ANNUAL REVIEWS

Annual Review of Food Science and Technology

Indole-3-Carbinol: Occurrence, Health-Beneficial Properties, and Cellular/Molecular Mechanisms

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Annu. Rev. Food Sci. Technol. 2023. 14:347–66

The *Annual Review of Food Science and Technology* is online at food.annualreviews.org

https://doi.org/10.1146/annurev-food-060721-025531

Keywords

indole-3 carbinol, 3,3′-diindolylmethane, occurrence, health benefits, mechanisms

Abstract

Indole-3-carbinol (I3C) is a bioactive phytochemical abundant in cruciferous vegetables. One of its main in vivo metabolites is 3,3′-diindolylmethane (DIM), formed by the condensation of two molecules of I3C. Both I3C and DIM alter multiple signaling pathways and related molecules controlling diverse cellular events, including oxidation, inflammation, proliferation, differentiation, apoptosis, angiogenesis, and immunity. There is a growing body of evidence from both in vitro and in vivo models that these compounds possess strong potential to prevent several forms of chronic disease such as inflammation, obesity, diabetes, cardiovascular disease, cancer, hypertension, neurodegenerative diseases, and osteoporosis. This article reviews current knowledge of the occurrence of I3C in nature and foods, along with the beneficial effects of I3C and DIM concerning prevention and treatment of human chronic diseases, focusing on preclinical studies and their mechanisms of action at cellular and molecular levels.

1. INTRODUCTION

Several epidemiological studies have investigated associations between consumption of cruciferous vegetables and diverse health outcomes. Notably, consumption of a high-glucosinolate diet is linked with a reduced incidence of chronic diseases, although large-scale placebo-controlled human trials including standardized glucosinolate supplements are needed (Connolly et al. 2021). Glucosinolates are natural components of many pungent plants; in particular, glucobrassicin can be found in almost all cruciferous plants, including cabbages, broccoli, and mustards. Hydrolysis of glucobrassicin by plant or bacterial myrosinase produces multiple indoles, predominantly indole-3-carbinol (I3C). Findings from preclinical models indicate that both I3C and its major in vivo product, 3,3′-diindolylmethane (DIM), are effective protective agents against many life-threatening human chronic diseases, and these compounds showed promise in clinical trials (Williams 2021).

A recent study examining causes of death and age-adjusted mortality rates in the United States identified the top ten causes of death, including six diet-related chronic diseases, heart disease, cancer, cerebrovascular disease, Alzheimer's disease, diabetes mellitus, and nephritis (Rana et al. 2021). Together, these six conditions are responsible for approximately 70–80% of deaths in older (≥65 years) adults.

Recent reviews have collated the diverse health-beneficial activities of I3C, including its anti-inflammatory (Ampofo et al. 2018) and anti-cancer (Williams 2021) activities. Most recently, the health-promoting effect of I3C has come under extensive investigation. However, despite interest in its diverse beneficial effects, there is no comprehensive collection of findings from preclinical and clinical studies investigating the use of I3C in the treatment of human diseases.

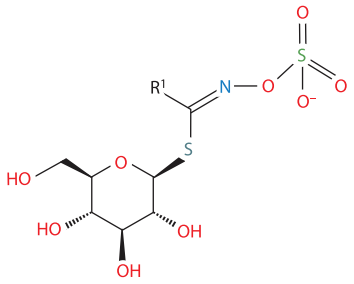
This review article describes the health-beneficial activity of I3C and its main in vivo metabolite, DIM, with a focus on prevention of the most commonly occurring human chronic diseases, including inflammation, obesity, diabetes, cardiovascular disease, cancer, hypertension, kidney disease, neurodegenerative diseases, and osteoporosis.

2. OCCURRENCE OF INDOLE-3-CARBINOL

2.1. Glucosinolates: The Precursors to Indole-3-Carbinol

Glucosinolates are commonly known as precursors to I3C that bring potential health benefits to humans. Glucosinolate biosynthesis starts with precursor amino acids to form the glucosinolate core structure and the side chain. The following describes the biosynthesis and presence of glucosinolates in plants.

2.1.1. Occurrence of glucosinolates in plants. Glucosinolates are a group of sulfur- and nitrogen-containing secondary metabolites found abundantly in cruciferous plants and are synthesized by all species in Brassicaceae; they are also moderately abundant in a significant number of noncruciferous species. Compounds in this group were first isolated in the nineteenth century from black and white mustard seeds (*Brassica nigra* and *Sinapis alba*) (Fahey et al. 2001, Holst & Williamson 2004). Among cruciferous plants, *Brassica oleracea* cultivars such as cauliflower, broccoli, brussels sprouts, and cabbage are well-known glucosinolate-rich food crops. Other Brassicaceae food crops rich in glucosinolates include root vegetables such as radish (*Raphanus raphanistrum*) and turnip (*Brassica rapa*). Meanwhile, most of the glucosinolate-rich noncruciferous plant species belong to Capparaceae and Caricaceae; a few species containing glucosinolates are found in more than ten other families, including Resedaceae, Moringaceae, and Euphorbiaceae. For centuries, glucosinolate-containing plants have been used by many cultures not just as foods but also as condiments, medicine, and feed because of their nutritional effects and biologically



Glucosinolate

Figure 1

Generic chemical structure of glucosinolates showing the glucosinolate skeleton structure along with the modifiable side chain (R^1).

active compounds. Examples of such plants include capers (*Capparis spinosa*), wasabi (*Wasabia japonica*), arugula (*Eruca sativa*), mustard (*Brassica* spp.), Abyssinian kale (*Crambe abyssinica*), and rapeseed (*Brassica napus*) (Barrett et al. 1998, Fahey et al. 2001).

2.1.2. Chemistry and biosynthesis of glucosinolates. From a chemical perspective, glucosinolates are primarily derivatives of glucose and amino acids. **Figure 1** depicts the generic chemical structure of glucosinolates. More than 200 glucosinolate compounds have been identified in cruciferous and noncruciferous plants (Fahey et al. 2001, Halkier & Gershenzon 2006, Hanschen et al. 2014, Wittstock & Halkier 2002). Glucosinolate biosynthesis occurs in three main steps: (*a*) chain elongation of selected precursor amino acids, (*b*) formation of the glucosinolate skeleton structure, and (*c*) modification of the side chain (R^1). The side chain is derived from one of the following amino acids: alanine, leucine, isoleucine, methionine, valine, phenylalanine, tyrosine, or tryptophan. Based on the chemical structure of R^1, glucosinolates are classified into the following classes: (*a*) sulfur-containing; (*b*) aliphatic straight chains; (*c*) aliphatic branched chains; (*d*) straight and branched chains; (*e*) aliphatic straight and branched chain alcohols; (*f*) aliphatic straight chain ketones; (*g*) aromatics, including aryl, benzoate, and indole groups; and (*h*) multiglycosylated. Of all these structures, the most widely observed are those having aliphatic straight or branched chains.

The concentration and form of glucosinolates in a given plant can vary depending on both endogenous and exogenous factors. The key endogenous factors are mainly the type of tissue and the age of the plant. For example, late vegetative or reproductive tissues of broccoli contain 4% or less of the glucosinolate concentration observed in younger broccoli tissues (1–4 μmol/g versus 70–100 μmol/g of fresh weight). Similarly, young broccoli tissues contain a low concentration of indole glucosinolates and high concentration of aliphatic glucosinolates, whereas late vegetative broccoli tissues contain almost equivalent concentrations of the two forms. Regarding differences between tissue types, higher glucosinolate concentrations are observed in roots and seeds [e.g., 30,000 ppm in horseradish (*Armoracia rusticana*) root and 60,000 ppm in mustard seed], whereas moderate concentrations are observed in foliage (e.g., 1,000–3,000 ppm in Brussels sprouts) (Agerbirk & Olsen 2012). Exogenous factors known to affect differences in glucosinolate concentrations and forms (such as aliphatic glucosinolates, S-containing glucosinolates, etc.) are environmental temperature, radiation, soil fertility, water availability, pathogens, plant growth regulators, tissue damage, and subsequent stresses (Agerbirk & Olsen 2012, Bodnaryk & Yoshihara 1995, Fahey et al. 1997, Velasco et al. 2007). Sulfur and nitrogen levels in soil impact a plant's glucosinolate profile when either of the two nutrients becomes a limiting factor. High sulfur

content can increase concentrations of methionine-derived glucosinolates, whereas high nitrogen content can increase indole glucosinolates (Hanschen et al. 2014). In addition, limited water availability in soil can increase levels of aliphatic glucosinolates (Mewis et al. 2012). Combined changes in temperature and radiation can also alter glucosinolate profiles; for example, decreasing temperature and increasing radiation can increment levels of sulfur-containing alkyl glucosinolates (Hanschen et al. 2014).

Inside a plant cell, glucosinolates occur as chemically stable, water-soluble compounds stored in vesicles; however, tissue damage during herbivory, harvest, or postharvest operations and subsequent cell rupture expose glucosinolates to an endogenous hydrolyzing enzyme and associated proteins. Consequently, the glucosinolates are hydrolyzed to form the metabolites discussed below (Fahey et al. 2001, Katz et al. 2018).

2.2. Glucosinolate Breakdown

Tissue damage followed by subsequent reactions triggers glucosinolates to be enzymatically broken down into I3C and other associated metabolites. The following describes the glucosinolate breakdown reaction in detail.

2.2.1. Generic reaction. When plant tissues containing glucosinolates are intact, glucosinolates and the enzyme that hydrolyzes them are stored separately from each other. When those tissues are ruptured (due to chewing, insect attack, or heating), the glucosinolate hydrolyzing enzyme—a β-thioglucosidase (also called myrosinase)—reacts with the glucosinolates and initiates their degradation. First, myrosinase degrades a glucosinolate molecule into two products: (*a*) β-D-glucose and (*b*) an aglucone, thiohydroximate-*O*-sulfonate, which is unstable. In the next step, thiohydroximate-*O*-sulfonate is converted to different chemical forms depending on the pH and other associated proteins. At a neutral pH, the aglucone is spontaneously rearranged to isothiocyanate. Alternately, epithiospecifier proteins (ESPs), nitrile specifier proteins, and thiocyanate-forming proteins (TFPs) can interact with the aglucone and form nitriles in a reaction induced by ferrous ions (Fe^{2+}). In addition, ESPs can convert aglucone into epithionitriles, and TFPs can convert it into thiocyanates (Burow et al. 2009, Williams et al. 2010). Conversely, epithionitrile and thiocyanate formation is hindered by the activity of epithiospecifier modifier proteins, which favor production of isothiocyanate (Zhang et al. 2006).

2.2.2. Formation of indole-3-carbinol in plants. Of the glucosinolates identified in Brassicaceae and non-Brassicaceae plants, indole glucosinolates are a major category and are abundant in *Brassica* and *Arabidopsis* spp. (Brown et al. 2003, Fahey et al. 2001, Petersen et al. 2002). These have side chains derived from the amino acid tryptophan. One predominant form in *Brassica* and *Arabidopsis* plants is indol-3-ylmethylglucosinolate (IMG), commonly known as glucobrassicin. In addition to IMG, its hydroxy and methoxy derivatives (e.g., 1-methoxyIMG, 4-methoxyIMG, 4-hydroxyIMG) are also commonly found in Brassicaceae plants, with higher quantities occurring in vegetative parts (Buskov et al. 2000, Fahey et al. 2001); moreover, *Brassica* leaves contain a higher concentration of IMG, whereas roots contain higher levels of 4-hydroxyIMG and IMG (Bennett et al. 2004, Cartea et al. 2008, Windsor et al. 2005). Just as in the generic glucosinolate reaction, IMG or its derivatives are hydrolyzed to a highly unstable aglucone (i.e., indol-3-ylacetothiohydroximate-O-sulfonate) upon tissue damage and subsequent exposure to myrosinase. The aglucone is then transformed into either the corresponding nitrile [i.e., indole-3-acetonitrile (IAN)] or an isothiocyanate (i.e., 3-indolylmethylisothiocyanate). These reactions are dependent on Fe^{2+}, pH, and ESP—the same factors that are known to intervene in the generic glucosinolate breakdown reaction. It is especially notable that the hydrolysis of IMG does not yield thiocyanates or epithionitriles (Agerbirk et al. 2009).

Indole-3-acetonitrile and isothiocyanates may undergo specific reactions. The former is hydrolyzed by nitrilases to produce indole-3-acetic acid (IAA), a plant hormone commonly known as auxin; the latter is highly reactive and spontaneously degrades into I3C in the presence of water. I3C then reacts with itself or other reactants to produce a range of plant metabolites, such as ascorbigen, DIM, indole-3-tryptophan, indole-3-cysteine, and indole-3-ylmethyl-glutathione. These metabolites are considered to have potential roles as signaling molecules in plant defense mechanisms and also with health benefits to humans, which are discussed below (Agerbirk et al. 2009, Fahey et al. 2001, Katz et al. 2018).

2.2.3. Indole-3-carbinol. Isotopic labeling in turnip roots has shown that IMG can be converted to its corresponding carboxaldehyde and carboxylate products. It is hypothesized that IMG is first converted to I3C, which is subsequently oxidized (Pedras et al. 2002). At neutral pH, I3C is the predominant species produced from hydrolysis of IMG. It is then slowly transformed into DIM (Agerbirk et al. 2009).

2.3. Significance of Indole Glucosinolate–Associated Metabolites in Plants

Indole glucosinolates are known to be involved in plant defense mechanisms against insects, pathogens, and associated extracellular metabolites. Studies have demonstrated the increment of indole glucosinolate concentrations and occurrence of systemic responses in plants following insect feeding (Agerbirk et al. 2009). Specifically, aphid feeding on Brassicaceae plants converts IMG to 4-methoxyIMG, which along with 1-methoxyIMG plays a major role in inhibiting aphid reproduction (Kim & Jander 2007). In addition, higher indole glucosinolate concentrations are associated with exposure to fungal pathogens and extracellular metabolites from bacterial pathogens. Interactions with plant hormones, especially those that act as plant defense signaling molecules, can also increase the concentration of indole glucosinolate (Agerbirk et al. 2009).

Notably, hydrolysis of indole glucosinolates is involved in clubroot symptom formation in cruciferous root vegetables. Infection of a root by the clubroot pathogen induces a series of reactions that ultimately lead to biosynthesis of IAA. In this sequence, indole acetonitrile is first produced through the hydrolysis of indole glucosinolates and then transformed into IAA (Ludwig-Müller et al. 1999).

2.4. Indole-3-Carbinol Metabolism in Humans

Chewing of fresh plant tissue exposes IMG to myrosinase, thereby initiating its hydrolysis into aglucone, which then is transformed into isothiocyanates and ultimately to I3C as described above. The acidity of the stomach triggers I3C molecules to aggregate with each other, resulting in a mixture of polycyclic aromatic compounds, including DIM, 5,11-dihydroindolo-(3,2-b) carbazole, and a cyclic triindole. Cooking inactivates the myrosinase enzyme and precludes the subsequent steps, allowing intact glucosinolates to travel into the colon and be metabolized by intestinal bacteria, releasing I3C to a lesser degree.

2.5. Food Processing Techniques and Indole-3-Carbinol

Unlike many other food matrix substances, glucosinolates and subsequent products of its hydrolysis are highly influenced by postharvest operations. These operations include domestic and industrial food processing techniques such as chopping, storage, cooking, and fermentation. The following subsections give a detailed summary of how food processing techniques affect indole glucosinolate concentration and its hydrolysis.

2.5.1. Cooking. Cruciferous vegetables are usually heat treated prior to consumption. The simplest method of heat treatment is cooking, which may inactivate proteins associated with IMG hydrolysis; however, it can also result in nonenzymatic degradation of IMG to I3C. Below is a summary of research efforts determining the effects of different cooking methods on concentrations of the indole glucosinolate I3C and other associated metabolites in Brassicaceae plants.

Boiling cruciferous vegetables can allow glucosinolates to be leached into water, which can be especially facilitated by the mechanical action of boiling water. According to studies by many groups, boiling broccoli for 2–5 minutes results in around 59% loss of indole glucosinolates and 23% loss of IMG. Similarly, microwaving broccoli has been found to result in a significant loss of IMG and other glucosinolates. Microwave treatment of foods increases the osmotic pressure difference in cells, thereby disrupting cellular structure and enabling enzymatic hydrolysis of glucosinolates by myrosinase (Vallejo et al. 2002, Yuan et al. 2009). Similarly, other domestic cooking methods are ineffective at preserving indole glucosinolates. For example, stir-frying and stir-frying associated with boiling, respectively, yielded a 67% and 64% reduction of indole glucosinolate (Yuan et al. 2009). Interestingly, these studies (along with some others) found that steam cooking resulted in lower levels of loss; accordingly, steaming has been suggested as a preferable heat treatment prior to consuming broccoli (Rungapamestry et al. 2006, Song & Thornalley 2007, Vallejo et al. 2002, Yuan et al. 2009). Other factors during cooking such as heating intensity and cooking time have shown inverse relationships with glucosinolate retention.

2.5.2. Chopping and subsequent storage. Verkerk et al. (2001) studied the indole glucosinolate concentration in white cabbage and broccoli after chopping the produce into small pieces and storing it at room temperature for different time periods. Interestingly, increases in concentration were observed for all four types of indole glucosinolates in white cabbage, namely IMG, 1-methoxyIMG, 4-methoxyIMG, and 4-hydroxyIMG, with total indole glucosinolate increasing by threefold and fivefold after storage for 23 hours and 48 hours, respectively. Meanwhile, in broccoli, concentrations of 4-methoxyIMG and 4-hydroxyIMG increased by 2.5- and 3.5-fold, respectively, after chopping and subsequent storage at room temperature for 48 hours. The researchers explained that slicing cabbage limited cell damage and subsequent hydrolysis at the cut surfaces; thus, the loss of indole glucosinolates was very minimal. Meanwhile, unlike cabbage, broccoli is a perishable vegetable; its yellowing causes cellular damage and subsequent hydrolysis of indole glucosinolates. Thus, the chopping-induced increase of indole glucosinolates in broccoli is not as prominent as that in white cabbage (Verkerk et al. 2001).

2.5.3. Fermentation and hydrothermal treatment. Cabbage fermentation is widely practiced in certain parts of Europe where fermented cabbage—commonly known as sauerkraut—is a popular food product. Studies have examined the concentrations of indole glucosinolate and its degradation products in fermented cabbage. Interestingly, no trace of indole glucosinolates was observed post fermentation in the fermented foods; instead, novel compounds had formed from their hydrolysis. For example, hydrolysis of IMG yielded a relatively high concentration of ascorbigen (8–14 μmol/100 g) and moderate to low concentrations of both I3C (1.5 μmol/100 g) and IAN (0.1 μmol/100 g). Many factors could affect the concentrations of these products, including the initial concentrations of indole glucosinolates, physicochemical properties of the fermented cabbage, and microbiological stability (Ciska & Pathak 2004, Ciska et al. 2009). Another study by the same research group demonstrated that the relatively low pH conditions in fermented cabbage trigger production of DIM from the condensation of I3C, although its final concentration was fourfold lower than that of I3C (Ciska et al. 2009). In addition, the authors investigated the effect of boiling on I3C degradation products in fermented cabbage. Prolonged boiling caused thermal hydrolysis of ascorbigen content, with reductions of approximately 30%

and 90% being observed after boiling for 10 and 60 minutes, respectively. Boiling of fermented cabbage similarly hydrolyzed IAN but was found to increase the total concentrations of I3C and DIM. Careful observation of these findings implies that ascorbigen can be broken down to I3C and its dimers such as DIM; however, detailed research is needed to confirm such speculations (Ciska et al. 2009).

3. HEALTH-BENEFICIAL PROPERTIES OF INDOLE-3-CARBINOL AND ASSOCIATED CELLULAR/MOLECULAR MECHANISMS

3.1. Anti-Inflammatory Activity of Indole-3-Carbinol and 3,3′-Diindolylmethane

Inflammation is a significant intermediate condition for many human chronic diseases. In vitro experiments have shown I3C and DIM to be effective in inhibiting an inflammatory response. In human dermal microvascular endothelial cells exposed to hypoxia and reoxygenation, I3C significantly reduced the transcriptional activity of a nuclear factor-κB (NF-κB) subunit and consequently inhibited the expression of E-selectin and intercellular adhesion molecule-1 (Ampofo et al. 2017). In lipopolysaccharide (LPS)-stimulated BV-2 microglia cells treated with I3C, the expression of several proinflammatory genes, namely *inducible nitric oxide synthase* (*i-NOS*), *interleukin* (*IL*)*-1β*, *NOD-*, *LRR-*, *and pyrin domain-containing protein* 3, *IL-6*, and *CC motif chemokine ligand 2* (*CCL2*), was diminished (Khan & Langmann 2020). In addition, I3C also suppressed the expression of IL-6, tumor necrosis factor alpha (TNF-α), and heat shock protein 70 in BV-2 cells (Prado et al. 2022). Similar results were observed in NMC460 cells (a normal human colorectal cell line), in which I3C significantly inhibited the expression of phosphorylated NF-κB, TNF-α, IL-1β, IL-6, and IL-8 (Peng et al. 2021). Also, in the LPS-stimulated BV-2 cells, DIM downregulated the binding activity of NF-κB with DNA (Kim et al. 2014). However, that study did not observe I3C to have any inhibitory activity on NF-κB binding affinity. In some cancer cell lines, both I3C and DIM have been shown to significantly inhibit inflammatory responses, which potentially indicates that both compounds can be used to treat cancers by relieving inflammation. In LNCaP human prostate cancer cells, I3C and DIM significantly suppressed CCL2 protein expression and THP-1 cell migration induced by dihydrotestosterone (Kim et al. 2013). Similarly, I3C and DIM have demonstrated inhibitory activity on macrophage cells, which can be strongly related to the improvement of a compromised immune system. In LPS-induced RAW 264.7 and THP-1 cells, I3C has been shown to significantly suppress inflammatory response by inhibiting several TIR-domain-containing adapter–inducing interferon-β-dependent signaling pathways and therefore reducing the production of nitric oxide (NO), IL-1β, and IL-6 (Jiang et al. 2013). Likewise, I3C reduces NO production, and TNF-α and IL-10 expression in LPS-stimulated RAW 264.7 macrophage cells (Tsai et al. 2010). Similar results have been reported in the same cells for DIM, namely suppressing LPS-induced increases in production of NO, prostaglandin (PG)E2, TNF-α, IL-6, and IL-1β and inhibiting the transcriptional activity of activator protein-1 induced by LPS (Cho et al. 2008); I3C also inhibited the expression of *IL-6* and *iNOS* and increased activity of peroxisome proliferator–activated receptor (PPAR)-γ in 3T3-L1 adipocyte cells cocultured with Raw 264.7 (Chang et al. 2011). In monocyte-derived macrophages from systemic lupus erythematosus patients, I3C has been shown to target the aryl hydrocarbon receptor (AhR) as an agonist, leading to downregulation of proinflammatory proteins, including IL-12, TNF-α, IL-23, interferon gamma (IFN-γ), and IL-6 and upregulation of an anti-inflammatory cytokine such as IL-10 (Mohammadi et al. 2018). Finally, it has been observed that DIM significantly inhibits the hypoxia-induced expression of *IL-1β*, *IL-6*, and *TNF-α* in H9c2 cells, suggesting that DIM can be used to treat heart disease by relieving inflammatory responses (Liang et al. 2017).

Several in vivo experiments have evaluated the anti-inflammatory activity of I3C and DIM. In mice with ischemia–reperfusion inflammation, I3C was found to significantly suppress leukocyte–endothelial cell interaction and leukocyte transmigration (Ampofo et al. 2017). In a study using male mice, those fed both a high-fat diet and I3C exhibited similar numbers of macrophages in epididymal adipose tissue as the control group, whereas the group fed a high-fat diet alone had more macrophages (Chang et al. 2011). In male rats born from female rats provided with bisphenol A (BPA), I3C was found to suppress the inflammatory response in the ventral prostate, as evidenced by decreased incidence of multifocal inflammation and inflammatory reactive atypia (Brandt et al. 2014). Similarly, in a mouse model of alcohol-induced liver injury, I3C significantly inhibited expression of the proinflammatory cytokine IL-1β (Choi et al. 2018). In a mouse model of colitis, I3C was reported to relieve symptoms by activating IL-22 expression, which led to production of anti-inflammatory butyrate by gut microbiota (Busbee et al. 2020). Likewise, I3C suppressed *Citrobacter* rodentium infection–caused physiological changes and tissue damage in mice. Infected mice fed with I3C maintained mRNA levels of the anti-inflammatory cytokine *IL-22* and exhibited decreased mRNA levels of the proinflammatory cytokines *IL-17A*, *IL-6*, *IL-1β*, *TNF-α*, and *IFN-γ* in colonic tissue; they also showed inhibited serum levels of the cytokines IL-17, TNF-α, IL-12p70, and granulocyte colony stimulating factor (Wu et al. 2020). Ulcerative colitis has also been demonstrated to be attenuated by I3C; namely, feeding the DSS-treated mice with I3C reduced the activation of NF-κB and mRNA levels of *TNF-α* and *IL-1β* (Peng et al. 2021). DIM has also evidenced anti-inflammatory activity via several pathways. In DSS-treated mice, DIM was found to suppress myeloperoxidase activity and the production of NO and PGE2, and other inflammatory cytokines (*TNF-α*, *IL-6*, and *IFN-γ*); it also inhibited expression of *iNOS* and the activation of NF-κB (Y. H. Kim et al. 2009). Similarly, DIM attenuated DSS-induced acute colitis in mice by suppressing the expression of *IL-1β*, *IL-6*, and *IFN-γ* (Jeon et al. 2016). When provided to mice alongside 12-*O*-tetradecanoylphorbol-13-acetate, DIM attenuated ear edema and inhibited the activities of cyclooxygenase-2 (COX-2), iNOS, CXC motif chemokine ligand 5, and *IL-6* possibly because of its suppression of NF-κB activation (Kim et al. 2010).

3.2. Anti-Obesity Activity of Indole-3-Carbinol and 3,3′-Diindolylmethane

In vitro experiments suggest that I3C and DIM could be effective in preventing and attenuating obesity. In the 3T3-L1 adipocyte cell line, I3C was observed to primarily target Sirtuin 1 (*SIRT1*), suppressing expression of the adipogenic genes (CCAAT-enhancer-binding protein) C/EBPα, PPAR-γ2, fatty acid synthase, and adipocyte protein 2 (aP2). Meanwhile, a cell line with *SIRT1* knocked out did not show any differences in expression of those genes (Choi et al. 2013). In addition, I3C has been shown to inhibit lipid accumulation in 3T3-L1 cells and repress adipocyte-stimulated tube formation in human endothelium–derived EA hy926 cells (Wang et al. 2016). In 3T3-L1 cells, it significantly suppressed the expression of *C/EBPα*, *PPARγ2*, and *aP2*, which are strongly connected to adipocyte differentiation (Choi et al. 2012). DIM has likewise been found to contribute to attenuation of obesity. In 3-isobutyl-1-methylxanthine, dexamethasone, and insulin (MDI)-induced 3T3-L1 cells, it significantly inhibited adipogenesis by suppressing expression of PPAR-γ and C/EBPα (Yang et al. 2017). However, in the same study, I3C did not show any inhibitory activity against adipogenesis (Yang et al. 2017). In a separate study of 3T3-L1 cells treated with adipogenic inducers, DIM was found to upregulate glucose uptake by enhancing insulin signaling pathways, including enhancing the activity of PPAR-γ2 and C/EBPα (Choi & Yoo 2018). Recently, we observed that DIM significantly reduced fat accumulation in 3T3-L1 adipocytes and expression of PPAR-γ and C/EBPα, fatty acid binding protein 4, and perilipin. In addition,

DIM activated adenosine monophosphate (AMP)-activated protein kinase α (AMPKα) (Lee et al. 2017). These observations were further confirmed in *Caenorhabditis elegans* as DIM treatment significantly reduced body fat accumulation, which was partly associated with aak-1, which is an ortholog of AMPKα (Lee et al. 2017). These findings suggest that I3C and its derivatives can potentially be used to reduce body fat and therefore play significant roles in weight loss.

Several in vivo studies have been conducted to identify the anti-obesity effect of I3C. Compared to mice fed a high-fat diet only, those fed a high-fat diet containing I3C (0.1% wt/wt) showed a smaller increase in body weight, visceral fat pad weight, and serum lipid levels. In addition, a high-fat diet was found to negatively affect the expression of *sirtuin 1*, *PPARα*, and *PPARγ coactivator α* in visceral adipose tissues relevant to thermogenesis; this effect was normalized by adding I3C (0.1% wt/wt) to a high-fat diet (Choi et al. 2012). Similarly, I3C significantly decreased body weight increase, fat accumulation, and macrophage infiltration in the epididymal adipose tissue of mice fed a high-fat diet; it also improved glucose tolerance and lowered blood glucose, insulin, and lipid levels (Chang et al. 2011). In addition, DIM has been shown to significantly inhibit high-fat-diet-induced body weight increase and the formation of adipose tissue (Yang et al. 2017).

Clinical research has additionally indicated an anti-obesity effect of I3C in humans. When consumed by overweight premenopausal women, I3C significantly enhanced the ratio of urinary estrogens 2OHE1 to E3, which is connected to increased estrogen 2-hydroxylation; values were similar to those in normal-weight women. This suggests that I3C can be used to treat and prevent obesity and obesity-related diseases in humans (Michnovicz 1998).

3.3. Anti-Diabetic and Anti-Atherosclerotic Activities of Indole-3-Carbinol and 3,3′-Diindolylmethane

Several studies support the possibility that I3C can be effective in treating diabetes. In mice fed I3C in addition to a high-fat diet, significantly lower blood glucose and insulin levels were observed relative to the group fed the high-fat diet alone. Moreover, I3C inhibited levels of the homeostasis model assessment of basal insulin resistance, indicating improved insulin sensitivity (Choi et al. 2012). The antioxidant capacities of I3C and DIM have also been shown to significantly attenuate diabetes-related symptoms in mice with symptoms similar to humans with the disease. Compared to the control animals, the treatment groups exhibited decreased levels of glucose, insulin, and glycated hemoglobin, along with increased hemoglobin levels. These effects are believed to be strongly connected to reduced oxidative stress; the antioxidant capacities of I3C and DIM were evident in the enhanced activity of related enzymes such as superoxide dismutase, catalase, and glutathione peroxidase, along with increased levels of vitamin C, vitamin E, and glutathione (Jayakumar et al. 2014). Similarly, I3C and DIM have been shown to significantly increase liver glycogen and hemoglobin content, and decrease serum glucose, insulin, and glycated hemoglobin in mice fed a high-fat diet, which induced expression of hexokinase and glucose-6-phosphate dehydrogenase and inhibited expression of glucose-6-phosphatase and fructose-1,6-biphosphatase in both liver and kidneys (Poornima & Mirunalini 2014). Regarding antioxidative activity and carbohydrate metabolism, DIM showed a greater degree of effect than did I3C.

Notably, the ability of I3C to inhibit lipid accumulation can be relevant to prevention of cardiovascular diseases such as atherosclerosis. In zebrafish larvae fed a high-cholesterol diet, provision of I3C resulted in autophagy-induced inhibition of lipid deposition, as evidenced by enhanced expression of cleaved light chain 3, beclin-1, hVps34, and m-cathepsin D protein. In addition, significant inhibition of protein expression was observed for the autophagy adaptor *p62* along with B-cell lymphoma 2 (Bcl-2), protein kinase B (Akt), p-Akt, mammalian target of rapamycin (mTOR), and p-mTOR. These effects are believed to be strongly related to the anti-atherosclerotic activity of I3C (Jiang et al. 2019).

3.4. Anti-Cancer Activity of Indole-3-Carbinol and 3,3′-Diindolylmethane

Cancer is the second leading cause of death in the United States, with 1,898,160 new cancer cases and 608,570 cancer deaths having been projected to occur in 2021 (Siegel et al. 2021). The primary hallmarks of cancer include self-sufficiency regarding growth signals, insensitivity to anti-growth signals, resistance to apoptosis and senescence, angiogenesis, and metastasis. Over the past 50 years, the anti-cancer activity of I3C and DIM has been extensively reported through hundreds of research articles, and many studies have demonstrated the utility of these compounds as chemopreventive and/or therapeutic agents. In this section, the anti-cancer activity of I3C and DIM in relation to hallmark-based mechanisms is reviewed.

3.4.1. Apoptosis. Apoptosis is a process of programmed cell death activated by either the mitochondria-dependent intrinsic pathway or the death receptor–dependent extrinsic pathway. The intrinsic pathway is triggered by internal factors such as DNA damage and cellular stress and mediated by mitochondrial membrane proteins, namely the pro-apoptotic *Bax* and *Bak* and anti-apoptotic *Bcl-2* and *BclxL*, with subsequent cytochrome c release. The death receptor pathway is mediated by binding of pro-apoptotic ligands to corresponding death receptors. Both pathways stimulate initiator caspases (such as caspase-8/-9/-10) and then executor caspases (caspase-3/-6/-7).

In an in vivo study, supplementation of a transgenic mouse prostate adenocarcinoma model (TRAMP) with 1% I3C resulted in significant decreases in palpable tumor formation and genitourinary weight (Wu et al. 2012). Likewise, in vitro studies suggest I3C has pro-apoptotic activity in diverse types of human cancer cell lines. In fact, I3C has been found to induce apoptosis by upregulating or downregulating apoptosis markers/proteins in key pathways. In colorectal cancer cell lines, it was shown to upregulate C*YP1A1* mRNA levels, act as an agonist for AhR activation, mitigate cell viability, and induce apoptotic activity in a dose-dependent manner (Megna et al. 2016). In a lung cancer cell line, I3C increased cellular reactive oxygen species (ROS) levels and expression of pro-apoptotic proteins (Bax and Bim) and downregulated both anti-apoptotic proteins (Bcl-2 and Bcl-xL) and the associated increases of caspase-3, -7, and -9, forkhead box O3, and cleaved poly(ADP-ribose) polymerase (PARP) (Lim et al. 2021). In pancreatic cancer cells, I3C suppressed phosphorylation of signal transducer and activator of transcription 3 and apoptosis in a concentration-dependent manner (Lian et al. 2004). In breast cancer cell lines, I3C was found to affect estrogen metabolism through AhR-dependent upregulation of *CYP1A1/A2*, *ATF-3*, and *NOXA* (a stress response transcription factor) (Caruso et al. 2014). Similarly, treatment of hepatocellular cancer cell lines with I3C resulted in a dose- and time-dependent increase of apoptosis, evidenced by increased DNA fragmentation, caspase-3/-7 cleavage, and PARP cleavage, along with decreased expression of p-Akt, Bcl-xL, thioredoxin-1, and peroxiredoxin-1 (Lee et al. 2019). Upregulation of caspase-3/-7/-9 and cleaved PARP and downregulation of Bcl-xL were likewise reported in osteosarcoma cells treated with I3C (Lee et al. 2018), and the upregulation of *Bax* and downregulation of *Bcl-2* were also observed in I3C-treated breast cancer cells (Sarkar et al. 2003). Regarding the pro-apoptotic signaling pathway, treatment of laryngeal cancer cells with I3C activated apoptosis by downregulating the *PI3K/Akt* signaling pathway, including p110α, p110β, PI3K class III, and p-Akt (Wang et al. 2013).

Oral DIM administration in an in vivo mouse xenograft model has been shown to suppress patient-derived colon tumor growth (Tian et al. 2019). Similarly, DIM suppressed the growth of hepatocellular carcinoma by stimulating endoplasmic reticulum (ER) stress–mediated mitochondrial apoptosis (Munakarmi et al. 2021) and stimulated *PTEN/Akt* signaling–mediated activation of mitochondrial apoptosis in malignant melanoma cells (Wang et al. 2020). DIM has also been reported to induce apoptosis in colon cancer cells via activation of caspase-8

(Kim et al. 2007). Regarding a novel mechanism, we reported that activating transcription factor 4 (ATF4)-mediated induction of ATF3 expression mediates DIM-induced apoptosis in human colorectal cancer cells (Lee et al. 2013).

3.4.2. Cell cycle arrest. The decision-making machinery involved in cell cycle arrest is one of the most significant targets for cancer prevention and therapy. This process is regulated by many molecules, including cyclins, cyclin-dependent kinases (CDKs), and CDK inhibitors. Cyclins (types A, B, D, and E) are associated with CDKs (CDK1/2/4/6) and activate their catalytic activities, which regulate the phospho-status of retinoblastoma (Rb) and subsequent activation of the E2F transcription factor. Meanwhile, CDK inhibitors (including p16, p21, p27, and p57) are molecules that inhibit the function of CDKs. All these cell cycle–regulating molecules constitute significant molecular targets for preventing cell cycle progression and treating cancer.

The combination of I3C and DIM resulted in upregulation or downregulation of certain miRNAs and directly reduced the expression of cell cycle–stimulating genes such as *CDK4/6* and *P27* (El-Daly et al. 2020). A study in breast cancer investigated the anti-cancer effects of I3C derivatives, including N-alkoxy I3C (the most effective in preventing breast cancer cell proliferation), N-ethoxy-I3C, N-propoxy-I3C, and N-butoxy I3C (Jump et al. 2008).

The anti-cancer effect of DIM has been found to be associated with G1 cell cycle arrest in esophageal squamous cancer (Kim et al. 2012) and nasopharyngeal carcinoma cells (Xu et al. 2013). In human ovarian cancer cells, DIM has been shown to suppress cell growth and division through activating G2/M arrest via the checkpoint kinase 2-dependent pathway (Kandala & Srivastava 2010). Similarly, in HT-29 human colon cancer cells, DIM induced both G1 and G2/M cell cycle arrests (Choi et al. 2009).

3.4.3. Senescence. Another hallmark of cancer is its limitless replicative potential. All normal cells have constraints on their capacity for cell division and doubling and eventually undergo senescence, which is associated with shortening and abrasion of telomeres. However, cancer cells overcome these limitations through overexpressing telomerase, which plays a crucial role in cell cycle progression. Accordingly, some anti-cancer agents downregulate the expression of *hTERT* (a component of the telomerase ribonucleoprotein complex), which leads to a significant reduction of cellular telomerase activity.

I3C has been shown to significantly inhibit telomerase activity and human telomerase reverse transcriptase (*hTERT*) mRNA expression in LNCaP and PC3 cells (Adler et al. 2011). Moreover, it downregulates *hTERT* in breast cancer cells by disrupting the endogenous interaction between ERα and Sp1, which are the main transcription factors driving *hTERT* expression in those cells (Marconett et al. 2011). DIM also reduces mRNA and protein expression of hTERT in a concentration-dependent manner, with consequent shortening of telomeres (F. Li et al. 2015).

3.4.4. Angiogenesis. Angiogenesis is the formation of new blood vessels and a particularly important process in cancer. As a tumor increases in size, cancer cells within it are subject to hypoxia, which is the main trigger of angiogenesis. In short, accumulation of the transcription factor hypoxia-inducible factor-alpha (HIF-1α) activates the expression of vascular endothelial growth factor (VEGF), which binds to the VEGF receptor (VEGFR) on the membranes of endothelial cells and stimulates proliferation, migration, and sprouting of blood vessels.

Anti-angiogenic activity of I3C has been observed in endothelial cells. In a coculture system involving endothelial cells and macrophages, I3C inhibited the LPS-induced formation of capillary-like structures by endothelial cells, decreasing secretion of VEGF, NO, IL-6, and matrix metalloproteinases (MMPs); this indicates a potential role for I3C in preventing inflammation-associated angiogenic diseases (Wang et al. 2012). Another study reported significant inhibition of

angiogenesis by I3C in mice, along with reduced serum levels of VEGF-A and MMP-9 through modulation of nuclear factor erythroid 2-related factor 2 (Nrf2)/antioxidant responsive element signaling pathway and increased expression of cytoprotective proteins such as heme oxygenase-1, NADH quinone dehydrogenase 1 (NQO1), and glutathione S-transferases (Hajra et al. 2018).

DIM has evidenced anti-angiogenic activity. In an in vivo matrigel plug angiogenesis assay using female mice, subcutaneous injection of DIM (5 mg/kg body weight, five times per week) led to 76% inhibition of neovascularization (Chang et al. 2005). DIM reduced HIF-α levels and HIF-1 activity in hypoxic-cultured human cancer cells (Riby et al. 2008) and suppressed angiogenesis in PC3 prostate cancer cells, which is associated with the platelet-derived growth factor D and the mTOR pathway (Kong et al. 2008).

3.4.5. Metastasis. Metastasis comprises invasion, extracellular matrix (ECM) degradation, and migration of malignant cancer cells. Cell invasion is the first step and is associated with the breakdown of cell adhesion factors such as the E-cadherin/catenin adhesion complex and focal adhesion mediated by integrins. ECM degradation is controlled by MMPs and tissue inhibitors of MMPs. Cell migration is controlled by the *Rho*, *Rac*, or *Cdc42* signaling pathway.

An in vitro study in MDA-MB-231 cells found I3C to significantly decrease migration via increased stress fiber formation and induced localization of the focal adhesion component, enhancing focal adhesions and stimulating Rho kinase enzymatic activity and cofilin phosphorylation (Brew et al. 2009). In estrogen-responsive breast cancer cells, I3C treatment resulted in significant inhibition of in vitro cell adhesion, migration, and invasion along with in vivo lung metastasis formation. These effects might be associated with the suppression of ER-stimulated migration and invasion (Meng et al. 2000).

Anti-metastatic activity has also been reported for DIM. Oral administration of DIM inhibited lung metastasis of 4T1 murine mammary carcinoma cells in BALB/c mice (E.J. Kim et al. 2009) and decreased the orthotopic liver tumor volume and suppressed lung metastasis in nude mice (W.X. Li et al. 2015). Similarly, DIM has been shown to suppress the growth of hepatocellular carcinoma by regulating invasion and migration (Munakarmi et al. 2021). DIM also inhibited the migration and invasion of human cancer cells through the combined suppression of extracellular signal-regulated kinases and Akt pathways (Rajoria et al. 2011) and suppressed the metastasis of squamous cell carcinoma through repressing epithelial–mesenchymal transition via the RhoA-mediated prostaglandin pathway (Zhu et al. 2020).

3.5. Preventive Effect of Indole-3-Carbinol and 3,3′-Diindolylmethane on Hypertension and Kidney Disease

Hypertension is a significant risk factor for cardiovascular diseases such as heart attack (myocardial infarction) and stroke. Blood pressure is regulated by elements of the cardiovascular system, including the heart and blood vessels, and the urinary system, including the kidneys. In particular, the renin–angiotensin system (RAS) plays a significant role in blood pressure regulation. Briefly, low blood pressure stimulates the central nervous system to trigger renin release from the kidneys, which converts angiotensinogen to angiotensin I by cleavage and activates subsequent conversion of angiotensin I to angiotensin II through cleavage by the angiotensin-converting enzyme. I3C contributes to this process by activating renin gene expression in the kidney and increasing blood pressure.

In rats, I3C treatment for two weeks resulted in dose-dependent increases in plasma renin concentration and arterial pressure. In addition, rats receiving 0.125% (w/w) I3C via their diet for 12 weeks exhibited significantly increased plasma prorenin concentration and mean arterial pressure (Peters et al. 2008). Notably, a new animal model of hypertension using dietary I3C

(Cyp1a1-Ren2 transgenic rats) has been developed that targets not only the onset of hypertension but also its magnitude (I3C dose dependency) (Leader et al. 2018). Dietary administration of 0.3% I3C to Cyp1a1-Ren2 rats generates angiotensin II–dependent malignant hypertension and increased renal vascular resistance (Cunningham et al. 2013). However, a recent study reported that treatment of the rats with 2,000 ppm/day of I3C prevented hypertensive remodeling, reduced reperfusion arrhythmias, and decreased oxidative-inflammatory markers; this indicates that I3C reduces the inflammatory–oxidative–proarrhythmic process of hypertension (Prado et al. 2022). An in vitro study using BV-2 glial cells subjected to LPS-induced oxidative-inflammatory damage likewise indicated a preventive effect of I3C against oxidative stress and inflammation (Prado et al. 2022). Further studies are required to elucidate in more detail the mechanism by which I3C induces renin production and consequent hypertension. Moreover, the effect of DIM on RAS activation has not yet been investigated, despite the conversion of dietary I3C to DIM after ingestion.

One of the main health complications of hypertension is chronic kidney disease, the incidence of which is rapidly increasing and becoming a worldwide epidemic. In addition, nephrotoxicity is a limiting problem associated with the clinical use of some drugs, such as cisplatin. El-Naga & Mahran (2016) investigated the potential protective effect of I3C against cisplatin-induced acute nephrotoxicity in rats and found that pretreatment with 20 mg/kg I3C mitigated cisplatin-induced acute nephrotoxicity; moreover, it significantly ameliorated the oxidative stress, inflammation, and apoptotic effects induced by cisplatin.

Kidney damage (nephropathy) is also a major complication of diabetes. Treatment of streptozotocin-induced diabetic mice with DIM enhanced insulin signaling and improved insulin-dependent diabetes; it also improved diabetic nephropathy by inhibiting expression of PKC-α, the marker of albuminuria, and TGF-β1, an indicator of renal hypertrophy. These data suggest that DIM may ameliorate hyperglycemia and diabetic nephropathy through the inhibition of PKC-α and TGF-β1 signaling (Choi & Yoo 2019).

3.6. Neuroprotective Effects of Indole-3-Carbinol and 3,3′-Diindolylmethane

Neurodegenerative diseases are a heterogeneous group of disorders characterized by progressive degeneration of the structure and function of neurons in the brain and peripheral nervous system. Microglial hyperactivation and neuroinflammation are implicated in the development and progression of neurodegenerative diseases. Kim et al. (2014) observed that DIM suppressed LPS-induced inflammation in brain cells (BV-2 microglia) by repressing expression of *iNOS* and *COX-2* and attenuating both the DNA-binding activity of NF-κB and phosphorylation of inhibitory proteins of κB; these findings suggest that DIM might inhibit microglial hyperactivation by attenuating inflammation. Similarly, in an in vivo mouse model of neuroinflammation, DIM suppressed LPS-induced inflammation in the hippocampus, as determined by the number of Iba-1-positive cells and mRNA expression of F4/80 (Kim et al. 2014).

The most common form of neurodegenerative disease is Alzheimer's, the hallmarks of which are β-amyloid plaques and neurofibrillary tangles. Interestingly, I3C has been shown to significantly increase the expression and enzyme activity of neprilysin, which is a major endogenous catabolic enzyme of β-amyloid (Qian et al. 2021).

The second most common progressive neurodegenerative disorder is Parkinson's disease, characterized by symptoms of motor dysfunction. Mohamad et al. (2022) investigated the effect of I3C in a rotenone-induced animal model of Parkinson's and found that it prevented rotenone-mediated motor dysfunction and amended striatal dopamine decrease, weight loss, neurodegeneration, tyrosine hydroxylase expression reduction, and *α-synuclein* expression increase in both the midbrain and striatum, with the most effective response being observed in the

group receiving the highest dose of I3C (100 mg/kg). These neuroprotective effects are partially associated with the anti-inflammatory and anti-apoptotic effects of I3C, mediated through activation of the sirtuin 1/AMPK pathway (Mohamad et al. 2022). Another study found that chronic administration of I3C (12.5, 25, 50 mg/kg/day) for 21 days in intranigral LPS-treated rats led to significant improvements in motor function, coordination, learning, and memory; these outcomes were associated with decreased activity of inflammatory cytokines such as TNF-α and IL-6. These results suggest a possible neuroprotective effect of I3C via amelioration of LPS-induced behavioral alterations, oxidative damage, and neuroinflammation (Saini et al. 2020).

3.7. Protective Effect of Indole-3-Carbinol and 3,3′-Diindolylmethane in Osteoporosis

Bone is the most dynamic organ in the human body. The synthesis of new bone, breakdown of old bone, and overall bone homeostasis are tightly regulated by two types of cells: osteoblasts, small

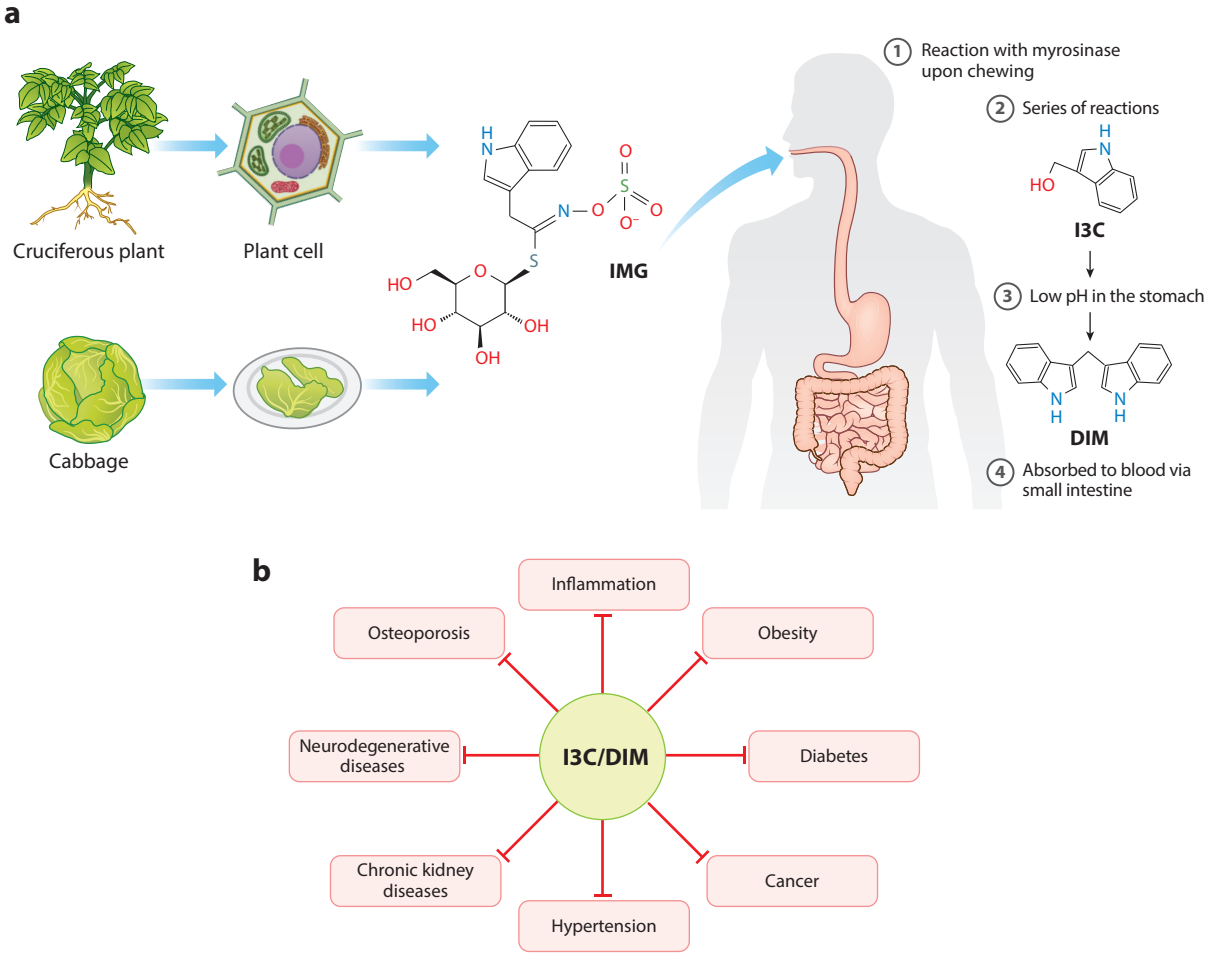


Figure 2

(*a*) Occurrence, metabolism, and (*b*) health benefits of indole-3-carbinol (I3C) and its main metabolite, 3,3′-diindolylmethane (DIM). Abbreviation: IMG, indol-3-ylmethylglucosinolate.

mononucleate cells that synthesize the bone matrix, and osteoclasts, large multinucleate cells that resorb the bone matrix. Osteoporosis is characterized by low bone mass and deterioration of bone structure. A major cause of osteoporosis, bone loss, and fractures is the glucocorticoid-induced apoptosis of osteoblasts; another important contributor is oxidative stress.

I3C has been shown to suppress glucocorticoid-induced cytotoxicity and apoptotic cell death in osteoblastic cells through reducing dexamethasone-stimulated sub-G1, activation of caspase-3/-8/-9 and subsequent cleavage of PARP, ROS production, and expression of *Nrf2*, *HO1*, and *NQO1* in a Nrf2-dependent manner (Lin et al. 2015).

DIM has also been shown to exert protective activity against osteoporosis. Intraperitoneal injection of DIM (0.1 mg/g body weight, twice a week for four weeks) significantly increased bone mass, as assessed by dual-energy X-ray absorptiometry and microcomputed tomography, and reduced bone resorption parameters, as indicated by histomorphometric analyses (Yu et al. 2015). In an additional experiment using an ovariectomy (OVX)-induced osteoporotic mouse model, administration of DIM prevented bone loss due to OVX-induced increase in bone resorption (Yu et al. 2015). These data indicate that DIM increases bone mass by suppressing osteoclastic bone resorption and has potential as a treatment for postmenopausal osteoporosis (Yu et al. 2015).

4. CONCLUSION

Glucosinolates are chemically diverse metabolites mainly concentrated in cruciferous plants. Indole glucosinolates constitute the most abundant form and exist as IMG or its derivatives. Upon chewing of plant tissue, IMG is enzymatically hydrolyzed; a series of subsequent reactions leads to the formation of I3C. Stomach acid aggregates I3C molecules and forms a mixture of polycyclic aromatic compounds that includes DIM. The concentration of I3C and its degradation products in foods is greatly affected by food processing and cooking methods. Emerging evidence from diverse experimental disease models supports I3C and its metabolites as having health benefits and furthermore suggests that both I3C and DIM could have promise as preventive and therapeutic agents for a variety of chronic human diseases; the proposed main mechanisms of their effects are described in **Figure 2**. More comprehensive mechanistic human clinical studies justifying the clinical and physiological relevance of these bioactive compounds are required to buttress the prospect of using them to combat metabolic diseases.

DISCLOSURE STATEMENT

The authors are not aware of any affiliations, memberships, funding, or financial holdings that might be perceived as affecting the objectivity of this review.

LITERATURE CITED

Adler S, Rashid G, Klein A. 2011. Indole-3-carbinol inhibits telomerase activity and gene expression in prostate cancer cell lines. *Anticancer Res.* 31:3733–37

Agerbirk N, De Vos M, Kim JH, Jander G. 2009. Indole glucosinolate breakdown and its biological effects. *Phytochem. Rev.* 8:101–20

Agerbirk N, Olsen CE. 2012. Glucosinolate structures in evolution. *Phytochemistry* 77:16–45

Ampofo E, Lachnitt N, Rudzitis-Auth J, Schmitt BM, Menger MD, Laschke MW. 2017. Indole-3-carbinol is a potent inhibitor of ischemia-reperfusion-induced inflammation. *J. Surg. Res.* 215:34–46

Ampofo E, Schmitt BM, Menger MD, Laschke MW. 2018. Targeting the microcirculation by indole-3-carbinol and its main derivate 3,3′-diindolylmethane: effects on angiogenesis, thrombosis and inflammation. *Mini Rev. Med. Chem.* 18:962–68

Barrett JE, Klopfenstein CF, Leipold HW. 1998. Protective effects of cruciferous seed meals and hulls against colon cancer in mice. *Cancer Lett.* 127:83–88

Bennett RN, Mellon FA, Kroon PA. 2004. Screening crucifer seeds as sources of specific intact glucosinolates using ion-pair high-performance liquid chromatography negative ion electrospray mass spectrometry. *J. Agric. Food Chem.* 52:428–38

Bodnaryk R, Yoshihara T. 1995. Structure-activity relationships of cyclopentane analogs of jasmonic acid for induced responses of canola seedlings, *Brassica napus* L. *J. Chem. Ecol.* 21:1735–43

Brandt JZ, Silveira LT, Grassi TF, Anselmo-Franci JA, Fávaro WJ, et al. 2014. Indole-3-carbinol attenuates the deleterious gestational effects of bisphenol A exposure on the prostate gland of male F1 rats. *Reprod. Toxicol.* 43:56–66

Brew CT, Aronchik I, Kosco K, McCammon J, Bjeldanes LF, Firestone GL. 2009. Indole-3-carbinol inhibits MDA-MB-231 breast cancer cell motility and induces stress fibers and focal adhesion formation by activation of Rho kinase activity. *Int. J. Cancer* 124:2294–302

Brown PD, Tokuhisa JG, Reichelt M, Gershenzon J. 2003. Variation of glucosinolate accumulation among different organs and developmental stages of *Arabidopsis thaliana*. *Phytochemistry* 62:471–81

Burow M, Losansky A, Müller R, Plock A, Kliebenstein DJ, Wittstock U. 2009. The genetic basis of constitutive and herbivore-induced ESP-independent nitrile formation in *Arabidopsis*. *Plant Physiol.* 149:561–74

Busbee PB, Menzel L, Alrafas HR, Dopkins N, Becker W, et al. 2020. Indole-3-carbinol prevents colitis and associated microbial dysbiosis in an IL-22-dependent manner. *JCI Insight* 5(1):e127551

Buskov S, Olsen CE, Sørensen H, Sørensen S. 2000. Supercritical fluid chromatography as basis for identification and quantitative determination of indol-3-ylmethyl oligomers and ascorbigens. *J. Biochem. Biophys. Methods* 43:175–95

Cartea ME, Velasco P, Obregón S, Padilla G, de Haro A. 2008. Seasonal variation in glucosinolate content in *Brassica oleracea* crops grown in northwestern Spain. *Phytochemistry* 69:403–10

Caruso JA, Campana R, Wei C, Su CH, Hanks AM, et al. 2014. Indole-3-carbinol and its N-alkoxy derivatives preferentially target ERα-positive breast cancer cells. *Cell Cycle* 13:2587–99

Chang HP, Wang ML, Chan MH, Chiu YS, Chen YH. 2011. Antiobesity activities of indole-3-carbinol in high-fat-diet-induced obese mice. *Nutrition* 27:463–70

Chang HP, Wang ML, Hsu CY, Liu ME, Chan MH, Chen YH. 2011. Suppression of inflammation-associated factors by indole-3-carbinol in mice fed high-fat diets and in isolated, co-cultured macrophages and adipocytes. *Int. J. Obes.* 35:1530–38

Chang X, Tou JC, Hong C, Kim HA, Riby JE, et al. 2005. 3,3′-Diindolylmethane inhibits angiogenesis and the growth of transplantable human breast carcinoma in athymic mice. *Carcinogenesis* 26:771–78

Cho HJ, Seon MR, Lee YM, Kim J, Kim JK, et al. 2008. 3,3′-Diindolylmethane suppresses the inflammatory response to lipopolysaccharide in murine macrophages. *J. Nutr.* 138:17–23

Choi HJ, Lim DY, Park JH. 2009. Induction of G1 and G2/M cell cycle arrests by the dietary compound 3,3′-diindolylmethane in HT-29 human colon cancer cells. *BMC Gastroenterol.* 9:39

Choi KM, Yoo HS. 2018. 3,3′-Diindolylmethane enhances glucose uptake through activation of insulin signaling in 3T3-L1 adipocytes. *Obesity* 26:1153–60

Choi KM, Yoo HS. 2019. Amelioration of hyperglycemia-induced nephropathy by 3,3′-diindolylmethane in diabetic mice. *Molecules* 24:4474

Choi Y, Abdelmegeed MA, Song BJ. 2018. Preventive effects of indole-3-carbinol against alcohol-induced liver injury in mice via antioxidant, anti-inflammatory, and anti-apoptotic mechanisms: role of gut-liver-adipose tissue axis. *J. Nutr. Biochem.* 55:12–25

Choi Y, Kim Y, Park S, Lee KW, Park T. 2012. Indole-3-carbinol prevents diet-induced obesity through modulation of multiple genes related to adipogenesis, thermogenesis or inflammation in the visceral adipose tissue of mice. *J. Nutr. Biochem.* 23:1732–39

Choi Y, Um SJ, Park T. 2013. Indole-3-carbinol directly targets SIRT1 to inhibit adipocyte differentiation. *Int. J. Obes.* 37:881–84

Ciska E, Pathak DR. 2004. Glucosinolate derivatives in stored fermented cabbage. *J. Agric. Food Chem.* 52:7938–43

Ciska E, Verkerk R, Honke J. 2009. Effect of boiling on the content of ascorbigen, indole-3-carbinol, indole-3-acetonitrile, and 3,3′-diindolylmethane in fermented cabbage. *J. Agric. Food Chem.* 57:2334–38

Connolly EL, Sim M, Travica N, Marx W, Beasy G, et al. 2021. Glucosinolates from cruciferous vegetables and their potential role in chronic disease: investigating the preclinical and clinical evidence. *Front. Pharmacol.* 12:767975

Cunningham MW Jr., Sasser JM, West CA, Milani CJ, Baylis C, Mitchell KD. 2013. Renal nitric oxide synthase and antioxidant preservation in Cyp1a1-Ren-2 transgenic rats with inducible malignant hypertension. *Am. J. Hypertens.* 26:1242–49

El-Daly SM, Gamal-Eldeen AM, Gouhar SA, Abo-Elfadl MT, El-Saeed G. 2020. Modulatory effect of indoles on the expression of miRNAs regulating G1/S cell cycle phase in breast cancer cells. *Appl. Biochem. Biotechnol.* 192:1208–23

El-Naga RN, Mahran YF. 2016. Indole-3-carbinol protects against cisplatin-induced acute nephrotoxicity: role of calcitonin gene-related peptide and insulin-like growth factor-1. *Sci. Rep.* 6:29857

Fahey JW, Zalcmann AT, Talalay P. 2001. The chemical diversity and distribution of glucosinolates and isothiocyanates among plants. *Phytochemistry* 56:5–51

Fahey JW, Zhang Y, Talalay P. 1997. Broccoli sprouts: an exceptionally rich source of inducers of enzymes that protect against chemical carcinogens. *PNAS* 94:10367–72

Hajra S, Patra AR, Basu A, Saha P, Bhattacharya S. 2018. Indole-3-carbinol (I3C) enhances the sensitivity of murine breast adenocarcinoma cells to doxorubicin (DOX) through inhibition of NF-κβ, blocking angiogenesis and regulation of mitochondrial apoptotic pathway. *Chem. Biol. Interact.* 290:19–36

Halkier BA, Gershenzon J. 2006. Biology and biochemistry of glucosinolates. *Annu. Rev. Plant Biol.* 57:303–33

Hanschen FS, Lamy E, Schreiner M, Rohn S. 2014. Reactivity and stability of glucosinolates and their breakdown products in foods. *Angew. Chem. Int. Ed.* 53:11430–50

Holst B, Williamson G. 2004. A critical review of the bioavailability of glucosinolates and related compounds. *Nat. Prod. Rep.* 21:425–47

Jayakumar P, Pugalendi KV, Sankaran M. 2014. Attenuation of hyperglycemia-mediated oxidative stress by indole-3-carbinol and its metabolite 3,3′-diindolylmethane in C57BL/6J mice. *J. Physiol. Biochem.* 70:525–34

Jeon EJ, Davaatseren M, Hwang JT, Park JH, Hur HJ, et al. 2016. Effect of oral administration of 3,3′-diindolylmethane on dextran sodium sulfate-induced acute colitis in mice. *J. Agric. Food Chem.* 64:7702–9

Jiang J, Kang TB, Shim DW, Oh NH, Kim TJ, Lee KH. 2013. Indole-3-carbinol inhibits LPS-induced inflammatory response by blocking TRIF-dependent signaling pathway in macrophages. *Food Chem. Toxicol.* 57:256–61

Jiang Y, Yang G, Liao Q, Zou Y, Du Y, Huang J. 2019. Indole-3-carbinol inhibits lipid deposition and promotes autophagy in hyperlipidemia zebrafish larvae. *Environ. Toxicol. Pharmacol.* 70:103205

Jump SM, Kung J, Staub R, Kinseth MA, Cram EJ, et al. 2008. N-Alkoxy derivatization of indole-3-carbinol increases the efficacy of the G1 cell cycle arrest and of I3C-specific regulation of cell cycle gene transcription and activity in human breast cancer cells. *Biochem. Pharmacol.* 75:713–24

Kandala PK, Srivastava SK. 2010. Activation of checkpoint kinase 2 by 3,3′-diindolylmethane is required for causing G2/M cell cycle arrest in human ovarian cancer cells. *Mol. Pharmacol.* 78:297–309

Katz E, Nisani S, Chamovitz DA. 2018. Indole-3-carbinol: a plant hormone combatting cancer. *F1000Research* 7:689

Khan AS, Langmann T. 2020. Indole-3-carbinol regulates microglia homeostasis and protects the retina from degeneration. *J. Neuroinflammation* 17:327

Kim EJ, Park H, Kim J, Park JH. 2010. 3,3′-Diindolylmethane suppresses 12-O-tetradecanoylphorbol-13-acetate-induced inflammation and tumor promotion in mouse skin via the downregulation of inflammatory mediators. *Mol. Carcinog.* 49:672–83

Kim EJ, Park SY, Shin HK, Kwon DY, Surh YJ, Park JH. 2007. Activation of caspase-8 contributes to 3,3′-diindolylmethane-induced apoptosis in colon cancer cells. *J. Nutr.* 137:31–36

Kim EJ, Shin M, Park H, Hong JE, Shin HK, et al. 2009. Oral administration of 3,3′-diindolylmethane inhibits lung metastasis of 4T1 murine mammary carcinoma cells in BALB/c mice. *J. Nutr.* 139:2373–79

Kim EK, Kim YS, Milner JA, Wang TT. 2013. Indole-3-carbinol and 3′,3′-diindolylmethane modulate androgen's effect on C-C chemokine ligand 2 and monocyte attraction to prostate cancer cells. *Cancer Prev. Res.* 6:519–29

Kim HW, Kim J, Lee S, Choi BR, Han JS, et al. 2014. 3,3′-Diindolylmethane inhibits lipopolysaccharide-induced microglial hyperactivation and attenuates brain inflammation. *Toxicol. Sci.* 137:158–67

Kim JH, Jander G. 2007. *Myzus persicae* (green peach aphid) feeding on *Arabidopsis* induces the formation of a deterrent indole glucosinolate. *Plant J.* 49:1008–19

Kim SJ, Lee JS, Kim SM. 2012. 3,3′-Diindolylmethane suppresses growth of human esophageal squamous cancer cells by G1 cell cycle arrest. *Oncol. Rep.* 27:1669–73

Kim YH, Kwon HS, Kim DH, Shin EK, Kang YH, et al. 2009. 3,3′-Diindolylmethane attenuates colonic inflammation and tumorigenesis in mice. *Inflamm. Bowel Dis.* 15:1164–73

Kong D, Banerjee S, Huang W, Li Y, Wang Z, et al. 2008. Mammalian target of rapamycin repression by 3,3′-diindolylmethane inhibits invasion and angiogenesis in platelet-derived growth factor-D-overexpressing PC3 cells. *Cancer Res.* 68:1927–34

Leader CJ, Clark BJ, Hannah AR, Sammut IA, Wilkins GT, Walker RJ. 2018. Breeding characteristics and dose-dependent blood pressure responses of transgenic Cyp1a1-Ren2 rats. *Comp. Med.* 68:360–66

Lee CM, Lee J, Nam MJ, Park SH. 2018. Indole-3-carbinol induces apoptosis in human osteosarcoma MG-63 and U2OS cells. *Biomed. Res. Int.* 2018:7970618

Lee CM, Park SH, Nam MJ. 2019. Anticarcinogenic effect of indole-3-carbinol (I3C) on human hepatocellular carcinoma SNU449 cells. *Hum. Exp. Toxicol.* 38:136–47

Lee J, Yue Y, Park Y, Lee SH. 2017. 3,3′-Diindolylmethane suppresses adipogenesis using AMPKα-dependent mechanism in 3T3-L1 adipocytes and *Caenorhabditis elegans*. *J. Med. Food* 20:646–52

Lee SH, Min KW, Zhang X, Baek SJ. 2013. 3,3′-Diindolylmethane induces activating transcription factor 3 (ATF3) via ATF4 in human colorectal cancer cells. *J. Nutr. Biochem.* 24:664–71

Li F, Xu Y, Chen C, Chen SM, Xiao BK, Tao ZZ. 2015. Pro-apoptotic and anti-proliferative effects of 3,3′-diindolylmethane in nasopharyngeal carcinoma cells via downregulation of telomerase activity. *Mol. Med. Rep.* 12:3815–20

Li WX, Chen LP, Sun MY, Li JT, Liu HZ, Zhu W. 2015. 3′3-Diindolylmethane inhibits migration, invasion and metastasis of hepatocellular carcinoma by suppressing FAK signaling. *Oncotarget* 6:23776–92

Lian JP, Word B, Taylor S, Hammons GJ, Lyn-Cook BD. 2004. Modulation of the constitutive activated STAT3 transcription factor in pancreatic cancer prevention: effects of indole-3-carbinol (I3C) and genistein. *Anticancer Res.* 24:133–37

Liang K, Qian WH, Zong J. 2017. 3,3′-Diindolylmethane attenuates cardiomyocyte hypoxia by modulating autophagy in H9c2 cells. *Mol. Med. Rep.* 16:9553–60

Lim HM, Park SH, Nam MJ. 2021. Induction of apoptosis in indole-3-carbinol-treated lung cancer H1299 cells via ROS level elevation. *Hum. Exp. Toxicol.* 40:812–25

Lin H, Gao X, Chen G, Sun J, Chu J, et al. 2015. Indole-3-carbinol as inhibitors of glucocorticoid-induced apoptosis in osteoblastic cells through blocking ROS-mediated Nrf2 pathway. *Biochem. Biophys. Res. Commun.* 460:422–27

Ludwig-Müller J, Pieper K, Ruppel M, Cohen JD, Epstein E, et al. 1999. Indole glucosinolate and auxin biosynthesis in *Arabidopsis thaliana* (L.) Heynh. glucosinolate mutants and the development of clubroot disease. *Planta* 208:409–19

Marconett CN, Sundar SN, Tseng M, Tin AS, Tran KQ, et al. 2011. Indole-3-carbinol downregulation of telomerase gene expression requires the inhibition of estrogen receptor-alpha and Sp1 transcription factor interactions within the hTERT promoter and mediates the G1 cell cycle arrest of human breast cancer cells. *Carcinogenesis* 32:1315–23

Megna BW, Carney PR, Nukaya M, Geiger P, Kennedy GD. 2016. Indole-3-carbinol induces tumor cell death: function follows form. *J. Surg. Res.* 204:47–54

Meng Q, Qi M, Chen DZ, Yuan R, Goldberg ID, et al. 2000. Suppression of breast cancer invasion and migration by indole-3-carbinol: associated with up-regulation of BRCA1 and E-cadherin/catenin complexes. *J. Mol. Med.* 78:155–65

Mewis I, Khan MA, Glawischnig E, Schreiner M, Ulrichs C. 2012. Water stress and aphid feeding differentially influence metabolite composition in *Arabidopsis thaliana* (L.). *PLOS ONE* 7:e48661

Michnovicz JJ. 1998. Increased estrogen 2-hydroxylation in obese women using oral indole-3-carbinol. *Int. J. Obes. Relat. Metab. Disord.* 22:227–29

Mohamad KA, El-Naga RN, Wahdan SA. 2022. Neuroprotective effects of indole-3-carbinol on the rotenone rat model of Parkinson's disease: impact of the SIRT1-AMPK signaling pathway. *Toxicol. Appl. Pharmacol.* 435:115853

Mohammadi S, Memarian A, Sedighi S, Behnampour N, Yazdani Y. 2018. Immunoregulatory effects of indole-3-carbinol on monocyte-derived macrophages in systemic lupus erythematosus: a crucial role for aryl hydrocarbon receptor. *Autoimmunity* 51:199–209

Munakarmi S, Shrestha J, Shin HB, Lee GH, Jeong YJ. 2021. 3,3′-diindolylmethane suppresses the growth of hepatocellular carcinoma by regulating its invasion, migration, and ER stress-mediated mitochondrial apoptosis. *Cells* 10(5):1178

Pedras MS, Nycholat CM, Montaut S, Xu Y, Khan AQ. 2002. Chemical defenses of crucifers: elicitation and metabolism of phytoalexins and indole-3-acetonitrile in brown mustard and turnip. *Phytochemistry* 59:611–25

Peng C, Wu C, Xu X, Pan L, Lou Z, et al. 2021. Indole-3-carbinol ameliorates necroptosis and inflammation of intestinal epithelial cells in mice with ulcerative colitis by activating aryl hydrocarbon receptor. *Exp. Cell Res.* 404:112638

Peters B, Grisk O, Becher B, Wanka H, Kuttler B, et al. 2008. Dose-dependent titration of prorenin and blood pressure in Cyp1a1ren-2 transgenic rats: absence of prorenin-induced glomerulosclerosis. *J. Hypertens.* 26:102–9

Petersen BL, Chen S, Hansen CH, Olsen CE, Halkier BA. 2002. Composition and content of glucosinolates in developing *Arabidopsis thaliana*. *Planta* 214:562–71

Poornima J, Mirunalini S. 2014. Regulation of carbohydrate metabolism by indole-3-carbinol and its metabolite 3,3′-diindolylmethane in high-fat diet-induced C57BL/6J mice. *Mol. Cell. Biochem.* 385:7–15

Prado NJ, Ramirez D, Mazzei L, Parra M, Casarotto M, et al. 2022. Anti-inflammatory, antioxidant, antihypertensive, and antiarrhythmic effect of indole-3-carbinol, a phytochemical derived from cruciferous vegetables. *Heliyon* 8:e08989

Qian C, Yang C, Lu M, Bao J, Shen H, et al. 2021. Activating AhR alleviates cognitive deficits of Alzheimer's disease model mice by upregulating endogenous Aβ catabolic enzyme neprilysin. *Theranostics* 11:8797–812

Rajoria S, Suriano R, Wilson YL, Schantz SP, Moscatello A, et al. 2011. 3,3′-Diindolylmethane inhibits migration and invasion of human cancer cells through combined suppression of ERK and AKT pathways. *Oncol. Rep.* 25:491–97

Rana JS, Khan SS, Lloyd-Jones DM, Sidney S. 2021. Changes in mortality in top 10 causes of death from 2011 to 2018. *J. Gen. Intern. Med.* 36:2517–18

Riby JE, Firestone GL, Bjeldanes LF. 2008. 3,3′-diindolylmethane reduces levels of HIF-1α and HIF-1 activity in hypoxic cultured human cancer cells. *Biochem. Pharmacol.* 75:1858–67

Rungapamestry V, Duncan AJ, Fuller Z, Ratcliffe B. 2006. Changes in glucosinolate concentrations, myrosinase activity, and production of metabolites of glucosinolates in cabbage (*Brassica oleracea* var. *capitata*) cooked for different durations. *J. Agric. Food Chem.* 54:7628–34

Saini N, Akhtar A, Chauhan M, Dhingra N, Pilkhwal Sah S. 2020. Protective effect of indole-3-carbinol, an NF-κB inhibitor in experimental paradigm of Parkinson's disease: in silico and in vivo studies. *Brain Behav. Immun.* 90:108–37

Sarkar FH, Rahman KM, Li Y. 2003. Bax translocation to mitochondria is an important event in inducing apoptotic cell death by indole-3-carbinol (I3C) treatment of breast cancer cells. *J. Nutr.* 133:2434S–39S

Siegel RL, Miller KD, Fuchs HE, Jemal A. 2021. Cancer statistics, 2021. *CA Cancer J. Clin.* 71:7–33

Song L, Thornalley PJ. 2007. Effect of storage, processing and cooking on glucosinolate content of *Brassica* vegetables. *Food Chem. Toxicol.* 45:216–24

Tian X, Liu K, Zu X, Ma F, Li Z, et al. 2019. 3,3′-Diindolylmethane inhibits patient-derived xenograft colon tumor growth by targeting COX1/2 and ERK1/2. *Cancer Lett.* 448:20–30

Tsai JT, Liu HC, Chen YH. 2010. Suppression of inflammatory mediators by cruciferous vegetable-derived indole-3-carbinol and phenylethyl isothiocyanate in lipopolysaccharide-activated macrophages. *Mediat. Inflamm.* 2010:293642

Vallejo F, Tomás-Barberán F, García-Viguera C. 2002. Glucosinolates and vitamin C content in edible parts of broccoli florets after domestic cooking. *Eur. Food Res. Technol.* 215:310–16

Velasco P, Cartea ME, Gonzalez C, Vilar M, Ordas A. 2007. Factors affecting the glucosinolate content of kale (*Brassica oleracea* acephala group). *J. Agric. Food Chem.* 55:955–62

Verkerk R, Dekker M, Jongen WM. 2001. Post-harvest increase of indolyl glucosinolates in response to chopping and storage of *Brassica* vegetables. *J. Sci. Food Agric.* 81:953–58

Wang ML, Lin SH, Hou YY, Chen YH. 2016. Suppression of lipid accumulation by indole-3-carbinol is associated with increased expression of the aryl hydrocarbon receptor and CYP1B1 proteins in adipocytes and with decreased adipocyte-stimulated endothelial tube formation. *Int. J. Mol. Sci.* 17(8):1256

Wang ML, Shih CK, Chang HP, Chen YH. 2012. Antiangiogenic activity of indole-3-carbinol in endothelial cells stimulated with activated macrophages. *Food Chem.* 134:811–20

Wang X, Zhao Y, Yu M, Xu Y. 2020. PTEN/Akt signaling-mediated activation of the mitochondrial pathway contributes to the 3,3′-diindolylmethane-mediated antitumor effect in malignant melanoma cells. *J. Med. Food* 23:1248–58

Wang YQ, Chen C, Chen Z, Xu Y, Wang Y, et al. 2013. Indole-3-carbinol inhibits cell proliferation and induces apoptosis in Hep-2 laryngeal cancer cells. *Oncol. Rep.* 30:227–33

Williams DE. 2021. Indoles derived from glucobrassicin: cancer chemoprevention by indole-3-carbinol and 3,3′-diindolylmethane. *Front. Nutr.* 8:734334

Williams DJ, Critchley C, Pun S, Chaliha M, O'Hare TJ. 2010. Key role of Fe^{2+} in epithiospecifier protein activity. *J. Agric. Food Chem.* 58:8512–21

Windsor AJ, Reichelt M, Figuth A, Svatos A, Kroymann J, et al. 2005. Geographic and evolutionary diversification of glucosinolates among near relatives of *Arabidopsis thaliana* (Brassicaceae). *Phytochemistry* 66:1321–33

Wittstock U, Halkier BA. 2002. Glucosinolate research in the *Arabidopsis* era. *Trends Plant Sci.* 7:263–70

Wu TY, Saw CL, Khor TO, Pung D, Boyanapalli SS, Kong AN. 2012. In vivo pharmacodynamics of indole-3-carbinol in the inhibition of prostate cancer in transgenic adenocarcinoma of mouse prostate (TRAMP) mice: involvement of Nrf2 and cell cycle/apoptosis signaling pathways. *Mol. Carcinog.* 51:761–70

Wu Y, Wang J, He Q, Yu L, Pham Q, et al. 2020. Dietary indole-3-carbinol alleviated spleen enlargement, enhanced IgG response in C3H/HeN mice infected with *Citrobacter rodentium*. *Nutrients* 12(10):3148

Xu Y, Zhang J, Shi W, Liu Y. 2013. Anticancer effects of 3,3′-diindolylmethane are associated with G1 arrest and mitochondria-dependent apoptosis in human nasopharyngeal carcinoma cells. *Oncol. Lett.* 5:655–62

Yang H, Seo SG, Shin SH, Min S, Kang MJ, et al. 2017. 3,3′-Diindolylmethane suppresses high-fat diet-induced obesity through inhibiting adipogenesis of pre-adipocytes by targeting USP2 activity. *Mol. Nutr. Food Res.* 61(10):1700119

Yu TY, Pang WJ, Yang GS. 2015. 3,3′-Diindolylmethane increases bone mass by suppressing osteoclastic bone resorption in mice. *J. Pharmacol. Sci.* 127:75–82

Yuan GF, Sun B, Yuan J, Wang QM. 2009. Effects of different cooking methods on health-promoting compounds of broccoli. *J. Zhejiang Univ. Sci. B* 10:580–88

Zhang Z, Ober JA, Kliebenstein DJ. 2006. The gene controlling the quantitative trait locus EPITHIOSPECIFIER MODIFIER1 alters glucosinolate hydrolysis and insect resistance in *Arabidopsis*. *Plant Cell* 18:1524–36

Zhu P, Yu H, Zhou K, Bai Y, Qi R, Zhang S. 2020. 3,3′-Diindolylmethane modulates aryl hydrocarbon receptor of esophageal squamous cell carcinoma to reverse epithelial-mesenchymal transition through repressing RhoA/ROCK1-mediated COX2/PGE2 pathway. *J. Exp. Clin. Cancer Res.* 39:113