

A Review of the Clinical Efficacy and Safety of Cruciferous Vegetable Phytochemicals

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Supplementation with the crucifer-derived phytochemicals indole-3-carbinol (I3C) and 3,3'-diindolylmethane (DIM) has been an area of active interest due to their role in estrogen metabolism. This review addresses the debate about which cruciferous compound to use clinically by evaluating their efficacy and safety. Significantly more clinical trials are available for I3C than for DIM. I3C leads to beneficial shifts in hormone markers, and limited evidence suggests that DIM may result in a similar effect. More research in humans is needed to further address whether DIM poses any safety risk. Current data do not suggest that DIM provides enhanced clinical benefits over I3C.

Key words: cruciferous vegetables, indole-3-carbinol, 3,3'-diindolylmethane, 2-hydroxyestrone, 16- α -hydroxyestrone

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WHAT IS INDOLE-3-CARBINOL?

Epidemiological and prospective clinical studies have shown that populations that consume higher amounts of cruciferous vegetables have lower incidences of cancer and improved biochemical indices (e.g., decreased oxidative stress).¹⁻³ In fact, the extent of evidence supporting the benefits of fruit and vegetable intake is rather convincing. In 1993, the US Food and Drug Administration (FDA) permitted the use of two health claims acknowledging the relationship between increased fruit and vegetable consumption and decreased cancer risk (an example of an allowable label statement: “Low fat diets rich in fruits and vegetables may reduce the risk of some types of cancer, a disease associated

with many factors.”). The health-promoting characteristics of many fruits and vegetables have been attributed to a number of nutritive components, including fiber, vitamins, and minerals. However, the substantial health benefits of cruciferous vegetables such as broccoli, brussels sprouts, cabbage, and cauliflower are believed to be imparted by a class of sulfur-containing compounds called glucosinolates, more specifically, indole-3-carbinol (I3C) (Figure 1),⁴⁻⁷ a phytochemical formed by the hydrolysis of the glucosinolate glucobrassicin and catalyzed by the enzyme myrosinase. Myrosinase and glucobrassicin, which are normally compartmentalized separately in intact plant cells, are brought together during the processes of cutting, cooking, freezing, or pressurizing crucifers, thus facilitating the formation of I3C.⁸

INDOLE-3-CARBINOL LEADS TO THE FORMATION OF MANY METABOLITES, INCLUDING 3,3'-DIINDOLYLMETHANE

Once consumed, I3C enters the acidic environment of the stomach, where it rapidly undergoes a condensation reaction leading to the formation of an estimated 15 or more other oligomeric compounds.⁹ Each of these compounds has not been thoroughly investigated for its bioactivity. However, the three main products of the reaction appear to have distinctive biological action.^{10,11} For example, hexahydrocyclohepta triindole binds to estrogen receptors and displays chemical structure similarities to tamoxifen, indolo[3,2-b]carbazole exhibits antiestrogenic activity and supports phase I detoxification activities, and 3,3'-diindolylmethane (DIM) (Figure 2) has been shown to be anti-proliferative in various cancer cell and animal models.¹²⁻¹⁷

To date, DIM has been researched more extensively than other I3C metabolites. Indeed, some researchers have proposed that I3C may simply serve as a precursor compound for DIM, which, unlike I3C, is present in the blood of subjects supplemented with I3C.¹⁸ Approximately 10% to 20% of I3C is metabolized to DIM.¹⁹⁻²¹ This number may fluctuate due to the possible variability of conversion of I3C to DIM.

The clinically studied dose of supplemental I3C is

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CLINICAL TRIAL COMPARISON: INDOLE-3-CARBINOL VS. 3,3'-DIINDOLYLMETHANE

Most of the clinical trials conducted to date have used I3C rather than DIM. A search using the US National Library of Medicine database without specific search limits reveals a total of 452 references for “indole-3-carbinol” and 82 for “3,3'-diindolylmethane.”²⁴ These references were subject to further scrutiny on an individual basis to identify clinical research publications. The results of the refined search are in the sections below.

I3C supplementation has been investigated in 13 published human studies (Table 1) during the past 15 years, totaling 333 study subjects (an estimated 293 subjects on I3C).²⁵⁻³⁷ Since 1991, I3C has been studied for its use in a number of conditions, including patients with human papillomavirus-induced diseases such as cervical cancer²⁶ and respiratory papillomatosis,²⁹⁻³¹ as well as in those subjects with increased risk for breast cancer,^{25,36} vulvar intraepithelial neoplasia,²⁷ and systemic lupus erythematosus.³² Favorable shifts in estrogen metabolism were observed in studies using these metabolites as clinical end points. It has been documented that I3C supplements have been taken for as long as 82 months (6.8 years) without reported side effects due to I3C, thereby supporting long-term safety.

In contrast to the number of clinical trials with I3C, there has been only one published human clinical trial using DIM. In this study, 10 postmenopausal women with a history of early-stage breast cancer took a DIM supplement (108 mg) for 30 days.³⁸ DIM supplementation led to a beneficial shift (although not statistically significant) in the metabolism of estrogen.

EFFECT OF INDOLE-3-CARBINOL AND 3,3'-DIINDOLYLMETHANE ON ESTROGEN METABOLISM BIOMARKERS IN HEALTHY VOLUNTEERS

In most of the clinical trials with I3C and DIM, one or more of the estrogen metabolites 2-hydroxyestrone (2-OHE₁), 4-hydroxyestrone (4-OHE₁), and 16- α -hydroxyestrone (16-OHE₁) was measured. In hepatocytes, estrogen converts primarily to either 2-OHE₁ or 16- α -OHE₁, with some formation of 4-OHE₁ also occurring.³⁹ It has been established that 2-OHE₁ has anti-estrogenic and anti-proliferative properties, while both 4-OHE₁ and 16- α -OHE₁ result in increased cell proliferation in tumor cell lines.^{40,41} Some (although not all) studies have observed a correlation between a low urinary 2-OHE₁ to 16- α -OHE₁ ratio and increased breast cancer risk⁴²⁻⁴⁵; therefore, this ratio is proposed as a measure of a person's risk for estrogen-dependent cancers. One of the key metabolites of estradiol, 4-OHE₁, is formed in estrogen-sensitive tissues such as mammary,

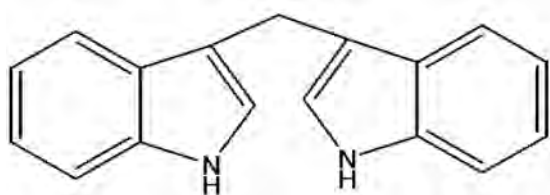
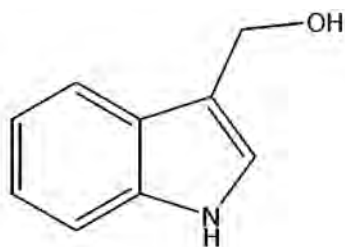


Figure 1. Structure of indole-3-carbinol.

often three to four times that of DIM. Since I3C is the precursor, or “parent,” to a variety of compounds that have differential actions, it is logical that a greater amount of I3C may be required for maximum biological activity relative to provision of just one of the I3C metabolites for a select action. There is some indication that these compounds may work in concert. Stresser et al.²² fed I3C to rats and quantified tissue levels of I3C metabolites. The findings revealed that individual levels of metabolites, including indolo[3,2-b]carbazole and DIM, were not alone sufficient for inducing hepatic enzymes such as cytochrome P450IA or for inhibiting microsomal activation of aflatoxin B1. The authors concluded that “the total hepatic mixture of I3C derivatives may be sufficient to provide both modulatory responses in the rat.”²²

As mentioned previously, each of the metabolites appears to have a targeted physiological effect. These individualized functions may coincide with the differential tissue compartmentalization of I3C metabolites. Anderton et al.²³ demonstrated that I3C appeared rather quickly in the plasma of female mice after administration, and other metabolites, including DIM, took longer to reach peak concentrations in plasma (2 hours) but persisted in tissues longer than I3C. Indolo[3,2-b]carbazole was not detected in plasma, lung, heart, kidney, or brain, whereas DIM and another I3C metabolite, [2-(indol-3-ylmethyl)-indol-3-yl]indol-3-ylmethane, were found in all of these tissues.

Arguably, providing I3C in supplemental form more closely approximates the whole food substance (i.e., cruciferous vegetables) than does reducing I3C to its individual condensation products. After all, it is difficult to say whether isolated I3C metabolites, compared with whole food or I3C, may function differently or even detrimentally if administered separately from the complex matrix from which they originate.

Table 1. Published Clinical Research on Indole-3-Carbinol (I3C) and 3,3'-Diindolylmethane (DIM) Listed Chronologically

Reference	Trial Design	Study Population	Study Period and Dose	Results Summary	Comments on Safety from Study Investigator
Naik et al., 2006 ²⁷	Prospective, open-label, randomized	12 women with vulvar intraepithelial neoplasia	200 or 400 mg daily for 6 mo	<ul style="list-style-type: none"> Significant improvement in symptoms, including reduced lesion size and severity Significant increase in 2-OHE₁/16-α-OHE₁ Tissue biopsy from worst affected vulvar areas revealed no improvement in grade of vulvar intraepithelial neoplasia at 6 mo 	One subject (on 400 mg) complained of "mild bowel upset".
Reed et al., 2005 ³³	Prospective, open-label, placebo run-in	17 women (1 postmenopausal and 16 premenopausal)	400 mg daily for 4 wks, followed by 800 mg daily for 4 wks	<ul style="list-style-type: none"> Maximal increase in urinary 2-OHE₁/16-α-OHE₁ found with 400 mg 	Authors stated that both doses were "well-tolerated" and that there were "no serious adverse events or trends related to ingestion of I3C." Only one adverse event (an episode of bronchospasm) led to discontinuation of the study product. This subject was on 800 mg I3C and did not disclose her prior history of asthma upon being admitted to the study (she had denied it at the time of enrollment).
De Bilderling et al., 2005 ³¹	Case report	8-year-old girl with respiratory papillomatosis	200 mg used daily as adjuvant therapy with intravenous Cidofovir for a total of 27 mo	Complete disappearance of lung lesions	Tolerance/safety findings not reported.
Rosen and Bryson, 2004 ²⁹	Prospective, open-label	33 patients with respiratory papillomatosis	Enrolled respiratory papillomatosis patients from 9/96 to 8/01; treated with 200 mg twice daily; continued follow-up over years (mean = 4.8 y)	<ul style="list-style-type: none"> 11 (33%) experienced remission of respiratory papillomatosis 10 (30%) had a decrease in papillomatous growth, resulting in less surgery 12 (36%) experienced no benefit No subjects experienced worsening of symptoms 	"No immediate or long-term side effects related to I3C were found, regardless of patients' responses to I3C as a treatment for respiratory papillomatosis (RRP)."
McAlindon et al., 2001 ²²	Prospective, open-label	17 women with systemic lupus erythematosus	1-wk study with 375 mg (n = 17), followed by a 3-mo observational period of taking I3C (n = 12)	<ul style="list-style-type: none"> Increase in 2-OHE₁/16-α-OHE₁ Mean SLEDAI (measure of disease activity) scores not significantly different at the end of 3 mo 	Authors concluded that "Our study provides preliminary evidence that I3C is well-tolerated in women with systemic lupus erythematosus with the exception that we observed the development of a skin rash in one participant."

Table 1 (Part 2). Published Clinical Research on Indole-3-Carbinol (I3C) and 3,3'-Diindolylmethane (DIM) Listed Chronologically					
Reference	Trial Design	Study Population	Study Period and Dose	Results Summary	Comments on Safety from Study Investigator
I3C (continued)					
Bell et al., 2000 ²⁶	Prospective, randomized, placebo-controlled, double-blinded	27 patients with biopsy-proven high-grade cervical intraepithelial neoplasia	Placebo (n = 10), 200 mg (n = 8), or 400 mg (n = 9) daily for 12 wks	<ul style="list-style-type: none"> No subjects on placebo had complete regression of symptoms 4 of 8 subjects on 200 mg/d and 4 of 9 subjects on 400 mg/d had complete regression based on 12-wk biopsy 	Multiple organ systems (blood, kidney, liver) were monitored throughout the study and no toxicity was observed. Authors concluded that "...indole-3-carbinol was well tolerated with no adverse events noted."
Taioli et al., 1999 ²⁸	Prospective, open-label	57 healthy women	400 mg/d for 5 days	Increase in 2-OHE ₁ /16- α -OHE ₁	Tolerance/safety findings not reported.
Rosen et al., 1998 ³⁰	Prospective, open-label	18 adults and children with respiratory papillomatosis	Minimum supplementation of 8 mo and mean follow-up of 14.6 mo; children's dose was determined by body weight; adults took 200 mg twice daily	<ul style="list-style-type: none"> 33% of subjects had a cessation of papilloma growth 33% of subjects had a decreased papilloma growth rate Beneficial effects on urinary 2-hydroxylation 	Authors state that "...indole-3-carbinol appears to be safe and well tolerated..." and "No major complications were noted in any of the study patients. Dysequilibrium symptoms developed in 3 patients when they took increased amounts of I3C. An adult male had imbalance and a slight tremor while taking oral I3C 400 mg twice daily for 10 days. His symptoms completely resolved when he returned to oral I3C 200 mg twice daily. A 2.5-year-old girl accidentally took triple her dose and experienced unsteadiness for a day, which entirely resolved. When a 12-year-old girl ingested 3 doses within a 12-hour period, unsteadiness with nausea developed and resolved in 8 hours."
Michnowicz, 1998 ³⁴	Prospective, open-label	5 healthy, overweight, premenopausal women (35-47 y)	400 mg daily for 2 mo	Increase in 2-hydroxylation of estrogen	Tolerance/safety findings not reported.
Michnowicz et al., 1997 ³⁵	Prospective, open label	Study I: 7 healthy men; study II: 10 healthy women	Study I: 500 mg daily for 1 wk; study II: 400 mg daily for 2 mo	Increase in urinary 2-OHE ₁ and decrease in urinary 16- α -OHE ₁ in both groups	Tolerance/safety findings not reported.
Wong et al., 1997 ²⁵	Prospective, placebo-controlled, double-blind	57 women at increased risk for breast cancer	Placebo (n = 10) or I3C 50 mg (n = 7); 100 mg (n = 10); 200 mg (n = 10); 300 mg (n = 10); 400 mg (n = 10) for 4 wks	<ul style="list-style-type: none"> 300-400 mg led to an increase in urinary 2-OHE₁/16-α-OHE₁ 300 mg considered minimum effective and safe dose for chemoprevention 	"Except for two participants who had unexplained small increases in the liver enzyme SGPT level (43 to 65, and 30 to 71), no other toxicity effects were encountered."

Table 1 (Part 3). Published Clinical Research on Indole-3-Carbinol (I3C and 3,3'-Diindolylmethane (DIM) Listed Chronologically

Reference	Trial Design	Study Population	Study Period and Dose	Results Summary	Comments on Safety from Study Investigator
I3C (continued)					
Bradlow et al., 1994 ²⁶	Prospective, placebo-controlled	60 women at increased risk for cancer	Placebo (n = 20), 20 g α-cellulose (n = 20), or 400 mg I3C (n = 20) daily for 3 mo	Increase in 2-OHE ₁ /16-α-OHE ₁ observed in I3C group over 3 mo	According to the authors, "...I3C can serve to increase the 2-OH-estrogen-estriol metabolite ratio in a sustained manner without detectable side effects...." "Careful questioning of the patients in the I3C arm revealed no significant differences other than a slight increase in gastrointestinal motility and a decrease in complaints of constipation in a few subjects." "There were no adverse effects in any subject at this dose."
Michnovicz and Bradlow, 1991 ²⁷	Prospective, open label	7 men and 5 women, healthy with no history of recent or chronic illness	5–7 mg/kg body weight (350–500 mg/d) for 7 days	Increase in 2-OHE ₁ in men and women	
DIM					
Delessandri et al., 2004 ²⁸	Prospective, placebo-controlled	19 postmenopausal women with a history of early-stage breast cancer (55–69 y)	108 mg absorbable DIM (BioResponse-DIM [®]) for 30 days (n = 10), placebo (n = 9)	DIM-treated subjects, relative to placebo, experienced: <ul style="list-style-type: none"> Increase in levels of 2-OHE₁, DIM, and cortisol Nonsignificant increase in 2-OHE₁/16α-OHE₁ 	Author lists specific adverse reactions: 1) "One DIM-treated subject developed a rash and arthralgias. The rash resolved with discontinuation of the supplements and taking antihistamines. The arthralgias improved with time"; 2) "Two DIM-treated subjects noted an increase in hot flashes, but this did not stop them from taking the capsules"; 3) "One DIM-treated subject noted some nausea when taking the capsules without food." Finally, "All other subjects took both the placebo and DIM capsules without significant side effects."
2-OHE ₁ , 2-hydroxyestrogen; 16-α-OHE ₁ , 16-α-hydroxyestrogen.					

	I3C	DIM
Source	Cruciferous vegetables	I3C
Number of references listed in the National Library of Medicine	452	82
Number of clinical trials	13	1
Longest duration that study subject was followed on supplement	82 months	30 days
Total number of subjects in clinical trials	333 (293 on I3C)	19 (10 on DIM)
Clinical end points	Symptoms related to human papillomavirus-induced diseases (respiratory papillomatosis lesions, cervical lesions) and SLE, estrogen metabolites (i.e., specifically urinary 2-OHE ₁ /16- α -OHE ₁)	Estrogen metabolites, cortisol
Number of adverse events possibly related (evaluated by study investigator) to supplement use as reported in clinical trials	10/293 subjects (3%)	3/10 subjects (30%)

2-OHE₁, 2-hydroxyestrone; 16- α -OHE₁, 16-alpha-hydroxyestrone.

ovary, and uterus. Through reductive-oxidative cycling to the semiquinone and quinone forms, 4-OHE₁ generates potentially mutagenic free radicals that may play a role in breast and endometrial carcinogenesis.⁴⁶ Additionally, these quinone intermediates may lead to further reaction with purine bases of DNA to form depurinating adducts that produce highly toxic apurinic sites.⁴⁷ Therefore, physiological levels of 4-OHE₁ have been given significant clinical attention.

The effect of I3C supplementation on estrogen metabolites has been measured in at least five clinical trials with a total of 108 healthy subjects.^{28,33-35,37} As will be discussed, these metabolites served as end points in many of the cancer trials with I3C. For I3C, the overall findings of all five published studies in relatively healthy individuals (in one study, subjects were overweight yet defined as “healthy”) have been consistently positive, indicating that I3C is able to increase levels of 2-OHE₁ and decrease levels of 16- α -OHE₁, thereby resulting in a more favorable 2-OHE₁ to 16- α -OHE₁ ratio. This result is not surprising considering that several *in vitro* studies have confirmed that I3C influences the activity of the cytochrome P450 isoenzyme CYP1A2.^{9,48-51}

The often-cited clinical trial conducted by Michnovicz et al.³⁵ has garnered much attention and perhaps even misinterpretation in the dietary supplementation arena. This study has been the impetus for comments in the lay press that oral I3C supplementation may lead to the production of “dangerous” estrogens. This study involved two groups of healthy research subjects comprising 7 men and 10 women. In the first part of the study, a group of men received I3C orally at a daily dose of 500 mg for 7 consecutive days. Urine samples collected before ingestion of I3C and after the last daily

dose of I3C were analyzed in a “blinded fashion.” In the second part of the study, women took 400 mg of I3C orally and had urine samples collected between day 3 and day 8 of the follicular phase of their menstrual cycle, before I3C ingestion and 2 months after I3C ingestion. These authors reported a wider range of estrogen metabolites than found in similar studies of this nature. The overall findings, as stated by the authors, were: “In both men and women, I3C significantly increased the urinary excretion of C-2 estrogens [i.e., 2-OHE₁, 2-OHE₂]. The urinary concentrations of nearly all other estrogen metabolites, including levels of estradiol, estrone, estriol, and 16 α -hydroxyestrone, were lower after I3C treatment.”

As part of their section called “Implications,” it was stated that “I3C may have chemopreventive activity against breast cancer in humans. . . .” Despite these positive findings, the clinical application of I3C has been questioned by a few researchers, since it has been suggested that levels of the “dangerous” estrogen metabolite 4-OHE₁ were increased in these subjects. Though the data suggest that there was a rise in 4-OHE₁ from 0.156 \pm 0.079 to 0.211 \pm 0.147 in men and from 0.339 \pm 0.226 to 0.430 \pm 0.260 in women, these modest changes in both groups were not statistically significant ($P = 0.25$ and $P = 0.16$, respectively).

EFFECT OF INDOLE-3-CARBINOL ON ESTROGEN METABOLISM BIOMARKERS IN SUBJECTS WITH RISK FACTORS OR DISEASES

The majority of clinical work on I3C is in the area of human papillomavirus-induced disease (including cervi-

cal cancer and respiratory papillomatosis), with four published studies consisting of 79 total subjects. In these studies, 200 to 400 mg of oral I3C was administered daily for 12 weeks (shortest) to 82 months (6.8 years) (longest). In these studies, 30% to 100% of subjects experienced remission or regression of their symptoms. Two studies explored the use of oral I3C in women with increased risk factors for breast cancer^{25,36}: approximately 67 women were studied for 4 weeks to 3 months with an oral I3C dose of 50 to 400 mg. Similar to the studies on human papillomavirus-induced diseases, the results were positive, with a beneficial shift in the ratio of estrogen metabolites. Recently, Naik et al.²⁷ published the results of oral I3C at either 200 or 400 mg/d in 12 women with vulvar intraepithelial neoplasia. I3C supplementation led to a significant reduction in symptoms as well as significant improvement in estrogen metabolites. Finally, a positive effect of I3C for systemic lupus erythematosus on hormone metabolism, specifically an improvement in the 2-OHE₁ to 16- α -OHE₁ ratio, was reported by McAlindon et al.³² in 17 subjects on a daily dose of 375 mg I3C for 3 months. The symptoms assessment (SLEDAI) did not change for these subjects.

In the single clinical trial on DIM supplementation, 10 postmenopausal women with a history of early-stage breast cancer ingested 108 mg of DIM (BioResponse-DIM[®]) daily for 30 days (Table 1).³⁸ The authors reported that DIM treatment showed a statistically significant increase in 2-OHE₁ relative to placebo ($P = 0.02$), and, in contrast to the studies on I3C, a non-significant increase in the clinically relevant 2-OHE₁ to 16- α -OHE₁ ratio ($P = 0.059$). In order to examine the influence of DIM on CYP3A activity, cortisol and 6 β -hydroxycortisol levels were measured (cortisol converts to 6 β -hydroxycortisol by CYP3A). The authors reported a significant increase in cortisol, suggesting that DIM stimulates the adrenal gland. The implications of this effect are unknown and require further exploration. Reed et al.³³ investigated cortisol levels in women supplemented with I3C as a measure of CYP3A activity. Although individual levels of cortisol were not reported, there was no statistically significant change in the ratio of 6 β -hydroxycortisol to cortisol.

SAFETY OF INDOLE-3-CARBINOL AND 3,3'-DIINDOLYLMETHANE SUPPLEMENTATION

Clinical data were reviewed for reported adverse reactions to I3C or DIM supplementation with special attention to study investigator's comments on whether these adverse reactions were possibly due to the supplement. Four out of 13 studies on I3C (31%) did not make any reference to safety or tolerance to the dose of I3C.^{28,31,34,35} In 3 out of 13 (23%) studies, either no

adverse effects were noted or I3C was well tolerated.^{26,29,37} Adverse reactions were reported in 6 of the 13 studies (46%), including: skin rash in one subject with systemic lupus erythematosus³²; small increases in the liver enzyme SGPT in two female subjects with increased risk for breast cancer²⁵; a slight increase in gastrointestinal motility and a decrease in complaints of constipation in a few female subjects (unspecified number in article, but counted as two subjects in the percentage tally of adverse reactions)³⁶; "mild bowel upset" in a patient with vulvar intraepithelial neoplasia²⁷; disequilibrium in three subjects with respiratory papillomatosis due to deliberate or accidental increased dosages of I3C³⁰; and an episode of bronchospasm in a woman with a previous history of asthma (subject denied at time of enrollment).³³ Therefore, of the total number of subjects in all studies on some dose of I3C ($N = 293$), about 10 (3%) experienced some degree of a reaction that was most likely due to I3C supplementation within a period of days to years, as reported by the study authors.

In the single DIM study, 4 subjects of the original 23 recruited discontinued participation due to various reasons.³⁸ One of the 4 participants was taking DIM and developed a rash and arthralgias. The subject decided to stop the supplement and the authors noted that "the rash resolved with discontinuation of the supplements and taking antihistamines. The arthralgias improved with time."

Of the 10 DIM-treated subjects who completed the study, two noted an increase in hot flashes, but they did not stop the capsules as a result, and one noted some nausea when taking the capsules without food. Therefore, a total of 3 out of 10 (30%) DIM-treated individuals had some reaction possibly related to the DIM supplement. Due to the limited number of published clinical studies and small subject number, it is not clear if supplementation solely with DIM results in greater side effects or not.

CONCLUSIONS

The available clinical data support the efficacy of I3C as an oral supplement for human papillomavirus-induced diseases such as cervical cancer and respiratory papillomatosis, individuals at increased risk for breast cancer, patients with vulvar intraepithelial neoplasia, and patients with systemic lupus erythematosus. In clinical studies, supplementation with I3C increases the 2-OHE₁ to 16- α -OHE₁ ratio, a marker associated with reduced risk of breast cancer. In the single clinical trial available for DIM, DIM supplementation led to a favorable (although not statistically significant) shift in the 2-OHE₁ to 16- α -OHE₁ ratio. DIM's effects on cortisol need further investigation.

A review of the safety data indicates that there is more clinical data on I3C than on DIM. Therefore, more study subjects have taken I3C compared with DIM. At least 13 clinical studies on I3C have been published, of which a total of approximately 300 participants took oral supplements of I3C. The longest duration that a study subject took I3C was 82 months (6.8 years). Only 3% of research subjects experienced adverse events possibly related to I3C. On the other hand, there is only one published clinical trial on DIM, in which 10 postmenopausal women took DIM for 30 days. In contrast to I3C, 30% of clinical trial subjects receiving DIM reported adverse events that may have been due to the DIM supplement. Further studies with DIM will be able to elucidate whether the increased number of adverse events are due to the small sample size of this study and overall limited clinical trial experience.

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