

Position Paper on I3C and DIM
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Deanna M. Minich, Ph.D., F.A.C.N.

Indole-3-carbinol (I3C) is a well-studied plant compound derived from glucosinolates, which are found in cruciferous vegetables, and is considered to be a primary ingredient responsible for the health benefits these foods provide. Researchers have indicated that I3C shows great promise as a cancer preventative and hormone-balancing agent.

Due to the extensive scientific research that has been generated on its safety and efficacy, Metagenics has chosen to provide I3C in supplement form. Metagenics takes the position that by marketing I3C in its natural form, we are consistent with a functional medicine approach to wellness as opposed to the drug model with the position of “one isolated compound for one specific activity.” By supplementing with the natural form of I3C, one has access to a wide variety of beneficial, active compounds that are formed as the result of I3C ingestion.

Recently, there have been some negative statements circulating about I3C by companies claiming to have a superior product. The product is known as 3,3'-Diindolymethane (DIM), which is a polycyclic aromatic condensation product formed from the interaction between I3C and stomach acid. Companies are selling DIM in a purified form and some claim that it is more absorbable, since it includes the addition of the synthetic material d-alpha-tocopheryl polyethylene glycol-1000 succinate. DIM is just one of the many products formed from I3C, and it appears to have some activity as shown in cell and animal studies. However, unlike I3C, there are no published human studies on the efficacy or safety of DIM. This is one of the primary reasons Metagenics recommends not to substitute DIM for I3C. Another reason is that supplementing with DIM alone would result in missing out on the other beneficial compounds formed by I3C after ingestion.

We at Metagenics would like to present the facts to you about I3C and DIM, which will allow you to make the most educated choice for yourself and your patients.

What is I3C?

I3C is a well-studied, plant-derived compound formed from glucosinolates, which are found in cruciferous (*Brassica*) vegetables such as broccoli, Brussels sprouts, cabbage, and cauliflower. In intact vegetable cells, the endogenous plant enzyme myrosinase is present in compartments separated from its substrate, the glucosinolates. If the cells are disrupted due to food processing (e.g., cutting, cooking, freezing, pressurizing) or chewing, I3C can be formed due to the enzymatic hydrolysis of glucosinolates by myrosinase. The stability of glucosinolates and myrosinase is strongly influenced by the presence of external factors. For example, the amount of glucosinolates can be reduced in vegetables during storage or degraded during processing. Alternately, glucosinolates can become less accessible by thermal inactivation of myrosinase.¹⁻³ Therefore, the amount of I3C found in the diet can vary greatly, ranging from 20 and 120 mg daily, and is dependent on dietary intake of cruciferous vegetables and their variable concentrations.^{4,5}

Clinical studies have demonstrated that 200 to 400 mg of supplemental I3C daily is the efficacious dose for chemoprevention.⁶⁻⁹ In order to obtain this approximate amount in the diet, one would have to consume, for example, roughly 130 raw Brussels sprouts or one-quarter of a head of raw cabbage on a daily basis, which is unreasonable for some individuals.^{5,7} Therefore, a dietary supplement of I3C is warranted in certain individuals.

I3C gives rise to a broad spectrum of beneficial compounds upon its consumption and digestion. Various researchers and reputable organizations, such as the National Cancer Institute and the Strang Cancer Prevention Center, have interest in I3C as a natural cancer prevention agent, particularly for breast, cervical, endometrial, and colorectal cancer.

The reason for the strong advocacy of I3C comes from the multitude of studies, which show that diets high in fruits and vegetables are associated with decreased risk of developing certain cancers. In fact, there is so much evidence that the FDA has allowed two health claims regarding the relationship between fruit and vegetable consumption and decreased cancer risk.

Several prospective clinical studies have shown that populations that consume higher amounts of cruciferous vegetables have lower incidence of cancer, or improved biochemical indices (e.g., decreased oxidative stress), compared to controls.¹⁰⁻¹² The National Research Council, Committee on Diet, Nutrition, and Cancer has recommended increased consumption of cruciferous vegetables as a measure to decrease the incidence of cancer. In fact, epidemiological studies provide evidence that the consumption of *cruciferous* vegetables protects against cancer more effectively than the total intake of fruits and vegetables.¹³ Researchers believe that the I3C found in cruciferous vegetables is one of the main components for this beneficial effect.^{6,14-16}

What is DIM?

As we eat a serving of cruciferous vegetables, the I3C in the food is released during chewing and is transported to the stomach. In the presence of adequate stomach acid, I3C rapidly undergoes a reaction leading to the formation of several other significant compounds in the stomach. The three most significant products of the reaction are: hexahydrocyclohepta triindole (HNTI), indolo[3,2-b]carbazole (ICZ), and 3,3'-diindolylmethane (DIM).^{17,18} The full spectrum of compounds generated by I3C has not been studied to a great extent; however, there is some evidence to suggest that each of them has their own unique action. For example, ICZ exhibits antiestrogenic activity and supports phase I detoxification activities; HNTI binds to estrogen receptors and shows chemical structure similarities to tamoxifen; and DIM has shown to be anticarcinogenic in various cell and animal models.¹⁹⁻²⁴

DIM forms about 10-20% of the breakdown products of I3C; therefore, the typical daily ingestion of I3C from the diet will provide between 2 and 24 mg of DIM.^{4,25,26} The fact that DIM has not been extensively studied implies that no known dosage for DIM has been verified for its health effects. Therefore, there is some risk in prescribing or recommending a specific dose for patients with health issues. As a result, taking I3C is important since it is the precursor to a variety of compounds, which have unique actions and may work synergistically. In isolation, these compounds may function differently, and even detrimentally.

What does the research say about I3C and DIM?

The clinical evidence definitely stacks in favor of I3C compared to DIM. Although cruciferous vegetables contain a number of compounds for cancer prevention, *I3C alone* shows efficacy for the prevention of breast, endometrial, and cervical cancers.^{7,15,27-29} In fact, the majority of studies done with human subjects that demonstrate the beneficial attributes of the phytochemicals contained in cruciferous vegetables have been conducted using I3C as a dietary supplement. Remarkably, the health benefits of I3C supplementation, particularly in women, have been demonstrated in at least **9 human clinical trials within the past 10 plus years.**^{7-9,30-34}

These studies were performed at reputable institutions such as the Boston University School of Medicine, New York University Medical Center, University of Pittsburgh Medical Center, and Strang Cancer Prevention Center. The data for I3C are compelling enough that the National Cancer Institute has nominated I3C for testing as a preventative for breast cancer.²⁵ Auburn et al. reported that I3C is a “promising agent for the prevention of estrogen-enhanced cancers.”³⁵

On the other hand, there are no published human clinical trials using DIM supplementation. There has been some reference to studies on DIM: “...well-controlled, independently-performed human studies have been completed and are awaiting publication.”³⁶ However, these papers have not appeared for publication in peer-reviewed scientific journals as indexed by the National Library of Medicine. Supposedly, these trials “...include the use of DIM showing statistically significant benefits for recurrent breast pain and improvement of cervical dysplasia.” It is worthwhile to note that even if there was a clinical trial pending publication for DIM and cervical cancer, I3C’s effects on cervical cancer have already been studied by others, including a placebo-controlled, clinical trial by Bell et al. in which there was a statistically significant regression of cervical intraepithelial neoplasia in patients treated with I3C orally compared with placebo.^{7,16,28}

What about the use of I3C for cancer prevention?

A balanced review of the literature on I3C reveals that *most* studies report inhibitory or chemoprotective effects of I3C *in vivo*. However, as is common in these types of studies—depending on the initiator, exposure protocol, and species—there are some animal studies demonstrating the promotion of cancer using I3C.³⁷ Findings from these studies suggest that I3C has a protective effect if given before or in conjunction with a carcinogenic agent, and a promoting effect if given after carcinogen exposure or tumor induction.³⁸⁻⁴⁰ Ultimately, the destiny of I3C or DIM probably depends largely on several factors, including lifestyle, diet, and duration and timing of exposure.

The fact that researchers have gone on to test I3C in human clinical trials for its chemoprevention ability demonstrates that those in the field trust in I3C’s use for cancer. In fact, a prominent I3C/DIM researcher stated in a recent review article on I3C, “Studies increasingly indicate that dietary indole-3-carbinol (I3C) prevents the development of estrogen-enhanced cancers including breast, endometrial, and cervical cancers. Epidemiological, laboratory, animal and translational studies support the efficacy of I3C.”³⁵

Substantial evidence exists that I3C can reduce the risk of cancers induced by several known carcinogens when administered to animals.^{41,42} Furthermore, in the Toxicological Data Report prepared by the National Institutes of Health (NIH), I3C was not found to

induce tumors, and even decreased the incidence of tumors in animals.”²⁵ In studies designed to assess inhibition, I3C did not induce tumors in target tissues when administered without an initiator in the following protocols (species/target tissue/I3C dose):^{14,43,44}

- Sprague-Dawley (SD) rats/mammary gland/100 mg/day 5 days a week by gavage for 107 days
- ACI/N rats/tongue/1000 ppm in the diet for 37 weeks
- ICR/Ha mice/forestomach/0.03 mmol/g diet for 63 days

Kojima and coworkers examined the inhibiting effect of I3C on spontaneous endometrial adenocarcinoma in female Donryu rats, a strain with a high incidence of endometrial cancer.¹⁵ Rats were fed 0, 200, 500, or 1000 ppm of I3C in the diet for 660 days. At the termination of the study, a dose-dependent decrease in uterine adenocarcinomas was observed (38% - control; 25 % - low-dose I3C; 16 % - mid-dose I3C; 14 % - high-dose I3C). The incidence in the high-dose group was significantly lower ($P < 0.05$) than that in controls. The researchers speculated that this chemopreventive effect of I3C might have been due to its induction of estradiol 2-hydroxylation.

In a three-generational study, Balb/cfC3H breeding pairs were exposed to a dose of 2000 mg/kg I3C *in utero* until 52 weeks of age. Mammary tumor incidence was not significantly different, however the tumor latency was reported at 36 weeks compared to 20 weeks in control animals. There was no body weight decrements observed in mice treated with I3C, and no clinical chemistry or histopathology was reported.”⁴⁵

Sharma et al. reported that I3C was one of 90 potential chemopreventive agents that were screened using six biochemical endpoints, and it was one of eight compounds found to be positive in all six assays.⁴⁶ I3C, and the multiple actives it produces, may be efficacious due to its ability to do the following activities:⁴⁷⁻⁵⁰

- Regulate cell-cycle progression
- Suppress the activation of invasion-promoting molecules associated with breast cancer cell metastasis
- Influence the transcription of estrogen receptors in human tumor cells
- Reverse the activation of multi-drug resistance in cancer cells

What about the role of I3C and DIM in estrogen metabolism?

With regard to estrogen-dependent cancers, I3C and estrogen have opposing effects: estrogen promotes the growth and survival of tumors, whereas I3C decreases the growth of tumors and increases cancer cell death.^{35,51} Human clinical trials have shown that I3C, at doses of 200-400 mg daily, can influence estrogen metabolism and promote the formation of 2-hydroxyestrone, which is considered to be the protective estrogen metabolite in regards to maintaining breast health.^{9,14-16,27,34,36}

In fact, according to an NIH Toxicology Data Report, “Indole-3-carbinol has been shown to shift metabolism of estradiol from 16 α - to 2-hydroxyestrone, thus offering a possible protective effect.^{16,25} It has been suggested that the ratio of 2- to 16 α -hydroxyestrone can be used as a prognostic indicator for some cancers. A high ratio of 16 α /2-hydroxyestrone in women with breast cancer and those at risk of developing breast cancer has been observed.”^{52,53}

On the other hand, there have been less studies conducted regarding the effects of DIM on cancer cells. In general, cell culture and animal studies, which are the only types of published studies currently available, demonstrate mixed findings on the effects of DIM, including its estrogen-related effects and influence on cancer.

There are a number of positive studies on DIM's (and I3C's) effects.²¹⁻²⁴ Although the available research on DIM states its favorable effects in cancer prevention, there are some studies that demonstrate other, less favorable results. For example, Leong et al. showed that DIM had stimulated estrogen-receptors similar to that of estrogen.⁵⁴ Additionally, DIM induced the expressions of the endogenous genes TGF- α , alkaline phosphatase, and progesterone receptor similar to levels induced by estradiol. It is well established that estradiol is the most potent and principal estrogen secreted by the ovaries, with the highest impact on health and longevity. The ambivalent nature of this steroid hormone, which can both sustain health and promote cancer, has become evident from its prolonged supplemental use by women and has also been extensively studied.⁵⁵

Additionally, both the effects of I3C and DIM were compared in a rat tumor model. The researchers found that compared to I3C, DIM did not increase the apoptotic activities in the rat mammary gland at the initiation of carcinogenesis.⁵⁶ As induction of apoptosis is an approach to suppress carcinogenesis, they conclude that I3C, and not DIM, would contribute to suppression of tumor development.

In light of the evidence to date, it has been postulated that DIM on its own may be limiting the complexity of reactions that can occur with all the I3C reaction products. Most of the studies on the I3C derivatives have been done in isolated cell model systems. It is fair to say that it is often difficult, and perhaps inaccurate, to make extrapolations from these study results to apply to humans. The findings in cell systems do not account for the complexity of the human body.

Does I3C upregulate the activity of beneficial enzymes?

I3C has been shown to upregulate, or enhance, detoxification enzymes so that the body can dispose of toxins more efficiently. It has been proposed that this mechanism is what causes I3C to beneficially affect estrogen metabolism. In fact, Leibelt et al. found that even when compared to absorption-enhanced DIM, I3C stimulated detoxification enzymes significantly better.⁵⁷ In our opinion, stimulating these enzymes is a necessary and beneficial process and not "unwanted," as they assist with the removal of cancer-causing toxins.

Is the safety of I3C and its breakdown products an issue?

The National Library of Medicine lists hundreds of studies on I3C and less than a hundred for DIM, indicating that DIM is less studied than I3C. (On May 26, 2004, 360 and 64 studies were listed in response to 'indole-3-carbinol' and 'diindolylmethane', respectively).⁵⁸ Additionally, according to the Natural Medicines Comprehensive Database, which is based on a review of scientific literature, "Indole-3-carbinol is likely safe for most people when used in amounts typically found in the diet. It seems safe for most people when used in medicinal amounts, under proper medical supervision."⁵⁹ On the other hand, "Diindolylmethane is safe when consumed in the small amounts found in foods. *There isn't enough information to know if supplements containing diindolylmethane are safe.*"

The NIH has published an extensive, six-page toxicology report on I3C, whereas no comparable report exists for DIM or other I3C breakdown products.²⁵ As is the nature of toxicology studies, high doses, unlike those that are found in the diet or advocated through supplementation, are used to evoke toxic effects in various cell or animal models. As supported by short- and long-term clinical trials, I3C was not found to be toxic in the amounts used for daily supplementation (200-400 mg/day). Furthermore, it is worthwhile to note in this toxicological report from the NIH that no significant toxic effects were found in any human clinical study using I3C.

The efficacy and safety of I3C has been demonstrated and reported in several studies by Bradlow and his associates.^{27,33} Their conclusion is that I3C has proven to be effective in improving the 2-hydroxylation of estrogen, and when given at doses of 400 mg/day to both women and men for periods up to 3 months, there have been no signs of adverse reactions. Additionally, upon completion of another clinical trial, Rosen et al. concluded that "...indole-3-carbinol appears to be safe and well tolerated and may be an efficacious treatment for recurrent respiratory papillomatosis."³²

Conversely, the safety of I3C breakdown products like DIM or ICZ, when given as sole compounds, has not been confirmed by rigorous toxicity studies or human clinical trials. It is thought that these compounds may all work together in concert, rather than alone in isolation. This is evidenced by the seemingly contradictory research in various cell models. For example, there has been some concern that ICZ is as toxic as the known carcinogen 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD); however, study results are mixed. When tested in the most TCDD-sensitive rat strain, TCDD elicited the expected toxicity syndrome, while ICZ failed to generate any toxic effects.⁶⁰

What about a highly absorbable form of DIM?

DIM on its own is not very bioavailable, nor is it very soluble. In order to render it more absorbable, the commercially available form of DIM has been artificially enhanced with d-alpha-tocopheryl polyethylene glycol-1000 succinate. A recent published study by Anderton et al. shows that this form is 50% more absorbable than the crystalline form of DIM.⁶¹ It is not known what the safety issues are associated with enhancing the absorption of DIM, as this approach violates the body's natural handling of DIM. Again, this issue relates back to the fact that no known dose has been established for DIM use.

The salient points from this article have been summarized for you in the following table.

Table 1. A comparison of I3C to DIM

	I3C	DIM
Source	Formed as a breakdown product of glucosinolates (compound found naturally in cruciferous vegetables)	Formed as a breakdown product of I3C ingestion, with adequate stomach acid
Amount required for health benefits	200-400 mg daily in supplemental form, as determined by clinical trials	Unknown (not tested to date)
Number of references listed in the National Library of Medicine	361	64
Evidence of safety	Extensive use in cell, animal, and human studies; NIH has published a toxicology report	Unknown, as studies are limited
Health benefits	Strong evidence in favor of chemoprevention and hormone (particularly estrogen) balance	Some scant evidence on its role in chemoprevention
Activity	A variety of unique compounds produced after ingestion	Does not break down into other valuable compounds

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