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COMMENTARY

Glucoraphanin: a broccoli sprout extract that ameliorates obesity-induced inflammation and insulin resistance

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ABSTRACT

Obesity is a low-grade sustained inflammatory state that causes oxidative stress in different metabolic tissues, which leads to insulin resistance and nonalcoholic fatty liver disease (NAFLD). Particularly, obesity-induced metabolic endotoxemia plays an important role in the pathogenesis of insulin resistance and inflammation. Nuclear factor erythroid 2-related factor 2 (Nrf2) is a key regulator of antioxidant signaling that serves as a primary cellular defense against the cytotoxic effects of oxidative stress. Pharmacological stimulation of Nrf2 mitigates obesity and insulin resistance in mice; however, Nrf2 activators are not clinically available due to biosafety concerns. A recent study demonstrated that glucoraphanin, a precursor of the Nrf2 activator sulforaphane, ameliorates obesity by enhancing energy expenditure and browning of white adipose tissue, and attenuates obesity-related inflammation and insulin resistance by polarizing M2 macrophages and reducing metabolic endotoxemia. Thus, this review focuses on the efficiency and safety of glucoraphanin in alleviating obesity, insulin resistance, and NAFLD.

Abbreviations: ALT, Alanine aminotransferase; AMPK, AMP-activated protein kinase; ATMs, Adipose tissue macrophages; BAT, Brown adipose tissue; CDDO-Im, 2-cyano-3,12-dioxoolean-1,9-dien-28-oic acid-imidazolide; CDDO-Me, CDDO-methyl ester; DIO, High-fat-diet-induced obese; FFA, Free fatty acid; FGF, Fibroblast growth factor; GTP, Glutamyl transpeptidase; HFD, High-fat diet; IKKβ, Inhibitor of κB-kinase β; IL, Interleukin; JNK, C-Jun N-terminal kinase; KD, Knockdown; Keap1, Kelch-like ECH-associated protein 1; KO, Knockout; LPS, Lipopolysaccharide; NADPH, Nicotinamide adenine dinucleotide phosphate; NAFLD, Non-alcoholic fatty liver disease; NF-κB, Nuclear factor-κB; Nrf2, Nuclear factor E2-related factor 2; ROS, Reactive oxygen species; T2D, Type 2 diabetes; TLR, Toll-like receptor; TNF, tumor necrosis factor; UCP, Uncoupling protein; WAT, White adipose tissue.

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Obesity activates inflammatory signaling and insulin resistance

Obesity has dramatically increased worldwide and leads to many adverse metabolic disorders including cardiovascular disease, type 2 diabetes (T2D), and nonalcoholic fatty liver disease (NAFLD). Caloric excess or obesity activates the innate immune system. Hotamisligil et al. found the level of tumor neurosis factor α (TNFα) increased in adipose tissue of obese mice compare with that in lean controls.[1] Moreover, inflammatory state of obesity is increased infiltration of immune cells, including adipose tissue macrophages (ATMs) and T cells, into the metabolic tissues.[2,3] Immune cell-derived cytokines and chemokines augment metabolic tissue inflammation, inducing insulin resistance.[4,5] In particular, macrophages represent a heterogeneous population of cells that are instrumental in initiating the innate and adaptive immune response to infection. In addition, they are crucial mediators of obesity-related insulin resistance, with a progressive infiltration of macrophages into obese adipose tissue and liver.[6] Importantly, ATMs play an essential role in the development of chronic inflammation during obesity. In obese subjects, ATMs are referred to as proinflammatory (M1) macrophages, which release proinflammatory cytokines such as interleukin (IL)-1β, IL-6, and TNFα, creating a proinflammatory environment that blocks adipocyte insulin action, contributing to the development of insulin resistance and T2D. By contrast, anti-inflammatory (M2) macrophages accumulate and modulate adipocyte lipid metabolism by secreting anti-inflammatory cytokines such as IL-10 and catecholamines.[7]

Insulin resistance is characterized by a decrease in insulin signaling mainly in the insulin receptor substrate/phosphatidylinositol 3 kinase-AKT/protein kinase

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B pathway, which is responsible for most of the metabolic actions of the hormone.[2,8] ATM-derived proinflammatory cytokines such as TNFα and IL-6 activate key regulators of inflammation such as c-Jun N-terminal kinase (JNK) and inhibitor of κB-kinase β (IKKβ) within insulin target cells. Under obese conditions, the activation of JNK and IKKβ stimulates proinflammatory transcription factors including activator protein 1 (c-Jun/Fos) and nuclear factor-κB (NF-κB), leading to serine phosphorylation of the insulin receptor substrate that interferes with insulin action.[9,10] In addition, toll-like receptors (TLRs) play a role in inflammation and insulin resistance during the development of obesity. Particularly, TLR4 expression is elevated in obese subjects and diabetic patients and is negatively correlated with insulin sensitivity. TLR4 activity activates both JNK and IKKβ, with the subsequent inhibition of insulin sensitivity.[11] Moreover, a deficiency in TLR4 protects obese mice from lipid flux-induced inflammation and insulin resistance by decreasing TNFα and IL-6 expression and reducing NF-κB activity.[12]

Numerous dietary factors including saturated fatty acids and glucose change the gut microbiota, causing dysbiosis. Dysbiosis may trigger metabolic inflammation in obese patients; the consequences are the production of proinflammatory cytokines and the recruitment of immune cells in metabolic tissues.[4] Inflammatory cytokines activate several kinases such as IKKβ, mTOR/S6 kinase, and MAP kinases that interfere with insulin signaling and action in adipocytes and hepatocytes.[13] Gut microbiota-derived lipopolysaccharide (LPS) induces low-grade chronic inflammation, leading to insulin resistance in obesity, termed metabolic endotoxemia.[14,15] Metabolic endotoxemia is caused by a moderate elevation of circulating LPS from Gram-negative bacteria and develops due to changes in the composition of gut microbiota and an increase in gut permeability.[16] Metabolic endotoxemia contributes to the development of inflammation and metabolic disorders by activating TLR4 in adipose tissues, liver, and skeletal muscles.[15,17] Furthermore, gut-derived LPS induced insulin resistance in adipose tissue in mice fed a high-fat diet (HFD). However, a deficiency of the LPS receptor CD14 enhances insulin sensitivity and reduces weight gain in HFD-fed mice. Thus, the pathogenesis of metabolic endotoxemia contributing to the inflammation and insulin resistance is, at least in part, dependent on the LPS/CD14 axis.[15]

The Nrf2-keap1 system serves as a primary cellular defense against oxidative stress

Nuclear factor erythroid 2 (NF-E2)-related factor 2 (Nrf2), a basic leucine zipper transcription factor, is encoded by the *Nfe2l2* gene in humans. Nrf2 is widely expressed in murine tissues and serves as a defense mechanism against extrinsic and intrinsic stressors. It belongs to the cap 'n' collar basic leucine zipper family, together with p45 NF-E2, Nrf1, and Nrf3, and acts by forming a heterodimer with one of the small Maf proteins. Under normal conditions, Nrf2 molecules mainly reside in the cell cytoplasm by associating with kelch-like ECH-associated protein 1 (Keap1) and cullin 3. Cullin 3 ubiquitinates Nrf2. Keap1 is a substrate of cullin 3, which facilitates the ubiquitination of cullin 3. The coupling between Nrf2 and Keap1 leads to Nrf2 proteasomal degradation (Figure 1).[18] However, when electrophilic and oxidative stress occurs, Keap1 senses cellular oxidative stress and releases Nrf2, leading to increased levels of free Nrf2 and Nrf2 nuclear translocation. Then, nuclear Nrf2 binds to the consensus nucleotide sequence, an antioxidant response element, in the promoter regions of a battery of genes that encode antioxidant enzymes (Figure 1).[18,19] The target antioxidants include nicotinamide adenine dinucleotide phosphate (NADPH)-oxidase, quinone oxidoreductase 1, hemeoxygenase-1, glutathione S-transferase, superoxide dismutase, catalase, and γ-glutamate cysteine ligase. In this manner, Nrf2 acts as a primary cellular defender against the cytotoxic effects of oxidative stress.

Nrf2-keap1 plays a vital role in anti-obesity

Oxidative stress is closely associated with energy metabolism, leading to metabolic syndromes, including obesity, diabetes, and NAFLD. Moreover, oxidative stress is a key factor involved in obesity-related comorbidities, such as insulin resistance, by increasing the production of cellular reactive oxygen species (ROS). Obese subjects have elevated levels of systemic oxidative stress, and show inhibition of energy metabolism in metabolic tissues.[20] Administration of NAPDH oxidase inhibitor reduces the concentration of ROS in adipose tissue of obese mice, resulting in improved hyperlipidemia and hepatic steatosis.[20] In addition, the Nrf2 pathway reportedly modulates a large number of genes involved in glucose and lipid metabolism. In the liver, the constitutive activation of Nrf2 via Keap1 knockdown (KD) represses the expression of genes involved in gluconeogenesis and lipogenesis, thereby alleviating obesity, diabetes, and hepatic steatosis.[21,22] These results suggest that the Nrf2 pathway may be a promising target for treating metabolic syndromes.

To elucidate the effects of Nrf2 on obesity, many researchers have generated Nrf2 knockout (KO) or Keap1 KD mice and Nrf2 activators for investigation; however, the results are mostly inconsistent. A study by Pi et al. demonstrated that a deficiency in Nrf2 suppresses adipose differentiation, reduces fat mass, and

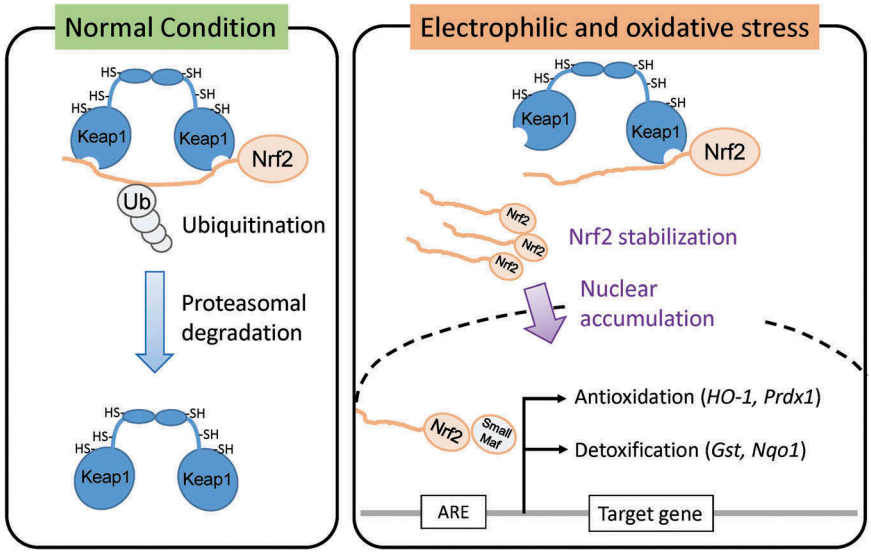

Figure 1. The Nrf2–Keap1 signaling pathway. Under normal conditions, Keap1 binds to Nrf2 and Nrf2 is polyubiquitylated by the Cullin3-based E3 ligase complex, leading to ubiquitination and subsequent degradation of Nrf2. Upon exposure to electrophilic and oxidative stress, Nrf2 detaches from its repressor Keap1, and is translocated from the cytoplasm into the nucleus. Nrf2 becomes stabilized, and then nuclear Nrf2 binds to the antioxidant response element (ARE) with members of the small Maf family in the promoter regions of a battery of genes that encode antioxidant and phase 2 detoxifying enzymes, including HO-1, Prdx1, Nqo1, and Gst.

protects against HFD-induced obesity by impairing lipogenesis. Transfection of Nrf2 directly stimulates Pparγ promoter activity, and stable knockdown of Keap1 enhances *Pparγ* expression in 3T3-L1 cells.[23] In Chartoumpekis et al., Nrf2 regulated fibroblast growth factor (*Fgf*) *21* gene expression and circulating FGF21 levels in response to an obesogenic diet, which suggests that increases in FGF21 are a potential mediator of the Nrf2-KO protection against obesity and insulin resistance in HFD-induced obese mice.[24] Moreover, an increase in Nrf2 activation deteriorates insulin resistance, decreases lipid accumulation in adipose tissues, and increases hepatic steatosis in Keap1-KD *ob/ob* mice.[25] Conversely, Zhang et al. found that activated Nrf2 decreases methionine- and choline-deficient diet-induced hepatic steatosis by inhibiting lipid deposition and *Cd36, Fgf21*, and *Pparα* expression in the liver.[26] Furthermore, a myeloid-specific deficiency of Nrf2 increases atherosclerosis and liver injury in mice fed an HFD/cholesterol diet, and does not change the fat percentage or body weight.[27] These results suggest that differences in the experimental design and the genetic background of the mice were responsible for the discrepancies regarding the role of Nrf2 in obesity.

To date, many Nrf2 activators have been used in investigations on the effects of Nrf2 on obesity and its comorbidities. Shin et al. first reported the effects of Nrf2 activator on obesity using triterpenoid 2-cyano-3,12-dioxoolean-1,9-dien-28-oic acid-imidazolide (CDDO-Im), a synthetic Nrf2 inducer.[21] The authors demonstrated that CDDO-Im effectively prevents HFD-induced weight gain, adipose mass, and hepatic fat accumulation in wild-type mice by enhancing energy expenditure and suppressing fatty acid synthesis. Subsequently, several natural and synthetic Nrf2 inducers have been proven effective against obesity. Treatment with CDDO-methyl ester (CDDO-Me), another Nrf2 agonist, reduces total body fat, plasma lipids levels, and improves glucose tolerance and insulin resistance in HFD-fed mice. CDDO-Me activates AMP-activated protein kinase (AMPK) via LKB1 activation both *in vivo* and *in vitro*.[28] Yu et al. showed that oltipraz stimulates Nrf2 activation, leading to the prevention of insulin resistance and obesity caused by an HFD.[29] Thus, the activation of Nrf2 by an Nrf2 activator could constitute the basis of a therapy for obesity. However, in clinical trials Nrf2 pathways enhanced by synthetic agonists exhibited adverse cardiac events and gastrointestinal toxicities.[30,31] Based on these observations, we explored a safer Nrf2 inducer for the treatment of obesity, insulin resistance, and NAFLD.

Sulforaphane, one of the most potent natural Nrf2 inducers derived from broccoli sprouts, has cancer-preventing effects by detoxifying chemical compounds absorbed into the body and enhancing antioxidation ability.[32] Treatment with sulforaphane induces pharmacological Nrf2 activation, subsequently affecting adipocyte differentiation and preventing adipogenesis and lipid accumulation.[26] Glucoraphanin, a stable glucosinolate precursor of sulforaphane, is mainly derived from broccoli sprouts.[30,33] In both rodents and humans, glucoraphanin is hydrolyzed by gut microbiota-derived myrosinase into bioactive sulforaphane before intestinal absorption.[33] Importantly,

emerging evidence has demonstrated the safety of orally administered glucoraphanin. In one study, the administration of 69 μmol/day glucoraphanin for 60 days decreased plasma levels of liver function enzymes, including ALT, γ-GTP and alkali phosphatase activity, suggesting the liver dysfunction was ameliorated.[34] In another study, doses of glucoraphanin up to 800 μmol/day did not cause clinically significant safety concerns or harmful adverse effects.[35] Therefore, in this review, we present the effects of glucoraphanin on obesity-related inflammation, insulin resistance, and energy homeostasis in HFD-fed mice.

Glucoraphanin decreases lipid accumulation and increases Nrf2-dependent energy expenditure

Abnormal or excessive energy is stored as triglycerides in adipocytes. Historical analyses have revealed that white adipose tissue (WAT) is primarily a fuel-storage depot. Moreover, it modulates appetite, inflammation, and insulin action by secreting free fatty acids (FFAs), hormones, and cytokines and/or chemokines into the circulation. Particularly, the release of excess FFAs from lipolysis of visceral adipose tissue into the circulation or portal vein inhibits the functions of other metabolic tissues, leading to the formation of adiposity and obesity.[36] Thus, inhibiting the accumulation of triglycerides or enhancing excess energy expenditure is the primary treatment for obesity.

Previous studies have demonstrated the protective effects of Nrf2 on obesity and obesity-related comorbidities using Nrf2-KO or Keap1-KD, as well as synthetic Nrf2 inducers. Consistent with previous reports that have demonstrated the anti-obesity effects of synthetic Nrf2 inducers, Nagata et al. showed that oral administration of glucoraphanin mitigates weight gain and attenuates fat mass in HFD-fed mice without affecting food intake. This suggests that glucoraphanin helps reduce body weight and adiposity and is independent of food intake.[37] The dose of glucoraphanin used in that study, approximately 12 μmol/mouse/day, was similar to the doses used in other experiments that have investigated the antitumor effects of glucoraphanin in mice.[32,38] This reduction did not cause gross toxicity. Moreover, when the concentration of sulforaphane in plasma was evaluated, glucoraphanin was absorbed as a sulforaphane after food consumption. However, the glucoraphanin-induced reduction of body weight and fat mass was abolished in HFD-fed Nrf2-deficient mice, which suggests that the anti-obesity effects of glucoraphanin are due to the activation of the Nrf2 pathway. Shin et al. showed the Nrf2 activator CDDO-Im enhanced energy expenditure and suppressed fatty acid synthesis in HFD-fed mice.[21] In one study, placing glucoraphanin-treated obese mice in indirect calorimetry cages revealed that supplementation of glucoraphanin lead to increased energy expenditure (Figure 2).[37] This indicates that the mechanism underlying weight reduction partially depends on the enhancement of energy expenditure.

In addition to energy storage in WAT, brown adipose tissue (BAT) is responsible for the energy consumption through heat production in response to cold or excess calories, termed adaptive thermogenesis. The thermogenic system of BAT depends on uncoupling protein (UCP) 1, a protein expressed in the inner mitochondrial membrane of brown fat cells that dissipates the proton gradient generated during oxidative phosphorylation. Mice deficient in UCP1 are more susceptible to DIO; by contrast, transgenic mice with increased UCP1 in WAT are resistant to obesity.[39,40] Several studies have shown that certain depots of WAT acquire a BAT phenotype when subjected to certain stimuli. Brown-like adipocytes, also known as beige cells, express UCP1 and contribute to thermogenesis.[41,42] Interestingly, a lack of Nrf2 enhances energy expenditure and increases *Ucp1* expression in mice, with subsequent resistance to obesity.[43] Furthermore, Nrf2 acts as a positive regulator of beige adipocyte differentiation. Nrf2 induces white adipocyte differentiation by increasing the expression of the *Pparγ* and *Cebpβ* genes, which regulate the differentiation of brown, beige, and white adipocytes.[23,44] Recently, Nagata et al. showed that glucoraphanin upregulates UCP1 expression in beige adipocytes, leading to increased energy expenditure and higher body temperature (Figure 2).[37] However, these effects of glucoraphanin on whole-body energy expenditure and protein levels in WAT were abolished in Nrf2-KO mice. These findings indicate that glucoraphanin ameliorates adiposity and obesity by elevating energy expenditure and tissue browning, and depends on Nrf2 activation (Figure 2).

Glucoraphanin administration attenuates HFD-induced steatosis and oxidative stress, providing further evidence of the response of glucoraphanin against obesity-related comorbidities.[37] A HFD causes hepatic ectopic fat deposition and inflammation, eventually leading to steatohepatitis and impairment of liver function and hepatic lipid metabolism. However, treatment with glucoraphanin improves diet-induced liver dysfunction and attenuates fatty acid accumulation in the liver, indicative of protective effects against hepatic steatosis. Moreover, glucoraphanin downregulates lipogenesis-related gene expression and suppresses ectopic fat peroxidation-induced oxidative stress, the mechanisms underlying the attenuation of steatosis.

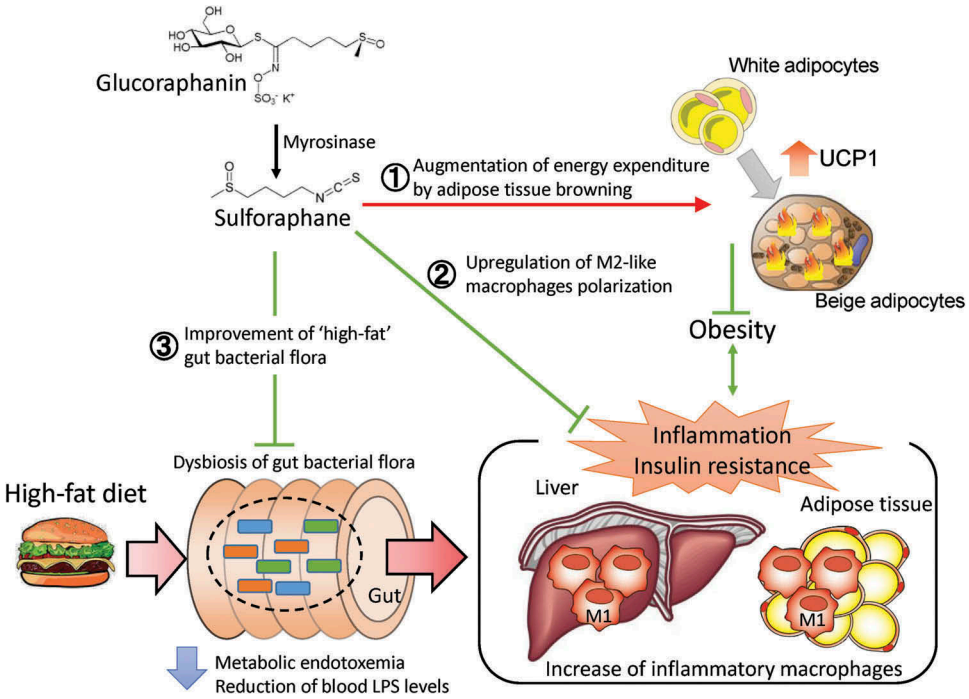

Figure 2. Schematic representation of the beneficial effects of glucoraphanin on obesity. Glucoraphanin is hydrolyzed by gut microbiota-derived myrosinase into bioactive sulforaphane, leading to the following: (1) Increased adipose tissue browning to augment energy consumption. (2) Enhanced M2 macrophage polarization resulting in M2-dominant shift of macrophages in the liver and white adipose tissue. (3) Improved 'high-fat' gut bacterial flora, with subsequent reduction of blood LPS levels and metabolic endotoxemia. Consequently, glucoraphanin attenuated the diet-induced adiposity and obesity-related inflammation and insulin resistance.

Glucoraphanin improves obesity-induced insulin resistance and inflammation by activating Nrf2

Oxidative stress is a main inducer of glucose metabolism and insulin resistance. Previous studies have shown that Nrf2 deficiency positively affects glucose homeostasis and insulin resistance. When modulating FGF21 expression in HFD-treated mice, greater glucose tolerance and insulin sensitivity is observed in Nrf2-deficient mice.[24] A lack of Nrf2 protects DIO mice from HFD-induced hyperglycemia and glucose intolerance.[26] Conversely, increased Nrf2 activity caused by knockdown of Keap1 expression in leptin-null mice leads to higher plasma glucose concentrations and deteriorated insulin resistance compared to *ob/ob* mice.[25] Interestingly, an *in vitro* study showed that expression of IL-1β in LPS- and TNFα-treated bone marrow-derived Nrf2-KO macrophages was lower compared to wild-type macrophages. Moreover, overall loss of Nrf2 protects obese mice from insulin resistance and adipose tissue inflammation. ATM infiltration and inflammatory gene expression are reduced in global Nrf2-deficient mice, resulting in improved insulin sensitivity.[27] However, myeloid-specific Nrf2-KO mice are less sensitive to insulin, which suggests that Nrf2 deficiency in myeloid cells is essential and negatively affects the development of insulin resistance by regulating inflammatory signaling.[27] Hongming et al. demonstrated that tenuigen extracted from the root of the Chinese herb *Polygala tenuifolia* suppresses inflammatory signaling by inhibiting the MAP kinase and NF-κB pathways, thereby inducing Nrf2 activation.[45] These findings suggest that the Nrf2-Keap1 pathway may play an important role in inflammatory signaling.

Nagata and colleagues found that glucoraphanin supplementation improves systemic glucose tolerance and insulin sensitivity in HFD-fed mice.[37] However, phosphorylation of AMPK and acetyl-CoA carboxylase in peripheral insulin target tissues was not significantly affected, which suggests that improvement of insulin resistance by glucoraphanin is AMPK-independent. Furthermore, glucoraphanin alleviates HFD-induced oxidative stress and chronic inflammation in the liver. Specifically, glucoraphanin administration attenuates the recruitment of macrophages and regulates the polarization of hepatic resident macrophages (i.e., Kuppfer cells) in the liver of DIO mice (Figure 2). Specific deletion of Kupffer cells or M1 macrophages alleviates diet-induced hepatic steatosis and insulin sensitivity.[46,47] Moreover, specific ablation of M1-like

macrophages restores insulin sensitivity in DIO mice. By contrast, suppressing M2 macrophage activation by blocking Pparδ predisposes lean mice to insulin resistance.[48] Therefore, the reduction of hepatic macrophage accumulation and M2-dominant polarization is partly responsible for the mitigation of hepatic steatosis and insulin resistance in glucoraphanin-treated mice. Notably, glucoraphanin fails to suppress the HFD-induced inflammatory signal pathway in the liver of Nrf2-KO mice, which suggests that glucoraphanin improves obesity-related insulin sensitivity and inflammation by stimulating Nrf2 in DIO mice.

Metabolic endotoxemia-related chronic inflammation eventually impairs insulin sensitivity in obesity. Studies have demonstrated a significant increase in Desulfovibrionaceae, potential endotoxin producers, in the gut microbiomes of obese subjects.[49,50] Notably, several studies have indicated that sulforaphane directly regulates the gut microbiota, because isothiocyanates (e.g., sulforaphane) have antibacterial activity against Proteobacteria.[51,52] Moreover, sulforaphane exhibits antibacterial activity against *Helicobacter pylori*, a member of the phylum Proteobacteria.[53] Importantly, glucoraphanin decreases the relative abundance of Gram-negative Proteobacteria, particularly the Desulfovibrionaceae family, while reducing circulatory LPS levels in DIO mice (Figure 2).[37] These findings indicate that glucoraphanin ameliorates insulin resistance, at least in part, by suppressing metabolic endotoxemia.

Conclusions and perspectives

Glucoraphanin acts against adiposity and hepatic steatosis by promoting energy utilization and preventing lipogenesis and oxidative stress in the liver. It increases UCP1 protein expression in white adipose depots and enhances browning in beige adipocytes (Figure 2). Furthermore, it attenuates obesity-induced inflammation and insulin resistance by regulating macrophage recruitment and M1/M2 status (Figure 2). Notably, the weight-reducing and insulin-sensitizing effects of glucoraphanin are abolished in Nrf2-KO mice, which suggests that the protective effects of glucoraphanin against obesity depend on Nrf2 signaling. Glucoraphanin-treated mice exhibit significantly low plasma LPS levels and a relatively low abundance of gut microbes of the Desulfovibrionaceae family of Gram-negative bacteria (Figure 2). Notably, results from glucoraphanin-treated lean mice indicate that glucoraphanin is safe for treating obesity. However, the main limitation of the present study is that the effects of glucoraphanin were evaluated on a preventive, not a therapeutic, treatment schedule. Thus extrapolating the results to humans is difficult. Therapeutic studies can benefit by utilizing experimental results regarding the anti-obesity effects of glucoraphanin in clinical settings.

Disclosure statement

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