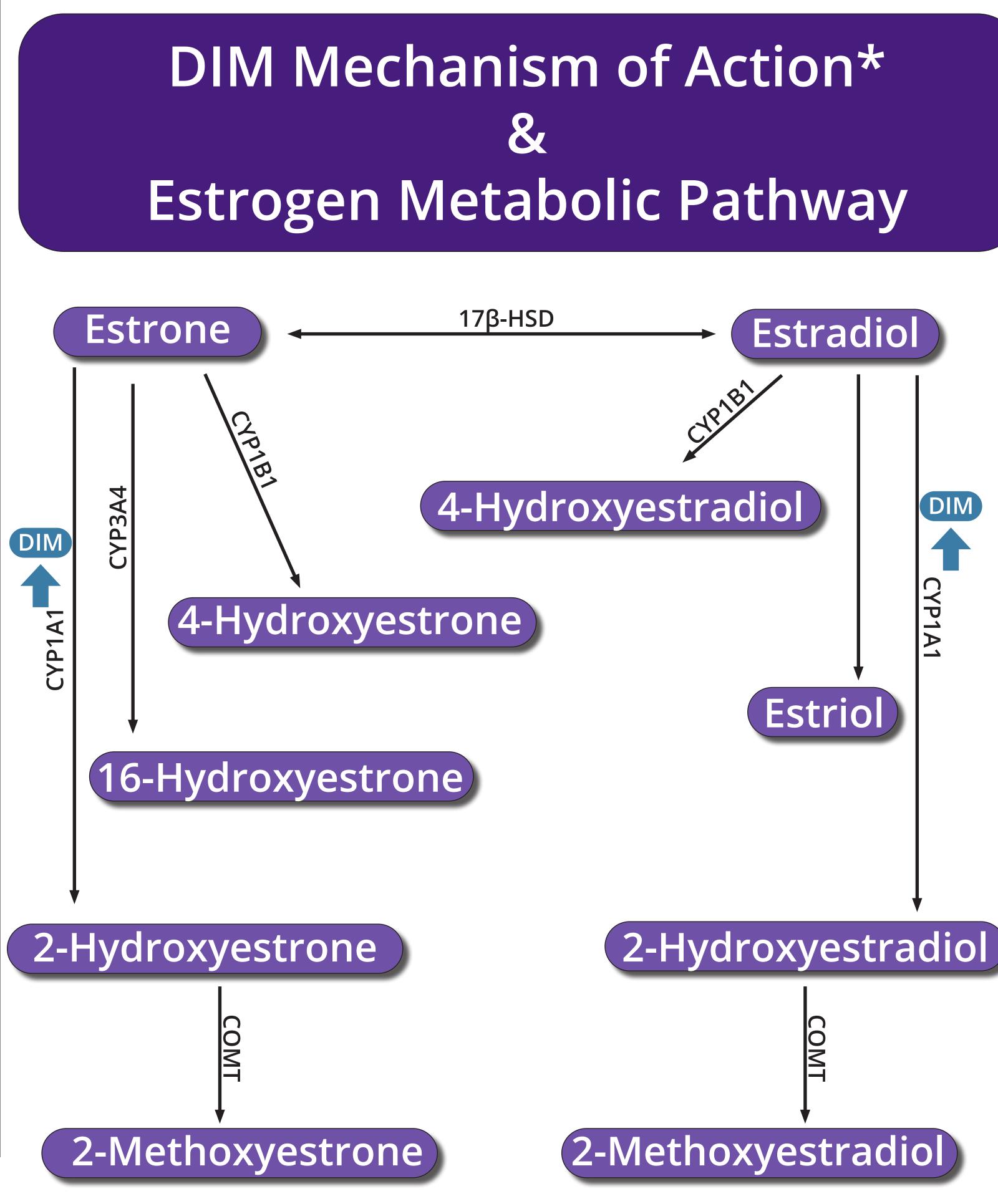


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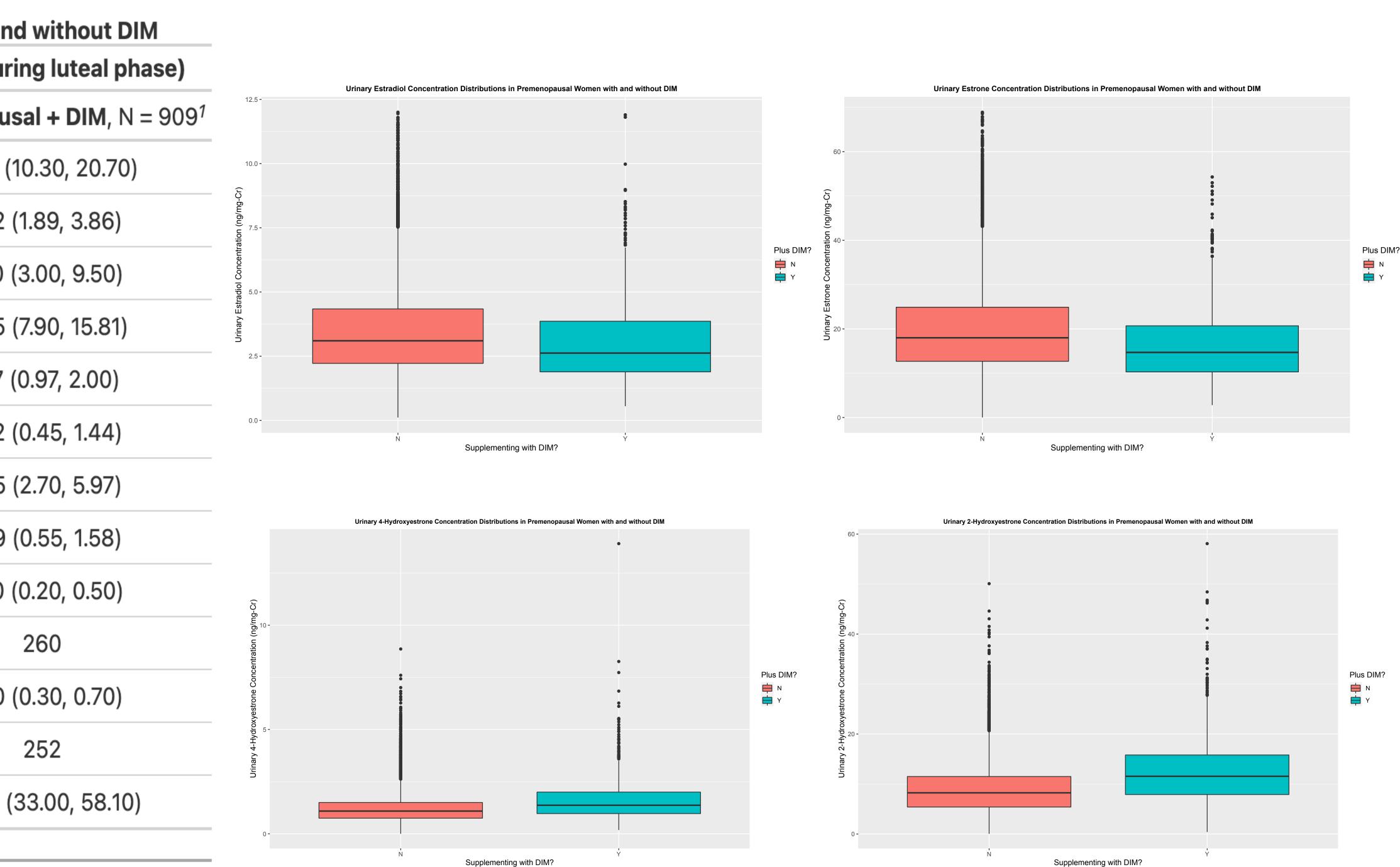
How does this compare to DIM use in Premenopausal Women?

Urinary Estrogen Metabolite Concentrations in premenopausal women with and					
	Supplementation Status (measured during				
Irinary Estrogen Metabolite (ng/mg-Cr)	Premenopausal , N = 18,385 ¹	Premenopaus			
strone	18.00 (12.70, 24.90)	14.70 (10			

Estradiol	3.10 (2.22, 4.34)	2.62 (1
Estriol	9.60 (5.90, 15.10)	5.20 (3
2-hydroxyestrone	8.24 (5.40, 11.50)	11.55 (7
4-hydroxyestrone	1.09 (0.75, 1.50)	1.37 (0
16-hydroxyestrone	1.21 (0.73, 1.99)	0.82 (0
2-methoxyestrone	4.20 (2.79, 5.91)	4.05 (2
2-hydroxyestradiol	0.66 (0.36, 1.09)	0.99 (0
4-hydroxyestradiol	0.30 (0.20, 0.40)	0.40 (0
Missing Values	5,560	2
2-methoxyestradiol	0.50 (0.30, 0.70)	0.40 (0
Missing Values	5,438	2
Total Estrogens	48.50 (36.50, 63.30)	44.20 (33
¹ Median (IQR)		



*This is DIM's MOA as it relates to the current study. DIM has many proposed mechanisms of action involving modulation of the aryl hydrocarbon receptor that are beyond the score of this work. As it relates to the modulation of sex hormones, DIM's effect on estrogen activity is thought to be primarily the result of altered CYP enzyme metabolism by DIM.



CONCLUSION

These results are a substantial addition to the literature as there are not many published studies investigating the effects of DIM on MHT, especially with large sample sizes. Postmenopausal women on transdermal E2 patch therapy (and potentially other MHT formulations) who choose to concurrently use a DIM/I3C supplement may have clinically significant changes in their urinary estrogen profiles, potentially as a result of altered estrogen metabolism. Simultaneous decreases in both E2 and 16-hydroxyestrone could combine to decrease the overall estrogenic mpact of therapy on key clinical endpoints such as bone mineral density and symptom improvement. The presence and magnitude of these changes suggests that providers treating postmenopausal women with MHT should ask their patients if they are taking a DIM/I3C supplement and, if so, potentially consider MHT dose adjustment or other therapy changes.

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