

## Harnessing Polyphenols for metabolic health

According to two recently published research papers:

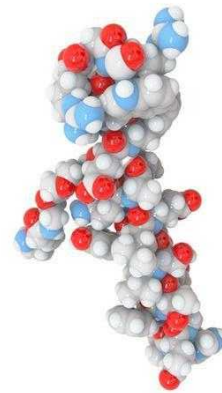
- **Physiological functions of poorly absorbed polyphenols via the glucagon-like peptide-1.**  
Yamashita Y., et al. (2024). *Bioscience, Biotechnology, and Biochemistry*, Oxford Press.
- **Grape pomace as a cardiometabolic health-promoting ingredient: Activity in intestinal environment**  
Taladrid D., et al. (2023) *Antioxidants*.

the polyphenols, fiber, and organic acids found in grape pomace provide significant health benefits when added to food or beverage formulas.

Polyphenols are plant-derived compounds with profound health benefits. For example, they play a key role in regulating glucagon-like peptide-1 (GLP-1), a hormone vital for glucose regulation, appetite suppression, and vascular health.

There are 20 known hormones that effect satiety and weight management. The other critical ones are:

- Peptide YY: also controls digestion and satiety
- cholecystikinin (CCK): controls digestion and satiety
- Polypeptide GIP: stimulates insulin secretion
- Incretin hormone: stimulates insulin secretion
- DPP-IV: inhibition can increase  
(Taladrid D., et al.)



GLP-1 molecule

Although *raw* polyphenols are often poorly absorbed, their activity within the gastrointestinal (GI) tract directly influences metabolic pathways.

**CDI puree**, derived from fermented grape pomace, serves as a rich source of these bioactive compounds, including procyanidins, anthocyanins, flavan-3-ols, and phenolic acids. The patented **CDI** fermentation process greatly enhances the bioavailability and potency of these compounds, particularly their ability to stimulate GLP-1 secretion.

### Polyphenol Activation of GLP-1 Secretion

GLP-1 is secreted by L-cells in the gut in response to nutrients and bioactive compounds. Procyanidins, especially tetrameric forms like cinnamtannin A2, are potent GLP-1 activators. These compounds stimulate the hormone's release by activating G-protein-coupled receptors (e.g., GPR40/120), which trigger calcium-dependent signaling pathways involving TRPM5 channels. Anthocyanins, such as delphinidin 3-rutinoside, enhance secretion through similar mechanisms. Together, these polyphenols amplify GLP-1 release, promoting better glucose regulation and metabolic health. (Taladrid D., et al.)

### CDI Fermentation Enhances Bioavailability

Fermentation enhances the efficacy of **CDI puree** by breaking down large polyphenols into smaller, more absorbable forms, such as catechins and phenolic acids. It also releases polyphenols bound to grape cell walls, improving their bioavailability. Once in the colon, gut microbiota metabolizes these polyphenols into bioactive compounds that further stimulate



GLP-1 secretion and provide systemic health benefits. This dual action highlights fermentation's role in unlocking the full potential of grape pomace (Yamashita Y., et al.)

### **Cardiometabolic and Gut Health Benefits**

Beyond GLP-1 secretion, polyphenols in **CDI** support cardiometabolic health by improving vascular function and regulating lipid profiles. They increase nitric oxide production, reduce oxidative stress, and inhibit enzymes involved in carbohydrate and lipid digestion, such as  $\alpha$ -amylase,  $\alpha$ -glucosidase, and lipase. Additionally, the dietary fiber in grape pomace modulates the gut microbiome, promoting short-chain fatty acid (SCFA) production. These SCFAs further enhance gut health, strengthen the intestinal barrier, and reduce systemic inflammation (Taladrid D., et al.; Yamashita Y., et al.)

### **Conclusion**

**CDI puree** combines the synergistic benefits of polyphenols and fermentation, creating a functional food with significant potential for managing metabolic health.

# Physiological functions of poorly absorbed polyphenols via the glucagon-like peptide-1

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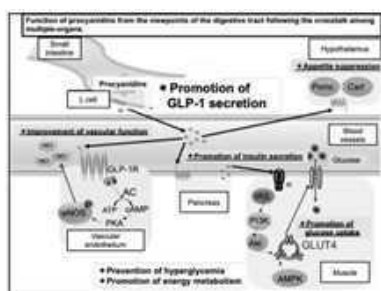
†This review was written in response to the author's receipt of the JSBBA Award for Young Scientists in 2022.

## Abstract

Polyphenols are compounds of plant origin with several documented bioactivities related to health promotion. Some polyphenols are hard to be absorbed into the body due to their structural characteristics. This review focuses on the health beneficial effects of polyphenols mediated by intestinal hormones, particularly related to the systemic functions through the secretion of glucagon-like peptide-1 (GLP-1), an enteric hormone that stimulates postprandial insulin secretion. **GLP-1 is secreted from L cells in the distal small intestine. Therefore, some poorly absorbed polyphenols are known to have the ability to act on the intestines and promote GLP-1 secretion. It has been reported that it not only reduces hyperglycemia but also prevents obesity by reduction of overeating and improves blood vessel function. This review discusses examples of health effects of polyphenols mediated by GLP-1 secretion.**

**Keywords:** polyphenols, glucagon-like peptide-1, hyperglycemia, obesity, vascular function

## Graphical abstract



The mechanisms of procyanidin of physiological functions.

**Abbreviations:** AMPK: AMP-activated protein kinase; BE: black soybean seed coat extract; C/EBP $\alpha$ : CAAT/enhancer binding protein  $\alpha$ ; cAMP: cyclic adenosine monophosphate; CLPr: cacao liquor procyanidin-rich extract; CVD: cardiovascular diseases; DP: degrees of polymerization; EGCG: epigallocatechin-3-gallate; ELISA: enzyme-linked immuno sorbent assay; eNOS: endothelial nitric oxide synthase; GI: gastrointestinal; GLP-1: glucagon-like peptide-1; GLUT4: glucose transporter 4; **GSPE: grape seed-derived proanthocyanidins**; ICR: Institute of Cancer Research; IR: insulin receptor; IRS: insulin receptor substrate; NO: nitric oxide; PKA: protein kinase A; PLC: phospholipase C; PPAR $\gamma$ : peroxisome proliferator-activated receptor  $\gamma$ ; TRPM5: transient receptor potential cation channel, subfamily M, member 5; UCP: uncoupling proteins

Polyphenols are secondary metabolites found in vegetables, fruits, grains, bark, tea, and wine. These compounds exhibit several health benefits such as immune modulators, vasodilators, antioxidants, and preventing life-style diseases. Based on their diverse chemical structures, polyphenols are classified into phenolic acids, flavonoids, stilbenes, and lignans (Pandey and Rizvi 2009). Phenolic acids are a group of compounds with a phenolic ring and carboxylic acid. Most dietary polyphenols exist naturally in the form of esters, glycosides, or polymers, many of which are poorly absorbed by the intestine. In general, poorly absorbable polyphenols are defined as those that are absorbed into the body at only

about 0.1%, and anthocyanins and condensed tannins belong to this category (Manach et al. 2004). One of the most extensively studied categories of polyphenols, are flavonoids. A majority of flavonoids are noncovalently bound to glucose in plants, and some are present as flavonoid oligomers such as epicatechin in cocoa. The hydrolysis of the glycoside form of flavonoids begins in the mouth where flavonoids are partially released from the food matrix by saliva (Walle et al. 2005). The low pH in the stomach facilitates the degradation of flavonoid oligomers into small units (Piskula 2000), but most flavonoids are hydrolyzed and further metabolized in the small intestine, where  $\beta$ -glucosidases cleave the

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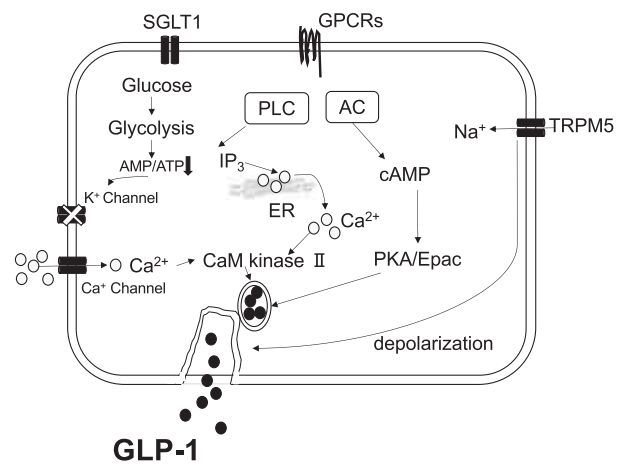
glucose moiety from flavonoids and generate the aglycone form (Day et al. 1998; Walle et al. 2000). A large proportion of ingested flavonoids do not undergo hydrolysis or conjugation in the small intestine (Crespy et al. 1999) and are not absorbed (Landete 2012). Instead, these unabsorbed flavonoids reach the colon where they are metabolized by colonic bacteria into smaller molecules such as phenolic acids, which can be partially absorbed into the circulation. Specifically, some intestinal bacteria strains have the enzymatic activity of glucuronidase and glucosidase that can deconjugate the glucuronidated or glycosylated forms of flavonoids, and yield simple deconjugated products that are partially absorbed and metabolized by colonic enterocytes (Feng et al. 2018). It is thus assumed that polyphenols exert various functions during their passage through the gastrointestinal (GI) tract (Lan et al. 2023; Osakabe, Fushimi and Fujii 2022). In fact, it has been reported that polyphenols exert effects such as secretion of various hormones, regulation of enzyme activities, and nerve stimulation, starting in the intestines. **Given the low bioavailability but relatively high concentrations of polyphenols in the GI tract following dietary ingestion, it is likely that the intestine might be a primary site for polyphenols to exert the observed metabolic actions (Barnes et al. 2011).** This review introduces the effect of polyphenol on health beneficial functions via the promotion of incretin hormone secretion.

The gut is the largest endocrine organ in the body and releases an array of hormones, which play many key roles in physiological and metabolic regulation. Specifically, GI hormones secreted after a meal play a critical role in regulating appetite, food intake, and glucose homeostasis (Koliaki et al. 2020). Glucagon-like peptide-1 (GLP-1) is a well-studied incretin hormone secreted by L cells in the gut, the second largest population of enteroendocrine cells. Incretin hormones are defined as gut peptides that are secreted after ingesting nutrients and stimulate insulin secretion (Nauck and Meier 2018). The role of GLP-1 in maintaining glucose homeostasis is mediated through its receptor (GLP-1R). GLP-1 binding to its receptor triggers cyclic adenosine monophosphate (cAMP) signaling that augments glucose-stimulated insulin secretion from pancreatic  $\beta$  cells while inhibiting glucagon release from  $\alpha$  cells. In addition, the activation of GLP-1 signaling promotes  $\beta$ -cell proliferation and survival, therefore preserving islet mass. Furthermore, GLP-1 slows the gastric emptying and gut mobility, and probably also targets its receptor in the hypothalamus to promote satiety, thereby reducing food intake. Moreover, it increases cardiac function as well as other physiological functions (Müller et al. 2019).

GLP-1 secretion is primarily stimulated by ingested nutrients. Glucose, fatty acid, and amino acid stimulation of GLP-1 secretion is largely mediated via their respective receptors. Schematic overview of nutrient-induced GLP-1 secretion from L cells is described in Figure 1. Emerging evidence shows that not only nutrients but also some polyphenols can induce GLP-1 secretion from L cells via various mechanisms. Specifically, polyphenols can function as ligands of G-protein-coupled receptors (GPCRs), or regulate intracellular signaling molecules to modulate GLP-1 secretion. Additionally, some polyphenols might indirectly cause GLP-1 secretion mediated by gut microbiota. In this way, polyphenols are also known to promote GLP-1 secretion and exert various physiological functions (Wang, Alkhalidi and Liu 2021).

## Effects of polyphenols on secretion of GLP-1

GLP-1, insulin, and the peripheral actions of insulin are strongly influenced by dietary components. Macronutrients are the most notable examples, but polyphenols and other micronutrients have also been documented as important regulators. It is well estab-



**Figure 1.** Schematic overview of nutrient-induced GLP-1 secretion from L cells. Abbreviations: AC: adenylyl cyclase; GLP-1: glucagon-like peptide-1; GPCRs: G-protein-coupled receptors; IP3: inositol trisphosphate; PLC: phospholipase C; PKA: protein kinase A; SGLT1: sodium/glucose cotransporter 1; TRPM5: transient receptor potential channel M5.

lished that the elevation of intracellular cAMP levels induces both transcription and secretion of GLP-1 via protein kinase A (PKA) and Epac, a camp-activated effector protein, so it is speculated that polyphenols that contribute to its regulation may be involved in promoting GLP-1 secretion. Several polyphenolic compounds have been found to regulate GLP-1 secretion via GPCRs or cAMP signaling pathway (Müller et al. 2019).

Specifically, GPCRs on the L cells might be activated by polyphenols to regulate GLP-1 secretion. Curcumin, a polyphenolic turmeric, has been found to induce GLP-1 secretion (Kato et al. 2017; Harada et al. 2022). It was reported that curcumin-induced GLP-1 secretion can be abrogated by the  $\text{Ca}^{2+}$  channel antagonist or IP3 receptor inhibitor, suggesting that both  $\text{Ca}^{2+}$  entry and release from intracellular store play a role in mediating curcumin-stimulated GLP-1 secretion, the latter of which may be due to the activation of phospholipase C (PLC). In addition, it has been already reported that GPR40/120 and 55 are involved in the induction of GLP-1 secretion of curcumin (Kato et al. 2017; Harada et al. 2022). Delphinidin 3-rutinoside, one of the anthocyanin derivatives from berry, increased GLP-1 secretion, via GPR40/120-mediated mechanism (Kato et al. 2015). As GPR40/120 are fatty acid receptors, it is unclear how above-discussed polyphenols are able to activate GPCRs.

The superfamily of bitter taste receptors (Tas2Rs) are expressed in the intestinal enteroendocrine cells (Wang, Alkhalidi and Liu 2021). Given that many dietary polyphenolic compounds are known to have a bitter taste, and they can interact with various taste receptors, in particular, Tas2Rsm, it is possible that Tas2Rs mediated polyphenols-induced GLP-1 secretion. While polyphenols have been found to activate several Tas2Rs, studies on whether polyphenols stimulate GLP-1 secretion via Tas2Rs are limited. Epigallocatechin-3-gallate (EGCG) is one of the most effective Tas2R39 activators among polyphenolic compounds. In addition, EGCG activated transient receptor potential ion channel A1 in the mouse clonal L-cell line (STC-1) (Kurogi et al. 2012), which was opened after Tsd2Rs activation to initiate the  $\text{Ca}^{2+}$  influx. EGCG has been reported to induce GLP-1 secretion in the mouse ileum segment (Song et al. 2015), and oral intake of EGCG stimulated GLP-1 secretion in mice and diabetic patients (Liu et al. 2014), suggesting that EGCG-stimulated GLP-1 might be mediated via Tas2R.

Recently, we also found that nonabsorbable polyphenols on procyanidin from cacao and black soybean seed coat induced GLP-1 secretion. Especially tetramer procyanidin on cinnamtannin A2 strongly increased GLP-1 secretion in the STC-1 cell line and in mice (Yamashita *et al.* 2013; Odongo *et al.* 2023). We extracted procyanidin oligomers from cacao liquor and acutely administered them individually to male Institute of Cancer Research (ICR) mice (10- $\mu$ g/kg body weight, p.o.). The animals were sacrificed an hour after the treatment, and Enzyme-Linked Immuno Sorbent Assay (ELISA) analyses were used to quantify GLP-1 and insulin. Among the studied compounds (epicatechin, procyanidin B2, procyanidin C1, and cinnamtannin A2), cinnamtannin A2 increased GLP-1 and insulin concentration, and was the most bioactive overall. The same compound also exerts significant changes on skeletal muscle, by increasing insulin receptor (IR)- $\beta$  and insulin receptor substrate (IRS)-1 phosphorylation. All compounds present in the cacao liquor samples are epicatechin and its oligomers, for example, procyanidin B1 is an epicatechin dimer, procyanidin C1 is an epicatechin trimer, and cinnamtannin A2 is an epicatechin tetramer. The evidence supports the hypothesis that the length of the procyanidin is key to the insulin-sensitizing results, as demonstrated by a lack of significant effects by the epicatechin monomer, dimer and trimer. In the STC-1 cell line, cinnamtannin A2 also induced GLP-1 secretion; however a low-degree of procyanidins, which are less than the tetramer, did not (Odongo *et al.* 2023). However, the mechanism of action of how cinnamtannin A2 promotes GLP-1 secretion is unknown, and more research is required. **Grape seed-derived proanthocyanidins (GSPEs), which are composed of monomers or flavan-3-ols, dimers, trimers, tetramers, and oligomers of procyanidins, were found to increase GLP-1 secretion from clonal L cells as well as mouse ileum and colon segments (Casanova-Martí *et al.* 2017).** This composition consists of compounds similar to the cacao polyphenols we used. **The stimulatory action of GSPE on GLP-1 secretion might result from a rise in intracellular Na<sup>+</sup> influx via cation channel, transient receptor potential cation channel, subfamily M, member 5 (TRPM5), which leads to the depolarization and the opening of voltage-gated calcium channel, resulting in an increase in Ca<sup>2+</sup> influx (González-Abuín *et al.* 2014), an essential step for exocytosis of GLP-1 granules.** As condensed tannins are ligands of Tas2R, procyanidins might also activate taste receptors to regulate GLP-1 secretion. However, the degree of polymerization of procyanidins might affect their affinity for taste receptors (Soares *et al.* 2013); for example, procyanidin trimer C2 activates Tas2R5, but procyanidin dimer B2 fails to activate any Tas2Rs. Thus, studies on specific procyanidin should be performed to verify the activity of certain procyanidin on Tas2Rs.

Some polyphenols may promote GLP-1 secretion via activating cAMP-mediated intracellular signaling. Genistein, an isoflavone, hispidulin, a flavone, and coffee-derived polyphenols were shown to stimulate GLP-1 secretion mediated via increasing intracellular cAMP content. This review focuses on polyphenols that are difficult to absorb, so a detailed explanation will be omitted. These reports suggest that poorly absorbed polyphenols contribute to GLP-1 secretion from the GI tract, and that the mechanism of action may involve binding to GPCRs or taste receptors. Depending on the structure of each compound, the receptors it binds to differ.

### Effect of procyanidins on antidiabetes and hyperglycemia through secretion of GLP-1

Since tetramer procyanidin induced GLP-1 secretion, we performed whether procyanidin prevent acute hyperglycemia. Procyanidin-rich compositions were extracted from cacao liquor

and black soybean seed coat (BE) and prepared procyanidin fractions with different degrees of polymerization (DP), namely low-DP (DP  $\leq$  3) and high-DP (DP  $\geq$  4) fractions, from a cacao liquor procyanidin-rich extract (CLPr). These extracts and fractions were administered orally to ICR mice and their antihyperglycemic effects were examined. We found that CLPr and its fractions prevent postprandial hyperglycemia accompanied by an increase in the plasma GLP-1 level with or without glucose load. In the absence of glucose load, both fractions increased the plasma insulin level and activated its downstream signaling pathway in skeletal muscle, resulting in promotion of the translocation of glucose transporter 4 (GLUT4). Phosphorylation of AMP-activated protein kinase (AMPK) was also involved in the promotion of GLUT4 translocation. High- and low-DP fractions showed a similar activation of insulin and AMPK pathways. In conclusion, cacao liquor procyanidins prevent hyperglycemia by promoting GLUT4 translocation in skeletal muscle, and both the GLP-1-activated insulin pathway and the AMPK pathway are involved in the underlying molecular mechanism. In addition, among the procyanidin monomers to tetramers, cinnamtannin A2 showed the strongest effect in ICR mice (Yamashita *et al.* 2016). Preadministration of cinnamtannin A2 suppressed postprandial hyperglycemia in a dose-dependent manner. When mice received 0.1-10  $\mu$ g/kg body weight of cinnamtannin A2, a significant effect was observed. Cinnamtannin A2 also showed a similar activation of insulin and AMPK pathways. Moreover, BE prevented hyperglycemia via GLP-1 secretion. The effect was stronger for procyanidin-rich fraction (PCBE) compared to BE (Odongo *et al.* 2023). Surprisingly, AMPK pathway as insulin-independent pathway was also activated by procyanidins. It was noteworthy that an antagonist for GLP-1 receptor exendin (9-39) canceled procyanidin-increased phosphorylation of AMPK (Hironao *et al.* 2020). These results suggest that procyanidin prevents hyperglycemia through GLP-1 secretion, and its underlying mechanisms contribute to both insulin and AMPK pathways. As described above, it is known that some polyphenol compounds prevent diabetes and hyperglycemia through the promotion of GLP-1 secretion.

### Effect of procyanidins on antiobesity through secretion of GLP-1

**GLP-1 is also known to contribute to obesity prevention by regulating energy metabolism and appetite.** AMPK is a nutrient and energy sensor, enhances lipolysis, fatty acid oxidation, and ketogenesis, and attenuates lipogenesis and synthesis of cholesterol, fatty acids, and triglycerides (Long and Zierath 2006; Hardie, Ross and Hawley 2012). AMPK also enhances energy consumption through mitochondrial biogenesis and its related functions. Moreover, AMPK regulates adipocyte differentiation through modulation of the expression of peroxisome proliferator-activated receptor  $\gamma$  (PPAR $\gamma$ ) and CAAT/enhancer binding protein  $\alpha$  (C/EBP $\alpha$ ) (Moseti, Regassa and Kim 2016). Thus, AMPK is a main target molecule for the prevention of obesity and hyperglycemia. Another important factor in regulation of obesity is uncoupling proteins (UCPs), which are key mitochondrial membrane transporters responsible for energy expenditure and thermogenesis. There are 2 types of adipocytes, white and brown adipocytes. White adipocytes store excess energy as fat, while brown ones play an important role in heat production because of having a large number of mitochondria expressing UCP1.

We have already reported the preventive effects of BE and CLPr on obesity and involvement of AMPK and UCPs using *in vivo* and

in vitro experiments (Kanamoto et al. 2011; Yamashita et al. 2012, 2020). Intake of BE for 2 weeks significantly increased phosphorylation of AMPK in the liver, skeletal muscle, and white adipose tissue (Yamashita et al. 2020). Long-term feeding of BE (Yamashita et al. 2020) and CLPr (Yamashita et al. 2012) prevented body weight gain and fat accumulation in white adipose tissue accompanied by upregulation of UCPs and downregulation of inflammatory cytokines in high-fat diet-fed C57BL/6 mice. Similar results were observed in KK-A<sup>y</sup> mice fed a normal diet containing BE and C57BL/6 mice fed a high-fat diet containing CLPr. **Procyanidins are also candidates for the active ingredients in BE because procyanidins from cacao and grape seed promoted AMPK phosphorylation in the liver, skeletal muscle, and adipose tissues in vivo and in vitro. As mentioned above, long-term feeding of BE increased UCPs in white and brown adipose tissue, suggesting that BE may increase energy expenditure through heat production.** Recently, it has been reported that GLP-1R analog activates AMPK (Yang et al. 2020). We also found that the activation of AMPK by CLPr was canceled by pretreatment with a GLP-1R inhibitor (Hironao et al. 2020). Although the details are not clear, GLP-1 may be involved as one of the mechanisms of preventive effects on AMPK-mediated obesity.

GLP-1 is a major player in the gut-brain axis regulation of energy balance (Salehi and Purnell 2019). Many studies also suggest that GLP-1 receptor analogs promote weight loss mainly due to their inhibitory effect on food intake through the gut-brain axis (Valassi, Scacchi and Cavagnini 2008). GLP-1 binding to the GLP-1 receptors on the surface of neurons initiates intracellular signal transduction and activation of target genes, resulting in the synthesis and release of neuropeptides (pro-opiomelanocortin [POMC] and cocaine- and amphetamine-regulated transcript [CART]) with anorectic effects. GLP-1 can also be expected to contribute to the prevention of obesity by suppressing feeding. We also found that BE intake suppressed hyperphagia in KK-A<sup>y</sup> mice and prevented obesity. Furthermore, at the end of the experiment, the concentration of GLP-1 in the plasma, as well as the expression levels of POMC and CART in the hypothalamus (preparing for paper submission), significantly increased in the BE-fed group. Thus, we also found that BE and CLPr, which are rich in procyanidins, prevent obesity through secretion of GLP-1. GLP-1 may play an important role in one of the mechanisms of the antiobesity effect of polyphenols.

### Effect of procyanidins on improvement of vascular function via GLP-1 secretion

Vascular function is important to the pathogenesis of cardiovascular diseases (CVDs) (Sun et al. 2019). Vascular dysfunction caused by aging and vascular stiffness is associated with a risk of CVD. In addition, injurious stimuli such as oxidative stress, inflammation, diabetes, and obesity result in the dysfunction of vascular endothelial cells (Ding, Hashem and Triggler 2007; Tabit et al. 2010). Because vascular dysfunction is recoverable, it is important to detect vascular dysfunction as early as possible and improve it.

Nitric oxide (NO) regulates vascular functions by inducing vasodilation and inhibiting platelet aggregation in blood vessels (Michel and Vanhoutte 2010; Ghimire et al. 2017). A reduction in NO levels can trigger the onset of CVD. Therefore, increasing NO production in the vascular endothelium might prevent CVD and improve vascular function. NO is produced by endothelial nitric oxide synthase (eNOS). eNOS activation is regulated by several molecular mechanisms, including Ca<sup>2+</sup>/calmodulin binding (Cai, Liu and Garcia 2008; Michel and Vanhoutte 2010),

cAMP-dependent protein kinase, AMP-activated protein kinase5, and Akt (Fulton et al. 1999; Michel and Vanhoutte 2010). Of note, Akt promotes the phosphorylation of eNOS at Ser1177 residues in response to various stimuli, including insulin (Fulton et al. 1999). In addition, GLP-1 induced endothelium-dependent vasodilation (Golpon et al. 2001; Nathanson et al. 2009). It was reported that GLP-1 affected vascular endothelial cells and increased eNOS phosphorylation and subsequent NO production via the cAMP/PKA pathway in vitro. We found that BE increased NO production in the aortas of rats (Domae et al. 2019). Regarding the underlying mechanism of BE-induced NO production, BE promoted the phosphorylation of eNOS in vascular endothelial cells through GLP-1 secretion from intestinal cells. We confirmed that a GLP-1 receptor antagonist (exendin 9-39) inhibited BE-induced NO production and eNOS phosphorylation. To the best of our knowledge, this is the first report revealing that food components increase NO production in the aorta through GLP-1 secretion from intestinal cells. Recently, we also investigated the effect of black soybean consumption on the vascular function and oxidative stress associated with the polyphenol concentrations in healthy women (Yamashita et al. 2020, 2020). There was an observed improvement of the vascular stiffness, an increase in the plasma and urinary NO level, and a decrease in the oxidative stress. Black soybean has the potential to improve vascular function through increasing NO accompanied by GLP-1 secretion, although GLP-1 levels were not measured in this study. In addition, a previous study reported that mean blood pressure decreased significantly following oral administration of flavan-3-ols extracted from cocoa for 2 weeks in normal rats (Yu et al. 2021). In addition, it was reported that coffee polyphenol consumption improves postprandial hyperglycemia and vascular endothelial function, which is associated with increased GLP-1 secretion and decreased oxidative stress in healthy humans (Jokura et al. 2015). These results suggest that polyphenols that promote GLP-1 secretion contribute to improving vascular function and reducing oxidative stress.

## Conclusion

Many polyphenols have low absorption efficiency due to their structure. However, recent research has revealed that it contributes to the action of the entire body, starting from the GI tract. Although this review only introduced the functionality of polyphenols mediated by the GI hormone GLP-1, there have been many reports targeting the intestine, such as functionality mediated by other hormones and intestinal microbiota. Although there is still hope that polyphenols may contribute to health, in the future it is necessary to develop research that takes into account signal transmission that occurs within the body, such as the network between multiple organs.

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## Disclosure statement

No potential conflict of interest was reported by the author.

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Review

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# Grape Pomace as a Cardiometabolic Health-Promoting Ingredient: Activity in the Intestinal Environment

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Review

# Grape Pomace as a Cardiometabolic Health-Promoting Ingredient: Activity in the Intestinal Environment

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**Abstract:** Grape pomace (GP) is a winemaking by-product particularly rich in (poly)phenols and dietary fiber, which are the main active compounds responsible for its health-promoting effects.

These components and their metabolites generated at the intestinal level have been shown to play an important role in promoting health locally and systemically. This review focuses on the potential bioactivities of GP in the intestinal environment, which is the primary site of interaction for food components and their biological activities. These mechanisms include (i) regulation of nutrient digestion and absorption (GP has been shown to inhibit enzymes such as  $\alpha$ -amylase and  $\alpha$ -glucosidase, protease, and lipase, which can help to reduce blood glucose and lipid levels, and to modulate the expression of intestinal transporters, which can also help to regulate nutrient absorption); (ii) modulation of gut hormone levels and satiety (GP stimulates GLP-1, PYY, CCK, ghrelin, and GIP release, which can help to regulate appetite and satiety); (iii) reinforcement of gut morphology (including the crypt-villi structures, which can improve nutrient absorption and protect against intestinal damage); (iv) protection of intestinal barrier integrity (through tight junctions and paracellular transport); (v) modulation of inflammation and oxidative stress triggered by NF- $\kappa$ B and Nrf2 signaling pathways; and (vi) impact on gut microbiota composition and functionality (leading to increased production of SCFAs and decreased production of LPS). The overall effect of GP within the gut environment reinforces the intestinal function as the first line of defense against multiple disorders, including those impacting cardiometabolic health. Future research on GP's health-promoting properties should consider connections between the gut and other organs, including the gut-heart axis, gut-brain axis, gut-skin axis, and oral-gut axis. Further exploration of these connections, including more human studies, will solidify GP's role as a cardiometabolic health-promoting ingredient and contribute to the prevention and management of cardiovascular diseases.

**Keywords:** grape pomace; (poly)phenols; dietary fiber; intestinal environment; digestive enzymes; nutrient transporters; enteroendocrine hormones; intestinal barrier integrity; inflammation; oxidative stress; gut microbiota



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## 1. Introduction

Grape pomace (GP) is the solid residue from the winemaking process and is composed mainly of grape skins and seeds [1]. Currently, GP is probably one of the food by-products most commonly used in the formulation of dietary supplements and fortified foods [2,3]. The potential health-promoting properties of GP are mainly attributed to its (poly)phenols and dietary fiber content, among other components. It is estimated that around 60–70% of the phenolic compounds of the grape remain in the pomace after winemaking [4], accounting for 4.8–5.4% of GP dry matter [5]. GP (poly)phenols encompass non-flavonoids such as hydroxybenzoic acids (C6-C1) (i.e., gallic, protocatechuic, and vanillic acids), hydroxycinnamic acids (C6-C3) (i.e., *p*-coumaric, caffeic, and ferulic acids) and stilbenes (C6-C2-C6)

(i.e., resveratrol, piceatannol, and resveratrol dimers), and flavonoids (C6-C3-C6) such as flavanols (i.e., catechins, procyanidins and polymeric proanthocyanidins or condensed tannins), anthocyanins (i.e., derivatives of malvidin, petunidin, cyanidin, peonidin and delphinidin) and flavonols (i.e., derivatives of quercetin, myricetin, and kaempferol) [6]. Dietary fiber is the major component of GP, ranging between 29 and 58% in dry weight [7,8]. The fiber present in GP is fundamentally insoluble and is mainly made up of structural polysaccharides such as cellulose, hemicelluloses (xyloglucans, arabinans, galactans, xy-lans, and mannans), pectins, and lignin [7]. These polysaccharides are commonly bound to (poly)phenols and other non-digestible compounds, forming what was denominated “antioxidant dietary fiber” [9].

For its use in the food industry, fresh pomace usually undergoes a process of extraction, resulting in the concentration of both (poly)phenols and dietary fiber. Extracts are further stabilized by adding encapsulation agents such as maltodextrins [10]. For example, Table 1 reports the composition of (poly)phenols and dietary fiber of four GP-derived extracts. In these products, the (poly)phenols content is normally referred to as the free phenolics solubilized in organic solvents used for their determination. In contrast, the fiber is determined as the percentage of soluble and insoluble fractions or as alcohol-insoluble residue (AIR) that might include phenolics bound to polysaccharides. These data give an idea of the GP’s compositional variability depending on the starting grapes’ diversity (variety, state of maturity, etc.), the winemaking process, and the extraction and stabilization processes [10].

**Table 1.** Some examples of industrially manufactured GP-derived extracts: RGPE and RGPE2 (red grape pomace extracts), ORGPE (organic red grape pomace extract), and WGPE (white grape pomace extract).

	RGPE	RGPE2	ORGPE	WGPE
Total (poly)phenols (mg/g) <sup>a</sup>	48.0 ± 4.1	13.0 ± 2.3	5.7 ± 0.5	3.5 ± 0.5
Phenolic acids (µg/g) <sup>a</sup>	1843.0 ± 23.2	652.5 ± 12.6	853.7 ± 15.2	1211.8 ± 20.1
Flavonols (µg/g) <sup>a</sup>	72.7 ± 3.5	nd	87.0 ± 7.6	nd
Flavan-3-ols (µg/g) <sup>a</sup>	276.3 ± 7.6	nd	682.8 ± 8.7	295.2 ± 6.1
Anthocyanins (µg/g) <sup>a</sup>	nd	nd	1706.2 ± 21.7	nd
AIR (mg/g) <sup>b</sup>	930.5 ± 18.1	819.4 ± 18.9	969.8 ± 22.3	613.1 ± 21.8
Glucose (mg/g AIR) <sup>b,c</sup>	446.6 ± 2.1	270.4 ± 0.6	598.4 ± 4.5	839.3 ± 1.7
Galactose (mg/g AIR) <sup>b,c</sup>	28.8 ± 1.1	32 ± 0.7	36.9 ± 2.8	47.7 ± 1.1
Mannose (mg/g AIR) <sup>b,c</sup>	5.9 ± 0.3	17.4 ± 0.2	4 ± 0.2	2.7 ± 0.1
Rhamnose (mg/g AIR) <sup>b,c</sup>	3.2 ± 0.2	5.7 ± 0.2	2.1 ± 0.1	6.3 ± 0.1
Uronic acids (mg/g AIR) <sup>b,c</sup>	62.6 ± 4.2	44.1 ± 2.9	58.1 ± 4.8	52.2 ± 2.6
Total sugars (mg/g AIR)	555.9	374.3	702.3	969.1
Klason lignin (mg/g AIR)	161 ± 9.3	22.8 ± 3.6	118.4 ± 13.3	193.6 ± 26.8

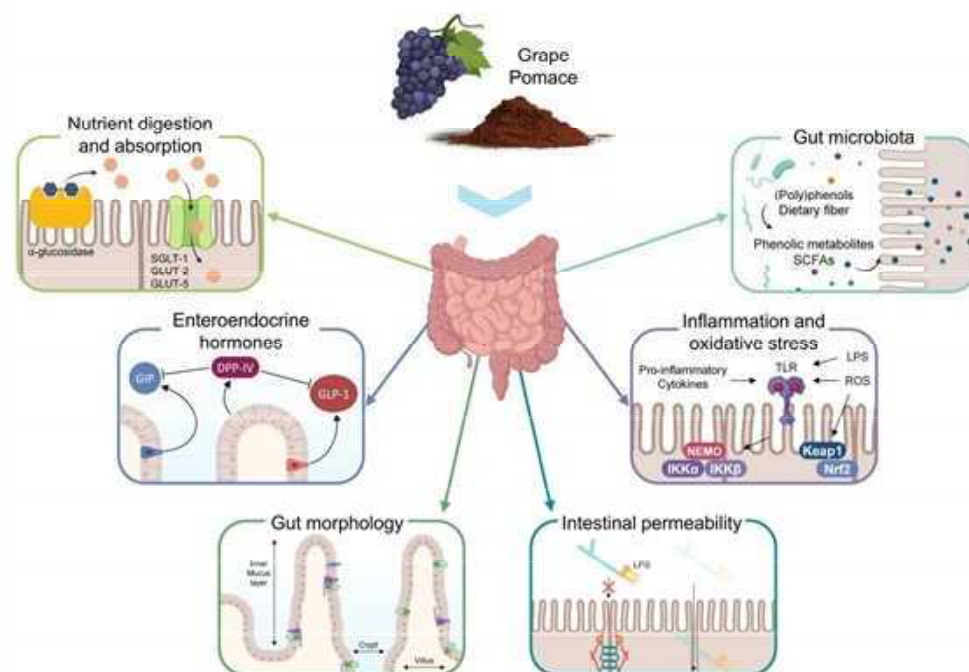
<sup>a</sup> Reported in Taladrid et al. [11]. <sup>b</sup> Unpublished results. <sup>c</sup> After hydrolysis (12 M H<sub>2</sub>SO<sub>4</sub>, 3 h and room temperature, followed by 0.6 M H<sub>2</sub>SO<sub>4</sub>, 3 h and 100 °C). nd: non-detected.

GP-derived products have been proposed to manage cardiovascular risk factors, including endothelial dysfunction, inflammation, hypertension, hyperglycemia, and obesity [12]. In this sense, some human intervention studies have evidenced reductions in blood pressure (BP) between 3 and 8% after GP supplementation in individuals with various symptoms of metabolic syndrome [13,14], which reached up to 14.5% in patients who suffered from hypertension [15,16]. However, other studies have reported almost no effects on BP after supplementation with GP (poly)phenols [17,18], which could be attributed to differences in the composition of the GP-derived products, doses, targeted population, etc. There is also a growing interest in evaluating the effect of GP on the regulation of baseline hyperglycemia, considered an early defect of type 2 diabetes and one of the primary anti-diabetic targets. Several intervention studies have suggested the ability of GP-derived products to reduce plasma glucose levels [14,18,19].

Nevertheless, in a certain way, it can be said that this activity of GP as a cardiometabolic health-promoting ingredient would begin in the gastrointestinal tract as GP components (i.e., (poly)phenols and fiber) undergo extensive catabolism, mainly by the action of the intestinal microbiota, that gives rise to low-molecular-weight bioactive compounds that can be absorbed and utilized by the body [20]. Dietary (poly)phenols are metabolized by the gut microbiota and concentrated in the gut lumen, reaching biologically relevant concentrations that allow them to exert local beneficial effects [21]. Regarding GP (poly)phenols, microbial catabolism pathways of the different flavonoid classes (anthocyanins, flavonols, flavan-3-ols, etc.) are known to share similar intermediate and end products such as benzoic, cinnamic, phenylacetic, and phenylpropanoic acids [22]. These phenolic metabolites are better absorbed than their precursors, occurring in plasma and urine as phase I and phase II-conjugated derivatives, and seem primarily responsible for the positive health activities in tissues and organs (systemic effects). Hepatic metabolism is a crucial step in the biotransformation of (poly)phenols, as it enables the formation of more bioavailable and biologically active metabolites that can exert beneficial effects on various tissues and organs [21,22]. Among other mechanisms, GP (poly)phenols and their metabolites appear to modulate the cell redox state by direct and indirect mechanisms such as inhibition of oxidant enzymes, activation of enzymatic and non-enzymatic antioxidant systems, and regulation of gene expression of antioxidants by interaction with redox signaling pathways [12]. The GP fiber fraction is fermented by colonic microbiota yielding short-chain fatty acids (SCFAs) such as acetate, butyrate, and propionate. SCFAs present cardioprotective effects, including modulating blood pressure and glucolipid metabolism, promoting post-infarction cardiac repair, anti-inflammation, and maintaining the gut barrier [23]. Notably, the two components, (poly)phenols and dietary fiber, interact in the body affecting their metabolism in the colon, which in turn may affect long-term systemic health effects [24].

Therefore, our goal in this paper was to disclose the potential bioactivities of GP -as a whole- in the intestinal environment, not only as a new target of health-promoting properties at the local level but also as the beginning for further effects at the systemic level (i.e., cardiometabolic effects). After a peer review, we have identified six main targets of potential bioactivity of GP in the gut: (i) nutrient digestion and absorption, (ii) enteroendocrine gut hormones release and satiety, (iii) gut morphology, (iv) intestinal barrier integrity, (v) intestinal inflammatory and oxidative status, and (vi) gut microbiome (Figure 1). The following sections (Sections 2–7) recompile the most recent studies concerning the effects of GP intake on each of these issues, and the final section (Section 8) compiles the overall conclusions from all of them.

The search strategy for the literature review focused on the effects of GP on the intestinal environment (nutrient digestion and absorption, enteroendocrine gut hormones release and satiety, gut morphology, intestinal barrier integrity, intestinal inflammatory and oxidative status, and gut microbiome). The review included publications from 2010 onwards, sourced from online databases such as Scopus, Web of Science, Wiley, ScienceDirect, SpringerLink, and Google Scholar. In instances where information was scarce or crucial to the discussion, a small number of older references were also included. The literature was searched using keywords such as grape pomace, grape marc, winemaking by-products, grape residues, nutrient digestibility, intestinal enzyme, intestinal transporter, enteroendocrine hormone, intestinal barrier integrity, inflammation, oxidative stress and gut microbiota. In vitro and in vivo studies and articles focused solely on specific molecules were excluded from the main text. The relevance of each study was evaluated using a hierarchical approach based on the title, abstract, and full manuscript.



**Figure 1.** Scheme of the potential bioactivity of grape pomace (GP) in the intestinal environment.

## 2. Effects of GP on Intestinal Nutrient Digestion and Absorption

In this section, we examine the impact of GP on intestinal nutrient digestion and absorption. Rich in dietary fiber and (poly)phenols, GP influences nutrient digestion and absorption through several mechanisms: physically impeding nutrient digestion and absorption, regulating intestinal enzymes, and modulating the expression of intestinal transporters. Gaining a deeper understanding of these mechanisms will offer valuable insights into the potential health benefits of GP and its role in modulating metabolic processes and promoting cardiovascular health.

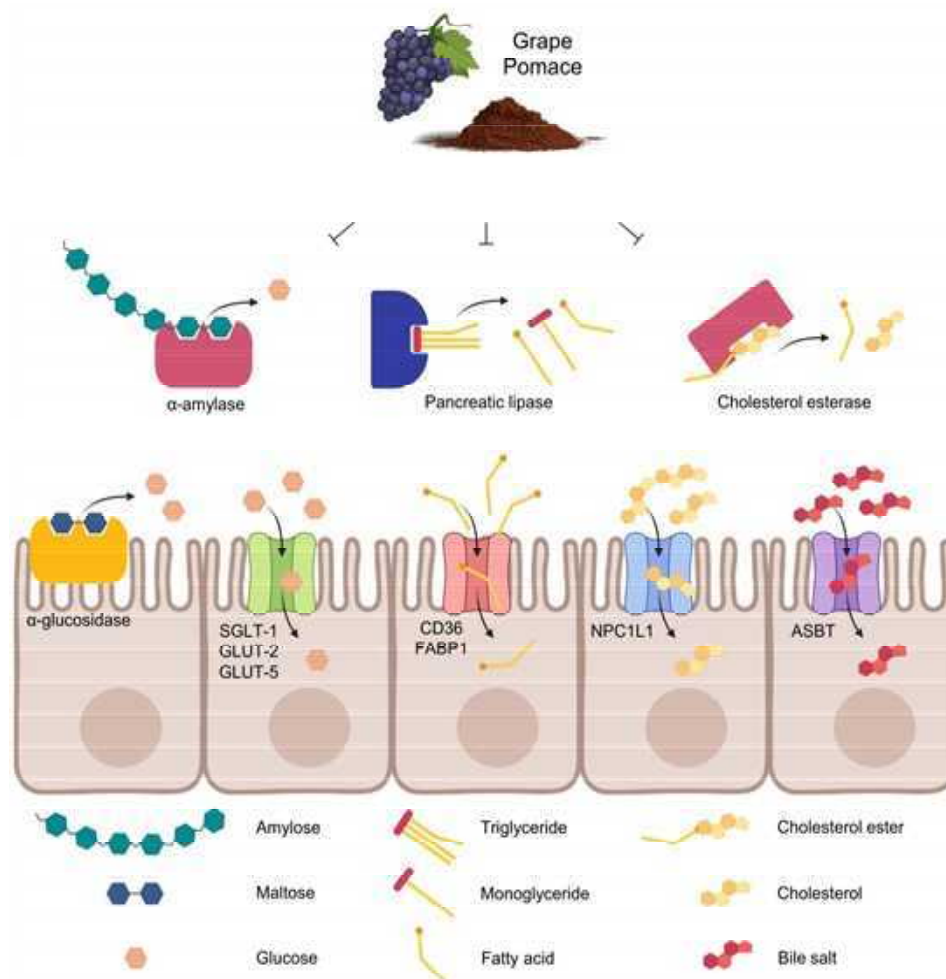
### 2.1. Physical Impediment on Nutrient Digestion and Absorption

GP is a rich source of both soluble and insoluble dietary fibers. The insoluble fraction can increase the bulk of the stool and promote food movement through the digestive tract, reducing the time available for nutrient absorption [25]. In contrast, soluble fiber can form a gel-like substance in the gut, leading to delayed gastric emptying and slower nutrient absorption [26]. While this effect can be beneficial for regulating blood sugar levels, it may also limit the bioavailability of some nutrients. The high dietary fiber content in GP can slow down the rate of glucose absorption in the small intestine by forming a viscous gel-like matrix. This hinders the accessibility of digestive enzymes to carbohydrates and glucose diffusion, resulting in a more gradual release of glucose into the bloodstream [27–30]. This mechanism contributes to better glycemic control and reduced insulin spikes [31].

The soluble fiber in GP can also interfere with the emulsification process, which is necessary for the hydrolysis of triglycerides and subsequent fat absorption [30,32]. In addition to its effects on fat absorption, GP has been shown to lower dietary cholesterol absorption through multiple mechanisms. Specifically, GP's soluble fiber and (poly)phenols can bind to bile salts in the intestine, reducing their reabsorption and promoting their excretion [33,34]. This leads to the liver utilizing more circulating cholesterol to synthesize new bile salts, ultimately resulting in decreased serum cholesterol levels. Moreover, (poly)phenols and dietary fibers in GP have been shown to inhibit intestinal cholesterol absorption by destabilizing cholesterol micelles, thereby reducing cholesterol solubility and availability [33,35]. These mechanisms have potential implications for reducing the risk of cardiovascular diseases and improving overall lipid profiles.

## 2.2. Regulation of Intestinal Enzymes

At the intestinal lumen, enzymes ( $\alpha$ -amylase and  $\alpha$ -glucosidase, protease, and lipase) are implicated in the digestion of primary macronutrients: carbohydrates, proteins, and lipids, respectively (Figure 2). Numerous studies have long shown the inhibitory capacity of (poly)phenols and dietary fiber separately against intestinal enzymes. It is widely known that (poly)phenols interact with enzymes, being these interactions through van der Waals, electrostatic forces, and hydrogen, as well as hydrophobic binding [36]. Concerning dietary fiber, enzyme-inhibitory capacity might be related to the inhibitors present on the surface of the insoluble fiber as well as the trapping capacity of the porous fiber network [32,37].



**Figure 2.** Schematic representation of the effects of GP on nutrient digestion and absorption.

However, studies about the GP capacity -as a whole- to inhibit the activity of intestinal enzymes are quite recent; it should be noted that they are mainly based on *in vitro* assays using microbial and animal enzymes, and GP-derived products are tested directly, without previous intestinal digestion. A recompilation of these studies for  $\alpha$ -amylase and  $\alpha$ -glucosidase has just been done by Cisneros-Yupanqui et al. [38]. From the ten studies recompiled [28,31,39–46], only that of Kato-Schwartz et al. [31] evaluated *in vivo* inhibition of  $\alpha$ -amylase and  $\alpha$ -glucosidase by running starch and maltose tolerance tests in rats with or without administration of a GP extract. From the revised studies, the authors concluded that GP, especially the red varieties, can be perceived as a possible source of  $\alpha$ -amylase and  $\alpha$ -glucosidase inhibitors. However, further investigations are necessary to understand key factors such as the bioavailability and the physiological responses to the GP components [38].

The action of GP on purified intestinal proteases and peptidases has been scarcely evaluated [40]. However, studies with complex enzymatic sources such as snake venoms indicated that the dried GP exerted inhibitory actions on proteolytic activity in conjunction with other phospholipase, hemolytic, and thrombolytic activities [47]. In relation to lipases, direct GP extracts were found to inhibit pure lipases obtained from porcine pancreas [41] and also cholesterol esterase [33], although no studies have been carried out to confirm these results in vivo.

In addition to its other effects, inhibiting carbohydrate-hydrolyzing enzymes such as  $\alpha$ -amylases and  $\alpha$ -glucosidases in the digestive system can help prevent glucose absorption, thereby reducing postprandial hyperglycemia. This is particularly important as postprandial hyperglycemia is a significant component of all forms of diabetes [48]. Concurrently, intestinal lipase plays an important role in triacylglycerols absorption, which is related to body weight control and obesity [49]. Therefore, this capacity of GP to inhibit intestinal enzymes has been suggested to be behind, at least partly, its cardiometabolic potential proved in vivo [50,51].

### 2.3. Regulation of Intestinal Transporters

Intestinal transporters are essential for the absorption of nutrients, including sugars, lipids, amino acids, and vitamins, from the gut lumen into the bloodstream (Figure 2). Modifying intestinal transporter expression can have significant implications for nutrient uptake, gut health, and overall cardiovascular health. GP has been reported to influence the expression of glucose transporters, particularly sodium-dependent glucose transporter 1 (SGLT1) [52], facilitative glucose transporter 2 (GLUT2) [52–54], and fructose transporter (GLUT5) [54], which are responsible for sugars absorption in the small intestine [24]. Studies have demonstrated that GP (poly)phenols and polysaccharide-(poly)phenol complexes can enhance the expression of these transporters, leading to improved glucose uptake and blood glucose regulation [24,55]. GP has been shown to impact the expression of lipid transporters (cholesterol and fatty acids), such as Niemann–Pick C1-like 1 (NPC1L1) [56], fatty acid binding protein 1 (FABP1), and fatty acid translocase (CD36) [57], and bile salt transporters [56,58]. Studies suggest that GP (poly)phenols may downregulate the expression of these transporters, resulting in reduced cholesterol and bile salts absorption [59], which can be beneficial in managing lipid levels and cardiovascular health.

In brief, GP's impact on nutrient digestion and absorption is multifaceted, mediated by its high content of dietary fiber and (poly)phenols, as summarized in Table 2. GP can physically impede nutrient absorption and regulate intestinal enzymes, as mentioned above. Furthermore, GP can modulate the expression of intestinal transporters, such as SGLT1, GLUT2, and NPC1L1, affecting the absorption of sugars and lipids. These mechanisms contribute to better glycemic control and reduced serum cholesterol levels, potentially lowering the risk of cardiovascular diseases. Gaining a deeper understanding of these mechanisms will provide valuable insights into the potential health benefits of GP and its role in modulating metabolic processes and promoting cardiovascular health.

**Table 2.** Effect of GP on nutrient digestion and absorption using in vitro and in vivo models.

Nutrient	Effect of GP on Physical Impediment	Refs.	Effect of GP on Intestinal Enzymes	Refs.	Effect of GP on Nutrient Transport	Refs.
Carbohydrates	↓ Glucose diffusion	[29]	$\alpha$ -amylase inhibition	[31,39–45]	SGLT1 downregulation GLUT2 downregulation	[52] [52–54]
	Starch digestibility	[27,28,30]	$\alpha$ -glucosidase inhibition	[28,31,40,42–46]	GLUT5 downregulation ↓ Intestinal glucose uptake	[54] [24]
Lipids	↓ Triglyceride hydrolysis	[30]	Pancreatic lipase inhibition	[33,39–41,46]	CD36 downregulation FABP1 downregulation	[57] [57]

Table 2. Cont.

Nutrient	Effect of GP on Physical Impediment	Refs.	Effect of GP on Intestinal Enzymes	Refs.	Effect of GP on Nutrient Transport	Refs.
Cholesterol	↓ Micellar cholesterol	[33]	Cholesterol esterase inhibitor	[33]	NPC1L1 downregulation ↓ Intestinal cholesterol uptake	[56] [59]
Bile salts	Binding	[33]	—	—	ASBT downregulation	[56,58]
Proteins	↓ Protein hydrolysis	[30]	Trypsin inhibition Chymotrypsin inhibition	[40]	PEPT1 downregulation	[54]

↓: Decrease in; SGLT1: sodium-glucose co-transporter 1; GLUT2: glucose transporter 2; GLUT5: fructose transporter; CD36: fatty acid translocase; FABP1: fatty acid-binding protein 1; NPC1L1: Niemann–Pick C1-like 1; ASBT: apical sodium-dependent bile acid transporter; PEPT1: H<sup>+</sup>/peptide transporter 1.

### 3. Effects of GP on Enteroendocrine Gut Hormones Release and Satiety

Numerous animal model studies have shown that GP can modulate gut hormone levels, such as glucagon-like peptide-1 (GLP-1), peptide YY (PYY), cholecystokinin (CCK), ghrelin, and glucose-dependent insulinotropic polypeptide (GIP), which play crucial roles in regulating satiety and regulating food intake [60–62] (Figure 3).

GP has been shown to stimulate the release of GLP-1 in vitro and in vivo [63,64], an incretin hormone that enhances insulin secretion, inhibits glucagon release and reduces glycemia [65], and promotes satiety, potentially through the activation of specific G-protein-coupled receptors [66]. Increased GLP-1 levels may contribute to enhancing satiety and reducing food intake, thereby promoting weight management. The phenolic content in GP may contribute to this effect [67,68], associated with an increased L-cell number [68].

GP has been reported to increase PYY levels [64,69], another gut hormone that plays a role in appetite regulation and energy balance. Both dietary fiber and (poly)phenols in GP may be responsible for this effect [68]. Additionally, CCK, a hormone involved in digestion and satiety, has been shown to increase following GP consumption [64,70]. GP's high (poly)phenol content may contribute to this effect by interacting with gut receptors and stimulating CCK release.

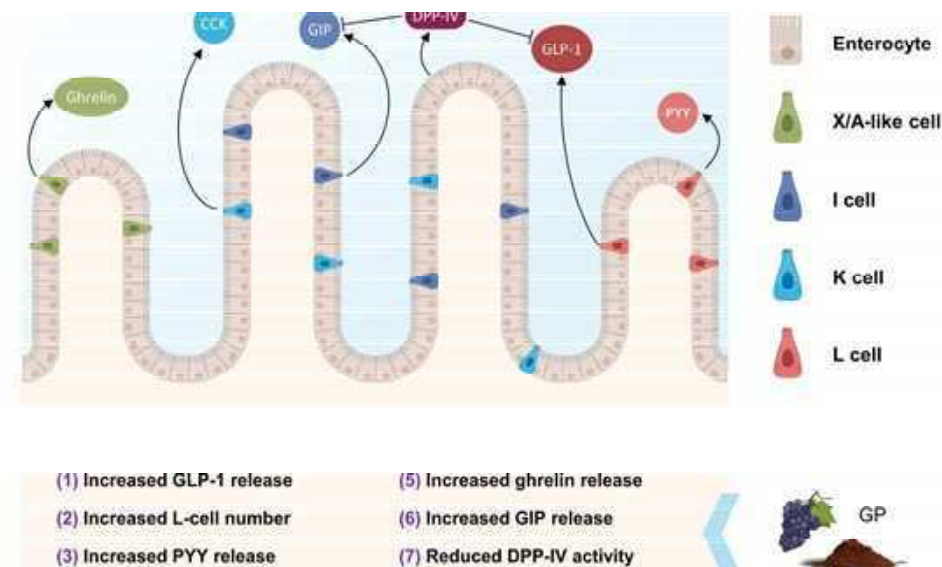
GP has been found to decrease ghrelin levels, an appetite-stimulating hormone [55,61]. Ghrelin stimulates gastrointestinal motility, reduces fat utilization and glucose-stimulated insulin release, increases body weight, and, notably, increases appetite [62]. The mechanisms underlying this ghrelin-reducing effect may include the influence of GP bitter-sensing (poly)phenols on ghrelin secretion [63] and the inhibitory effects of dietary fiber on ghrelin release [64].

GIP is an incretin hormone secreted by the K-cells in the proximal small intestine. GIP plays a role in stimulating insulin secretion in response to food intake, particularly carbohydrate-rich meals, and modulating lipid metabolism [71]. The potential mechanism through which GP might affect GIP secretion could be via the fermentation of its dietary fiber content by gut microbiota, producing SCFAs such as acetate, propionate, and butyrate. SCFAs have been implicated in the regulation of gut hormones, including GIP [72,73]. Furthermore, GP contains bioactive (poly)phenols, which may influence GIP release [63].

Dipeptidyl peptidase-IV (DPP-IV) is an enzyme that plays a crucial role in regulating incretin hormones, such as GLP-1 and GIP. DPP-IV rapidly degrades these incretins, reducing their insulin-stimulating and appetite-suppressing effects. Inhibition of DPP-IV activity can lead to increased levels of GLP-1 and GIP, improving glucose control and promoting satiety [65]. GP and grape seed proanthocyanidin extracts have demonstrated DPP-IV inhibitory activity in vitro and in vivo [63,74], suggesting a potential role in modulating incretin hormone levels and improving satiety and glycemic control.

In summary, although the evidence on humans is still scarce, accumulating in vitro and in vivo research indicates that GP modulates gut hormones involved in regulating food intake and satiety. GP's phenolic and fiber content can increase GLP-1, PYY, and CCK levels while decreasing ghrelin levels, potentially promoting weight management. GP may

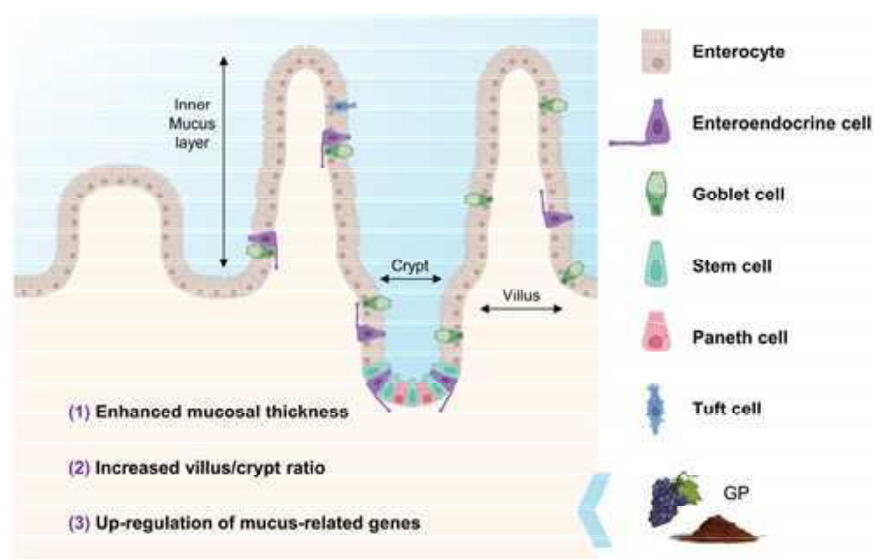
also improve glycemic control by inhibiting DPP-IV and promoting GIP secretion. Overall, these findings suggest that GP has a potential as a dietary intervention for managing weight and improving glucose control.



**Figure 3.** Schematic representation of the effects of GP on enteroendocrine hormone secretion. GLP-1 is secreted in L-cells in the distal ileum and colon, PYY in L-cells in the distal ileum and colon, CCK in I-cells in the duodenum and jejunum, ghrelin in X/A-like cells in the fundus of the stomach, GIP in K-cells in the duodenum and jejunum, and DPP-IV in the brush border membrane of the enterocytes primarily in the duodenum and jejunum. Numbers represent those activities associated with GP in several in vitro and in vivo experiments: (1): Li et al. [63]; Casanova-Martí et al. [64]; (2): Casanova-Martí et al. [68]; (3): Casanova-Martí et al. [64,68]; Casanova-Martí et al. [69]; (4): Casanova-Martí et al. [64]; Ginés et al. [70]; (5): Serrano et al. [75]; (6): Li et al. [63]; (7): Li et al. [63]; González-Abuín et al. [65,74]; (8): Serrano et al. [60,62,64].

#### 4. Effects of GP on Gut Morphology

The gut morphology (i.e., crypt-villi structures) is a key aspect of the absorptive capacity of the intestine. The intestinal tract is lined by a single layer of epithelium originating from stem cells at the base of each crypt, giving rise to four major types of epithelial cells: absorptive enterocytes, enteroendocrine cells, which export peptide hormones, goblet cells, responsible for mucus segregation, stem cells, responsible for maintaining and repairing the tissue that lines the gut, paneth cells which secrete antimicrobial defensins, digestive enzymes, and growth factors, and tuft cells, with a role in the immune system, nutrient sensing, and gut motility regulation (Figure 4) [76]. Presumably, an impaired villus/crypt ratio implies reduced digestive capacity and imbalance in the intestinal barrier. In fact, decreased villus height/crypt depth ratio is a common response in animals treated with dextran sodium sulphate (DSS) or 2,4,6-trinitrobenzene sulfonic acid (TNBS), two inductors of intestinal damage [52,77].



**Figure 4.** Schematic representation of villus/crypt structures in the duodenum epithelium. Numbers represent those activities linked to gut morphology associated with GP in several *in vivo* models: (1): Bibi et al. [52]; Wang et al. [78]; (2): Wang et al. [78]; Wang et al. [79]; Gessner et al. [80]; Zhang et al. [81]; (3): Bibi et al. [52]; Lu et al. [82]; Pistol et al. [83].

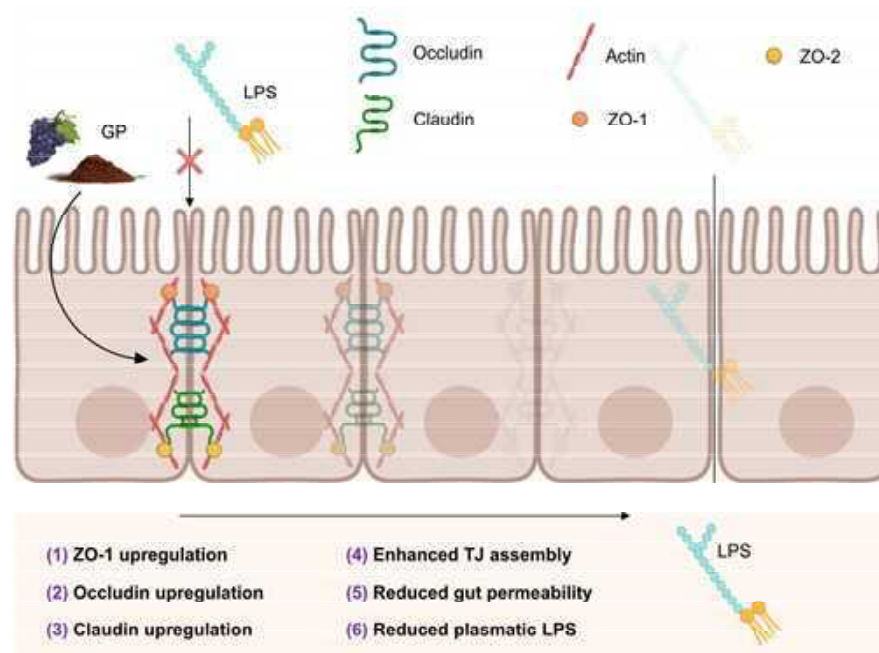
In this line, several authors reported increased villus length [52,78] or villus height/crypt depth ratio [78–81] and decreased crypt depth [81,84] in the jejunum, ileum, and colon cells from rodents, lambs, pigs, and broilers fed with GP-derived products. These products were also responsible for reducing the colonic lesion and colon shortening in rats subjected to stressful DSS and TNBS treatments [77,85], with the dietary fiber and fiber-bound (poly)phenols of the GP being more effective than free (poly)phenols [85]. This response may be a consequence of the increase in villin, a calcium-regulated actin-binding protein inhabiting the intestinal brush border and related to epithelial differentiation, which in turn was responsible for the rise in goblet cell density [52]. In contrast, these outcomes enhance mucosal thickness promoted by GP products [52] and upregulation of genes involved in extracellular mucin secretion (i.e., Muc1, Muc2, and Muc3) [82,83]. Furthermore, as a secondary issue derived from the consumption of GP (poly)phenols, it has also been described the enhanced expression of genes related both to nutrient transporters in general [52] but specifically to (poly)phenols absorption [82].

The reduction in the inner surface area due to the defective development of crypt-villi structures might result in various intestinal dysfunctions and disorders such as celiac disease, inflammatory bowel disease, colorectal cancer, and brush border-related enteropathies [86]. The evidence reviewed herewith indicates that GP-derived products might help in the management of these pathologies, although, to our knowledge, there are still no specific human interventions with GP concerning them.

## 5. Effects of GP on Intestinal Barrier Integrity

As mentioned above, the intestinal barrier is a dynamic structure that separates the internal part of the host from the intestinal lumen. Of particular importance for the integrity of the intestinal barrier are the tight junctions (TJ) formed between neighboring epithelial cells, including several proteins such as occludins, claudins, junctional adhesion molecules, and plaque proteins [87] (Figure 5). The intestinal barrier selectively allows or restricts the exchange of water, ions, and macromolecules between the intestinal lumen and the underlying tissues. This exchange occurs through both transcellular pathways, governed by the cell-specific profile of transporters and channels, and paracellular pathways, mainly controlled by TJ. However, the disruption of the intestinal barrier results in what is

known as the “leaky gut” and has been related to inflammation processes and intestinal dysfunction [88].



**Figure 5.** Schematic representation of intestinal epithelium (enterocytes). LPS: lipopolysaccharide; ZO: Zonula occludens or tight junction protein. Numbers represent those activities associated with GP in several in vitro and in vivo models: (1): Pistol et al. [83]; Gil-Cardoso et al. [89]; (2): Pistol et al. [83]; (3): Maurer et al. [85]; Pistol et al. [83]; (4): Nallathambi et al. [90]; Reiche and Huber [91]; (5): Hidalgo-Liberona et al. [92]; Yang et al. [93]; Cremonini et al. [94]; Gil-Cardonso et al. [95]; González-Quilen et al. [96]; (6): Taladrid et al. [97].

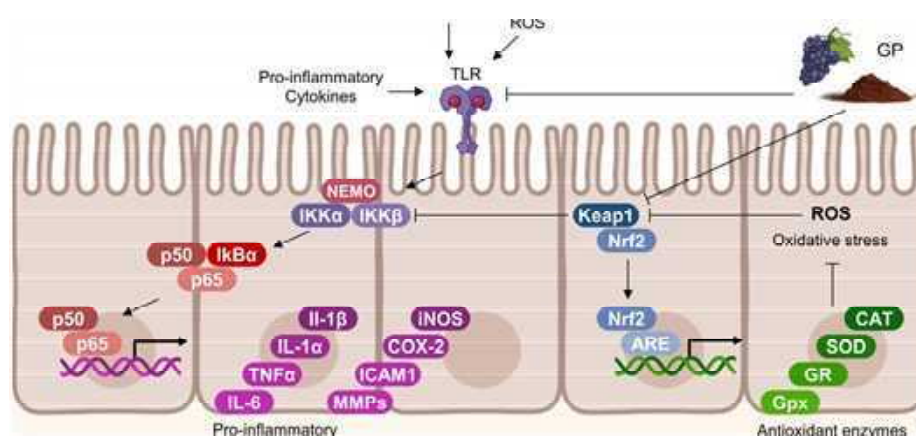
In recent years, there has been growing interest in how food-derived molecules, particularly (poly)phenols, affect the gut barrier by influencing the TJ and paracellular transport [92]. Numerous studies have investigated the effect of various grape-derived products on in vitro intestinal models such as Caco-2 cells or animal digestive tracts. These studies have demonstrated that grape-derived compounds can counteract permeabilization by studying paracellular flux (e.g., fluorescein isothiocyanate-dextran (FITC-dextran)) or transepithelial electrical resistance (TEER) [93–96]. In our most recent research, we observed a decrease in paracellular transport of FITC-dextran after incubating Caco-2 monolayers with different GP-digested samples [97]. We also noted that GP-digested samples resulted in increased production of SCFAs and phenolic acids during colonic fermentation compared to control samples. This increase in SCFA and phenolic acid production may partially explain the observed decrease in permeabilization [97]. The mechanisms underlying the enhancement of the gut barrier exerted by GP are varied, including the upregulation of key genes encoding for diverse TJ proteins, such as occludins [83], claudins [83,85], ZO-1 [83,89], and TJ protein 1 [90]. Moreover, higher expression of the same proteins without variations in mRNA levels has also been reported [82,98]. Therefore, GP compounds may mediate a boost in intercellular unions through different routes than the transcription of TJ proteins, such as post-translational modifications required in TJ assembly and their association with the cytoskeleton [91] (Figure 5). However, the physiological impact of all these mechanisms is still difficult to assess.

In summary, GP-derived molecules, such as (poly)phenols, have been shown to influence the gut barrier through TJ and paracellular transport. GP (poly)phenols have a counteracting effect on permeabilization, upregulating key genes encoding for TJ proteins

and mediating a boost in intercellular unions. These mechanisms result in a reduction in paracellular transport and enhanced gut barrier function.

## 6. Effects on Intestinal Inflammatory and Oxidative Status

The intestinal lumen is continuously exposed to harmful stimuli that may cause oxidative stress, inflammation, and injury. Thus, reactive oxygen species (ROS) can damage cell membranes, disturb barrier integrity, and lead to enhanced intestinal permeability, inflammation, and endotoxemia [99]. The antioxidant and anti-inflammatory properties of grape/wine (poly)phenols are widely known [100] and can be expected to be evident in the GP. Similar behavior can be said for dietary fiber, whose intake has been associated, in general, with lower systemic inflammation [101]. In this review, among the multiple and complex signaling routes involved in inflammation and oxidative stress, we have focused on those concerning the nuclear factor  $\kappa$ B (NF- $\kappa$ B) for inflammation and the nuclear factor erythroid 2-related factor 2 (Nrf2) for oxidative stress since their activation cascades are considerably susceptible to GP components (Figure 6).



**Figure 6.** Effects of GP on intestinal inflammatory and oxidative status. A schematic representation of relevant signaling cascades mediating the gene expression of key mediators of inflammation and oxidative stress. Numbers represent those activities associated with GP in several in vitro and in vivo experiments: (1): Pistol et al. [83,102]; Sheng et al. [98]; Maurer et al. [103]; (2): Bibi et al. [52]; Wang et al. [104]; (3): Boussenna et al. [77]; Wang et al. [78]; Gil-Cardoso et al. [89]; Nallathambi et al. [90]; Pistol et al. [102]; Maurer et al. [103]; (4): Wang et al. [78]; Gil-Cardoso et al. [89] (5): Boussenna et al., [77]; (6): Pistol et al. [83]; (7): Gessner et al. [80]; (8): Wang et al. [78]; Maurer et al. [103]; (9): Gessner et al. [80]; Sheng et al. [98]; Kafantaris et al. [105].

### 6.1. Regulation of the NF- $\kappa$ B Signaling Pathway

Under ordinary conditions, NF- $\kappa$ B is restricted to the cytoplasm forming a complex with its inhibitor (I $\kappa$ B $\alpha$ ). However, several responses may initialize their decoupling through the inhibitors of kappa kinase  $\beta$  (IKK $\beta$ ) and  $\alpha$  (IKK $\alpha$ ), which phosphorylate I $\kappa$ B $\alpha$  for polyubiquitination and subsequent degradation [106]. Once the NF- $\kappa$ B becomes free, it begins its activation and translocation to the nucleus, where it joins to specific DNA regions, the NF- $\kappa$ B sites [106]. This binding to DNA is responsible for expressing cytokines, adhesion molecules, and inflammatory enzymes. Among the principal stimuli that trigger the NF- $\kappa$ B cascade are the Toll-Like Receptors (TLR), transmembrane proteins mainly present in immune cells which react to cytokines, ROS, and, notably, to lipopolysaccharide

(LPS) [107]. Overexpression of TLR is clearly recognized in various cases of intestinal bowel disease (IBD) [108]. Furthermore, through the p300 coreceptor, phosphorylated and activated by mitogen-activated protein kinase (MAPK), this enzyme participates in the inflammatory cascade since the phosphorylated NF- $\kappa$ B forms an active complex with the p300, which facilitates their binding to DNA [109].

Some trials feeding rodents or pigs with GP-derived products have evidenced reductions in NF- $\kappa$ B expression, mRNA levels, and transactivation in the duodenum [80], jejunum [52], and colon [102] cells. Moreover, Maurer et al. [103] reported downregulation of IKK $\beta$ , the kinase responsible for NF- $\kappa$ B liberation prior to its transport to the nucleus, and of the TLR-4 signaling, concluding that fiber-bound (poly)phenols of grape peels were mainly responsible for both responses. In accordance, Yang et al. [93] reported reduced levels of MAPK in mice colonic cells, while Pistol and collaborators observed the same pattern accompanied by the suppression of TLR2 and TLR4 genes after feeding piglets with a grape seed meal [83,102]. In addition, the last authors emphasized that not only (poly)phenols may exert an inflammatory activity, highlighting the important amount of fiber and unsaturated fatty acids in grape seed products [102]. In fact, it has been described that butyrate may suppress LPS-induced NF- $\kappa$ B due to G-protein coupled receptors (GPCRs) both in vitro in colonic cells and ex vivo in rodents' large intestine [110], and specific dietary fibers present in GP voided the TLR2 cascade in human dendritic cells [111].

In addition to modulating the transcription factor NF- $\kappa$ B, GP may also mitigate the progression of associated inflammatory processes. Following the intracellular increase in inflammatory mediators, these molecules are released into the intestinal mucosa, initiating the breakdown of the extracellular matrix and the recruitment of innate immune cells such as neutrophils and macrophages. This cascade of events can lead to significant intestinal impairment, a pattern commonly observed in patients with IBD. In studies investigating the effects of GP products on intestinal inflammation, researchers often induce ulcerative colitis in animal models. As a primary outcome, there is a significant increase in pro-inflammatory cytokines, including interleukin (IL)-1 $\beta$ , IL-1 $\alpha$ , IFN- $\gamma$ , tumor necrosis factor (TNF) $\alpha$ , and IL-6 [89,103], adhesion molecules such as intercellular adhesion molecule 1 (ICAM1) [77], and proteins involved in the breakdown of the extracellular matrix, like those belonging to the matrix metalloproteinase (MMP) family [83]. Upon administering GP in various formulations, researchers observed a reduction in cytokine levels and a downregulation of their gene expression [77,89,103]. Additionally, anti-inflammatory cytokines such as IL-10 increased [102], and ICAM-1 and MMP9 gene expression was suppressed [77]. Neutrophil migration and infiltration into the intestinal mucosa are closely associated with myeloperoxidase (MPO) activity, a potent oxidative inducer that catalyzes the production of hypochlorous acid from Cl<sup>-</sup> and H<sub>2</sub>O<sub>2</sub> in neutrophils [112]. In line with this, some studies reported reduced colonic MPO levels in animal models of ulcerative colitis after GP consumption [78,89,103], with Maurer et al. [103] attributing this response to the fiber-bound (poly)phenols fraction. Moreover, the anti-inflammatory effects of GP have been observed in healthy animals, resulting in reduced pro-inflammatory cytokine levels and gene expression [78,102], decreased MMP2 and MMP9 activity [83], and increased levels of anti-inflammatory IL-10 and IL-4 [102,103].

### 6.2. Regulation of the Nrf2 Signaling Pathway

Oxidative stress is a physiological state that arises from an imbalance between the production of reactive oxygen species (ROS) and the body's ability to detoxify them. ROS are highly reactive molecules that can damage lipids, proteins, and nucleic acids, leading to cellular dysfunction, tissue damage, and inflammation. Oxidative stress and inflammation are closely intertwined processes that play important roles in the development of various diseases, including gut inflammation [113]. Neutrophil infiltration into the mucosa, for example, leads to increased ROS production in the area, which in turn facilitates the migration of other immune cells to the epithelium and enhances the establishment of a

niche for pro-oxidant enzymes, such as inducible nitric oxide synthase (iNOS). This enzyme catalyzes the generation of the potent oxidative peroxynitrite radical [114]. Moreover, ROS are effectors of TLRs, which can stimulate the translocation of NF- $\kappa$ B into the nucleus [104].

As previously mentioned, another crucial signaling pathway involves antioxidant responses mediated by Nrf2 [115]. Similarly to NF- $\kappa$ B, Nrf2 is sequestered in the cytoplasm by binding to a Kelch-like ECH-associated protein 1 (Keap1) [116] (Figure 6). Keap1 is an oxidation-sensitive protein that dissociates from Nrf2 when ROS levels increase in the cytoplasm, enabling Nrf2 to translocate to the nucleus and bind to the antioxidant response elements (ARE) in its target genes. This process induces the expression of antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT), or glutathione peroxidase (GPx) [117]. Apart from regulating Nrf2, Keap1 has been found to inhibit the activity of IKK $\beta$ , which is responsible for initiating the NF- $\kappa$ B cascade. This provides another intersection point between oxidation and inflammation. Then, the activation of Nrf2 can enhance the gut epithelium's antioxidant capacity and reduce inflammation. Many studies examining the effects of GP on inflammation have also reported increased SOD, CAT, and GPx activity and gene expression [78,103], as well as reduced levels of ROS, nitric oxide (NO), or iNOS protein [89]. Specifically, Gessner et al. [80] conducted a study involving pigs in which they observed that the increase in antioxidant enzymes was accompanied by enhanced transactivation of both Nrf2 and NF- $\kappa$ B.

Considering that obesity and other associated metabolic disorders are characterized by chronic low-grade intestinal inflammation—also mediated by factors such as Nrf2 and NF- $\kappa$ B [118]—it is plausible that the protective effects of GP against these disorders might be achieved through the regulation of these molecular pathways at the intestinal level.

In conclusion, the antioxidant and anti-inflammatory properties of GP (poly)phenols and dietary fiber have been widely studied and shown to have potential benefits in reducing oxidative stress and inflammation in the gut. Specifically, the activation cascades of the NF- $\kappa$ B and the Nrf2 are susceptible to GP components, as demonstrated by studies in rodents, pigs, and humans. GP-derived products have been shown to reduce NF- $\kappa$ B expression and transactivation, downregulate TLR signaling, and mitigate the progression of associated inflammatory processes. Moreover, GP consumption has been associated with an increase in antioxidant enzyme activity and gene expression, as well as reduced levels of ROS, nitric oxide, or iNOS. Given the association between chronic low-grade intestinal inflammation and metabolic disorders, the regulation of these molecular pathways at the intestinal level may play a crucial role in the protective effects of GP against these disorders.

## 7. Effects of GP on the Gut Microbiome

Because of its health implications, the gut microbiome is now a thriving area of research integrating basic and clinical sciences and a priority topic worldwide [119]. Alterations in the state of the gut microbiota (dysbiosis) are increasingly linked to the incidence of non-communicable diseases [120]. Among the factors that determine our gut microbiota, diet is one of the most important and, possibly, the one we can influence the most. The dietary fiber fraction has long been recognized as a food component that most modulates gut microbiota [121]. Regarding (poly)phenols, current research suggests that they have great potential for action against microbial dysbiosis, with a favorable impact on other aspects of health related to the gut microbiome [122].

As seen in gastrointestinal digestion simulations of GP, gut microbiota extensively degrade their constituents leading to a great battery of bioaccessible phenolic metabolites (i.e., benzoic, phenylacetic, and phenylpropionic acids) as well as SCFAs mainly derived from the dietary fiber fraction [123]. In turn, these GP components and their microbial-derived metabolites can modulate the composition and functionality of the gut communities [123]. The mechanisms behind this phenolic-mediated modulation are intricate and still scarcely known, which gives rise to a considerable amount of pending work in the area. For the time being, some hypotheses point to the interference of the quorum sensing mediated by (poly)phenols, limiting the formation of pathogens biofilms [124]; others suggest that

competition for nutrients favors those bacteria capable of extracting the sugars attached to (poly)phenols and fiber to obtain sugars and SCFAs, generally species associated with a probiotic effect [125].

Table 3 reports the in vivo studies reported in the literature concerning the effects of GP-derived products on gut microbiota composition. Other studies dealing with other grape products (grapes, raisins, grape seeds, wine, etc.) have not been considered in this review as they show different chemical compositions, especially concerning the fiber fraction.

**Table 3.** Animal and human studies concerning the effects of GP on gut microbiota composition.

Study PI Design/Intervention	Microbiota-Related Measurements	Changes in Microbiota Composition	Relation to Other Biomarkers	Refs.
<b>Broiler chicks</b>				
Cobb broiler chicks; 1-day old <i>n</i> = 25 ♂ animals/group Duration: 21 days Diets:	Plate counting 16S rRNA sequencing	<ul style="list-style-type: none"> <li>Ileum</li> <li>↑ <i>Enterococcus</i></li> <li>↓ <i>Clostridium</i></li> <li>Cecum</li> <li>↑ <i>E. coli</i></li> <li>↑ <i>Lactobacillus</i></li> <li>↑ <i>Enterococcus</i></li> <li>↑ <i>Clostridium</i></li> <li>↑ Bacterial diversity</li> </ul>	=Weight gain	[126]
<ul style="list-style-type: none"> <li>Antibiotic-free (control)</li> <li>Antibiotic-included (50 mg/kg of avoparcin)</li> <li>Antibiotic-free diet + GP concentrate (60 g/kg)</li> </ul>			<ul style="list-style-type: none"> <li>Jejunum</li> <li>↑ Villus height/crypt depth ratio</li> </ul>	
Cobb-500 broiler chicks; 1-day old <i>n</i> = 192 animals/group Duration: 42 days Diets:	16S rRNA sequencing SCFAs by GC	<ul style="list-style-type: none"> <li>↑ <i>Bacteroidetes</i></li> <li>↓ <i>Firmicutes</i></li> <li>↑ <i>Bacteroides</i></li> <li>↑ <i>Lactobacillus</i></li> <li>=SCFAs</li> </ul>	<ul style="list-style-type: none"> <li>↑ Feed intake</li> <li>=Feed conversion ratio</li> <li>Duodenum</li> <li>↑ Villus height</li> <li>↑ Villus height/crypt depth ratio</li> <li>Jejunum</li> <li>↑ Villus height</li> <li>↑ Villus width</li> <li>Ileum</li> <li>=Crypt depth</li> <li>=Villus height/crypt depth ratio</li> </ul>	[127]
<ul style="list-style-type: none"> <li>Antibiotic-free (control)</li> <li>Antibiotic-included (0.05% BMD)</li> <li>Antibiotic-free + 2.5% GP</li> </ul>				
<b>Rodents (rats, mice)</b>				
Wistar rats; <i>n</i> = 6 animals/group Duration: 14 months Diets:	qPCR Urine phenolic metabolites by LC-QTOF	<ul style="list-style-type: none"> <li>↑ <i>Bifidobacterium</i></li> <li>=<i>Bacteroides</i></li> <li>=<i>Clostridium leptum</i></li> <li>=<i>Enterococcus</i></li> <li>↓ <i>Clostridium</i> Cluster I</li> </ul>		[128,129]
<ul style="list-style-type: none"> <li>0.1% DMSO (control)</li> <li>GP phenolic extract (2.5, 5, 10, and 20 mg/kg/day)</li> </ul>				
C57BL/6J mice; 9-week old <i>n</i> = 14 animals/group Duration: 8 weeks Diets:	16S rRNA sequencing SCFAs by GC	<ul style="list-style-type: none"> <li>↓ <i>Desulfovibrio</i></li> <li>↓ <i>Lactococcus</i></li> <li>↑ <i>Allobaculum</i></li> <li>↑ <i>Roseburia</i></li> </ul>	<ul style="list-style-type: none"> <li>↓ Fat mass gain</li> <li>↓ Adipose tissue inflammation</li> <li>=Food intake</li> <li>↑ Glucose tolerance</li> <li>↓ Insulin resistance index</li> <li>↑ Antimicrobial peptides</li> <li>↑ Tight junction proteins</li> </ul>	[57]
<ul style="list-style-type: none"> <li>Control</li> <li>High-fat diet</li> <li>High-fat diet + GP phenolic extract (8.2 g/kg)</li> </ul>				

Table 3. Cont.

Study PI Design/Intervention	Microbiota-Related Measurements	Changes in Microbiota Composition	Relation to Other Biomarkers	Refs.
C57BL/6J mice; 9-week old <i>n</i> = 10 ♂ animals/group Duration: 3-week antibiotics + 1 week diet Diets: <ul style="list-style-type: none"><li>Saline solution (control)</li><li>GP extract 200 mg/kg</li></ul>	16S rRNA sequencing	<ul style="list-style-type: none"><li>Fecal microbiota</li></ul> ↑ Relative abundance ↑ Diversity ↑ <i>Akkermansia</i>		[130]
Piglets				
Landrace × Large White–Duroc <i>n</i> = 12 animals/group Duration: 15 days Diets: <ul style="list-style-type: none"><li>Control</li><li>4% GP</li></ul>	Plate counting	↑ Facultative probiotic bacteria ↑ Lactic acid bacteria ↓ <i>Enterobacteriaceae</i> ↓ <i>Campylobacter jejuni</i>	↑ Weight gain ↑ GSH ↑ H <sub>2</sub> O <sub>2</sub> decomposition activity ↑ Total antioxidant capacity ↓ MDA (TBARS) ↓ Protein carbonyls	[105]
			=Growth	
Songliao black pigs; 28-day old <i>n</i> = 6 animals/group Duration: 28 days Diets: <ul style="list-style-type: none"><li>Control</li><li>5% GP</li></ul>	16S rRNA sequencing	↑ <i>Lactobacillus delbrueckii</i> ↑ <i>Olsenella umbonate</i> ↑ <i>Selenomonas bovis</i>	<ul style="list-style-type: none"><li>Jejunum</li></ul> ↑ Villus height ↑ Villus height/crypt depth ratio  <ul style="list-style-type: none"><li>Caecum</li></ul> ↓ Pro-inflammatory cytokines (IL-1β, IL-8, IL-6, TNF-α) =SCFAs receptors (GPR41/43) ↑ Serum IgG	[78]
Lambs				
Chios lambs; 15-day old <i>n</i> = 12 ♂ animals/group Duration: 55 days Diets: <ul style="list-style-type: none"><li>Control</li><li>45% GP</li></ul>	Plate counting	↑ Facultative probiotic bacteria ↓ <i>Enterobacteriaceae</i> ↓ <i>Escherichia coli</i>	↑ CAT ↑ GSH ↓ MDA (TBARS) ↓ Protein carbonyls	[131]
Humans				
Healthy women ( <i>n</i> = 10) Duration: 3 weeks Diet: 1.4 g/day of a red GP extract	qPCR SCFAs by GC-MS Phenolic metabolites by UPLC-ESI-MS/MS	=Faecal bacteria populations =Faecal/urine phenolic metabolites ↑ Fecal SCFAs	↓ Blood fasting glucose Glucose metabolism-related miRNA modulation	[19]
≥2 metabolic syndrome factors subjects ( <i>n</i> = 49) Duration: 6 weeks Diet: 8 g/day of GP extract	qPCR SCFAs by GC	=Microbiota profile ↑ Lactobacilliales ↑ <i>Bacteroides</i> =SCFAs	↑ insulin levels (responders)	[132]
Responders ( <i>n</i> = 23) and non-responders ( <i>n</i> = 26) to insulin reduction Duration: 6 weeks Diet: 8 g/day of GP extract	16S rRNA sequencing Sequencing SCFAs by GC	↓ Prevotella ↓ Firmicutes	↑ miRNA-222 levels (responders)	[133]

Table 3. Cont.

Study PI Design/Intervention	Microbiota-Related Measurements	Changes in Microbiota Composition	Relation to Other Biomarkers	Refs.
High-risk cardiometabolic ( $n = 17$ ) healthy ( $n = 12$ ) subjects Duration: 6 weeks Diets: <ul style="list-style-type: none"> <li>• Control</li> <li>• 2 g/day of GP seasoning</li> </ul>	16S rRNA sequencing SCFAs by GC-MS Phenolic metabolites by UPLC-ESI-MS/MS	= $\alpha$ and $\beta$ diversity ↓ <i>Peptoniphilus</i> ↓ <i>Clostridiaceae 1</i> ↓ <i>Clostridium sensu stricto 1</i> ↓ <i>Ezakiella</i> ↓ <i>Streptococcus</i> ↓ <i>Lachnospiraceae ND3007</i> ↓ <i>Paraprevotella</i> ↓ <i>Senegalimassilia</i> ↑ Streptococcaceae ↓ Eggerthellaceae ↓ Coribacteriales <i>Incertae Sedis</i> ↓ Propionic acid ↑ Protocatechuic acid	↓ Blood pressure ↓ Fasting blood glucose Bacterial communities associated with cardiovascular and metabolic data	[16]

↓: Decrease in; ↑: Increase in.

The first studies reporting GP's effect on gut microbiota aimed to assess the possible application of (poly)phenols as alternatives to antibiotics in broiler chicken production [126]. These authors found that GP concentrates seemed to be effective in increasing the ileal populations of beneficial bacteria as well as markers of healthy gut morphology (Table 3). Recently, other researchers have found similar results, further corroborating that the inclusion of GP-derived products in the diet of broiler chickens favors intestinal health without affecting their blood biochemical and immune profiles [127] (Table 3).

In rodents, Chacar et al. [128,129] specifically assessed the impact of a GP phenolic extract on gut microbiota, finding that, at the conditions used, GP supplementation could inhibit non-beneficial bacteria and potentiate the growth of probiotic ones, counteracting the adverse outcomes of aging on the gut bacterial population (Table 3). Experiments have also been conducted in mice fed high-fat diets, finding that GP supplementation appeared to ameliorate the overall metabolic profile derived from a high-fat diet [57] or to facilitate the recovery of gut microbiota after antibiotic treatment [130], both effects related to changes in some key microbial genera (Table 3).

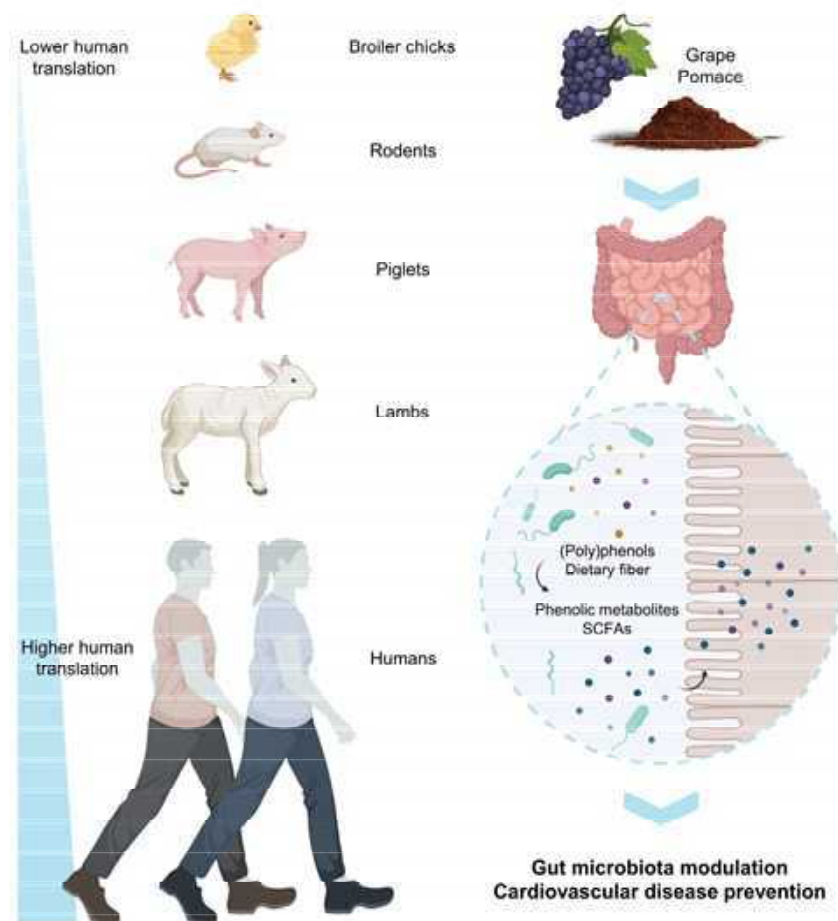
Other studies in larger animals such as pigs [78,105] and lambs [131] corroborated that GP supplementation has no adverse effects on animal growth and can also promote the content of some beneficial bacteria in the caecum [78,105,131], as well as the enhancement of particular antioxidant [105,131], and anti-inflammatory mechanisms [78] (Table 3).

Concerning human intervention studies (Table 3), the only three studies carried out to date reported slight changes in the composition of the gut microbiota after GP supplementation in healthy women [19]; subjects exhibited at least two factors for metabolic syndrome [132,133] and subjects at high-risk cardiovascular or not [16]. No significant increase in GP-derived metabolites such as SCFAs and phenolic acids after supplementation was reported [19,132,133]. A key aspect of these studies was the high inter-individual variability observed in the body response to GP supplementation in terms of biomarkers such as blood pressure [16], fasting glucose [16,19], or insulin levels [132] (Table 3). This inter-individual variability in clinical trials with GP-derived products was related to differences in gut microbiota and miRNA [19,133]. On the other hand, it happens that the relationship between gut microbiota composition and clinical and metabolic changes derived from GP supplementation may rely more on metabolic-related bacterial communities rather than bacterial genera/family, emphasizing the ecological/functional aspects of the different communities over taxonomic aspects [16].

The differences in the extent and impact of these in vivo effects may be attributable to differences in study design (animal model, GP-derived product, doses, etc.), although it seems clear that the human gut microbiota is more stable and, therefore, more resilient to

diet-induced changes. However, all these studies highlight the potential modulatory effects of GP-derived products on gut microbiota composition and open the door to studies on how GP supplementation impacts gut microbiota metabolism and functionality in relation to different pathologies. For instance, gut microbiome-modulating properties of GP seem to be behind the reduction of trimethylamine-*N*-oxide (TMAO) [134], a gut microbiota-derived metabolite recognized as strongly related to cardiovascular diseases, mainly increasing the risk of atherosclerosis development [135].

Hence, gut microbiota can degrade GP components, producing bioaccessible phenolic metabolites and SCFAs, which can modulate gut communities' composition and functionality (Figure 7). In vivo studies have shown that GP supplementation can promote intestinal health, ameliorate metabolic profiles, enhance antioxidant and anti-inflammatory mechanisms, and increase the content of beneficial bacteria in animals. Clinical trials with GP-derived products show inter-individual variability in terms of biomarkers, emphasizing the importance of metabolic-related bacterial communities over bacterial genera/family. GP-derived products have significant potential for modulating gut microbiota composition and functionality in relation to different pathologies, including reducing the risk of non-communicable diseases such as cardiovascular disease. Further research is needed to explore the therapeutic applications of GP-derived products in promoting gut health.



**Figure 7.** Schematic representation of the potential of GP in modulating gut microbiota composition and functionality in vivo and in clinical studies.

## 8. Conclusions

GP is a fascinating food by-product that has long been studied for its potential use as a dietary supplement and its protective properties against cardiovascular diseases. The health-promoting properties of GP are primarily attributed to its rich content of

(poly)phenols and dietary fiber, as well as the interactions between these components. Considering that the intestinal tract is the first site of interaction for food components and their potential biological activities, this review focuses on GP's bioactivity within the gut environment and its possible implications for cardiovascular health. **Based on in vitro and in vivo studies, substantial evidence supports GP's ability to (i) regulate nutrient digestion and absorption (modulating digestive enzyme action in the intestinal lumen and the expression of intestinal transporters), (ii) modulate gut hormones (GLP-1, PYY, CCK, ghrelin, and GIP) levels and satiety, (iii) reinforce gut morphology (crypt-villi structures), (iv) protect intestinal barrier integrity through tight junctions and paracellular transport, (v) modulate intestinal inflammation and oxidative stress via NF- $\kappa$ B and Nrf2 signaling pathways, and (vi) positively impact gut microbiota composition and functionality.** Hence, GP promotes cardiovascular health within the intestinal environment by regulating blood lipid and glucose levels, supporting appetite regulation, reducing inflammation and oxidative stress, and fostering a beneficial gut microbiota composition. The current state of knowledge does not clearly define a primary mechanism of action for GP at the intestinal level, which may vary among individuals and pathological conditions. However, these mechanisms are evidently interconnected. For example, GP-mediated changes in microbiota composition may lead to increased production of SCFAs such as acetate, propionate, and butyrate or decreased production of bacterial LPS, which are known disruptors of gut barrier integrity and inflammation stimulants. Thus, GP's overall effect reinforces the intestinal function as a crucial first line of defense against multiple disorders, including those impacting cardiometabolic health. In a sense, GP's systemic bioactivity begins in the gut, which can contribute to the prevention and management of cardiovascular diseases. Future research on GP's health-promoting properties should consider connections between the gut and other organs (gut-heart axis, gut-brain axis, gut-skin axis, oral-gut axis), further solidifying its role as a cardiometabolic health-promoting ingredient.

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## Abbreviations

BMD	bacitracin methylene disalicylate
CCK	cholecystokinin
GIP	glucose-dependent insulintropic polypeptide
GLP-1	glucagon-like peptide-1
GP	grape pomace
LPS	lipopolysaccharide
PYY	peptide YY
qPCR	quantitative real-time PCR
NF- $\kappa$ B	nuclear factor $\kappa$ B
Nrf2	nuclear factor erythroid 2-related factor 2
SCFAs	short-chain fatty acids

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