ANALYSIS OF FULVIC ACID – NATURE'S MOST POTENT METABOLIC REGULATOR

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Abstract:

Fulvic companies commonly claim that fulvic acid is a nutrient transport molecule, inferring that it increases the body's ability to absorb nutrients from food. Mineral Logic, a fulvic manufacturer, has asked me to review the science supporting this notion.

The effects of dietary fulvic acids (e.g. flavonoids, phenolic acids, and polyphenols) has been attributed to several concerted molecular events, as shown in many published studies reviewed for this paper.

Absorbing nutrients involves the digestive process, or complex biochemical processes taking place throughout the body. It is very easy to eat excessive carbohydrates which over time and frequency can lead to insulin-insensitivity and subsequent disruption of glucose homeostasis. At a minimum, our modern diet places an unnatural load of simple sugars, and chemically-altered molecules on our digestive system.

The studies I've reviewed show that phytochemicals modulate intracellular signaling, which allows glucose to be shuttled away from fat production to an increase uptake in muscle and other tissues that provide an increase of muscle energy and subsequent up-regulation of mitochondrial biogenesis.

Polyphenols have antioxidant activity and they inhibit advanced glycation end product formation. Advanced glycation end products (AGEs) are proteins or lipids that become damaged as a result of exposure to sugars. These damaged proteins or lipids can be a factor in aging and in the development or worsening of many degenerative diseases, such as diabetes, atherosclerosis, chronic kidney disease, and Alzheimer's disease.

Presented below are studies supporting the mechanisms of action that fulvic acids perform throughout the body by regulating the metabolism of nutrients in the following ways:

- The regulation of glucose homeostasis and insulin sensitivity.
- The regulation of glucose absorption preventing the shuttling of glucose towards the production of adipose tissue.

- The regulation of carbohydrate digestion allowing for stimulation of insulin secretion, and protection of pancreatic β-cells against glucotoxicity.
- Prevents the dysregulation of glucose absorption leading to the accumulation of fat, including adipose and the liver storing an excess of glucose as glucagon and hepatic fat.
- Increases mitochondrial biogenesis
- Stimulates AMPK, a master regulator of cellular energy
- Maintains a healthy gut microbiome

Fulvic acids modulate the mechanisms necessary to maintain these complex digestive processes, thus they can be classified as a potent metabolic regulator.

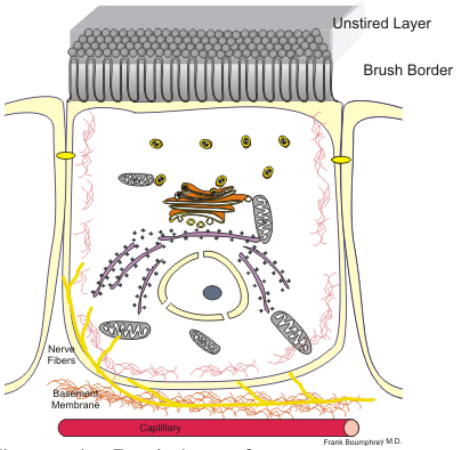

Figure 1. Depiction of an enterocyte (intestinal absorptive cells) where glucose is taken into cells via transporters (e.g. SGLT1) located in the brush border membrane.

The Background

Mineral Logic harvests a full-spectrum fulvic micronutrient from a prehistoric compost deposit in the United States which contains over seventy trace minerals and over thirty organic acids that are also known as phenolic acids, which certainly contain flavonoids. Across the board, flavonoids are the powerhouse of nutritional activators and fulvic acids appear to be the most complex assortment of flavonoids available. Testing is currently underway by Invitrox to identify and characterize these specific flavonoids in the product MLG-50™.

Polyphenols and Nutrient Absorption- Focus on Glucose and Carbohydrate Metabolism

Fulvic acids modulate carbohydrate digestion and glucose absorption in the intestine thereby regulating glucose homeostasis and maintaining healthy blood glucose levels. The key enzymes involved in digestion of dietary carbohydrate are α-amylase (breaks down starch and glycogen yielding the sugars maltose, maltotriose and α-dextrins) and α-glucosidase (breaks down starch and disaccharides to glucose). Further digestion occurs in the small intestine by α-glucosidase which is a class of brush-border bound enzymes which hydrolyze the terminal -1, 4-linked glucose residues [1, 2]. Glucose is taken into cells via transporters, predominately sodium-dependent glucose transporter (SGLT1) located in the brush border membrane (Figure 1.) at the apical side of the small intestine absorptive cells (enterocytes) [3]. Inhibition of α-amylase and α-

glucosidase activities *in vitro* (e.g. regulation of glucose absorption) and the corresponding positive health benefits have been demonstrated with dietary fulvic acids from berries (strawberries, raspberries, blueberries and blackcurrants) [4], vegetables (pumpkin, beans, maize and eggplant) [5, 6], black rice [7], legumes [8], green and black tea [2], tea polyphenols [9] and red wine [10]. Regulation of glucose transport has been shown with flavonoids and phenolic acids [11-15]

Dietary Fulvic Acids Modulate Tissue Uptake of Glucose

Enhanced insulin-mediated glucose uptake *in vitro*, a glucose transporter 4-facilitated process, has been shown with dietary fulvic acids including epicatechin [16], epigallocatechin-3-O-gallate (EGCG) [17], grape seed-derived procyanidins [18, 19], bitter melon [20] , blueberry [21], canna indica root [22] and black soy bean [23].

Gut Microbiota

Only 5%–10% of the total intake of dietary fulvic acids are directly absorbed through the stomach and the small intestine [24]. The majority of the ingested fulvic acids reach the colon, thereafter, undergoing intensive metabolism prior to absorption [24, 25]. Some flavonoids are thought to exert a prebiotic effect by stimulating the growth and activity of some bacteria in the digestive tract [25]. After absorption, fulvic acids undergo phase I and II biotransformation (sulfation, glucuronidation, methylation and glycine-conjugation) by enterocytes in the liver to increase hydrophilicity favoring urinary secretion [24]. Fulvic acid metabolites derived from liver metabolism interact with adipose tissue, pancreas, muscle and liver, and may exert anti-diabetic effects [25]. Absorption of fulvic acids can be affected by dosage, size of the phenolic compound, prior diet, food matrix, gender and differences in the gut microbial populations. An increased level of fecal *Bifidobacteria* has been associated with improved glucose tolerance and diminished inflammatory markers such as the interleukins IL-6, IL-1 and IL-1, tumor necrosis factor and monocyte chemoattractant protein-1 [25-27]. Clinical trials [28-33] have shown the potential prebiotic effects of dietary fulvic acids to increase the population of *Bifidobacteria*.

Liver Glucose Homeostasis

The liver is a key regulator of blood glucose levels in coordination with muscle and adipose tissues [1]. After eating a meal the liver stores glucose as glycogen via the glycogenesis pathway and glucokinase (GK) which is a key enzyme in the regulation of glucose utilization in the liver, along with glycogen synthase (GS). GK is an enzyme that facilitates phosphorylation of glucose to glucose-6-phosphate. GK occurs in cells in the liver and pancreas of humans and most other

vertebrates. In each of these organs it plays an important role in the regulation of carbohydrate metabolism by acting as a glucose sensor, triggering shifts in metabolism or cell function in response to rising or falling levels of glucose, such as occur after a meal or when fasting. Mutagens of the gene for this enzyme can cause unusual forms of diabetes or hypoglycemia (low blood glucose). GS is a key enzyme in glycogenesis, the conversion of glucose into glycogen that is a form of polymerized glucose stored in the liver. Under fasting conditions, the liver produces glucose via two routes; either glycolysis -the breakdown of stored sugars in the liver- or gluconeogenesis- the polymerization of glucose for storage in the liver [1]. Ingesting fulvic acids also activates AMPK. The net effect of AMPK activation is stimulation of hepatic fatty acid oxidation, ketogenesis, stimulation of skeletal muscle fatty acid oxidation and glucose uptake, and inhibition of cholesterol synthesis.

Study: Black soybean seed rich in the fulvic acids, anthocyanins (cyanidin 3-glucoside) and procyanidins (PCs) lowered glucose levels and improved insulin sensitivity by activation of 5'-adenosine monophosphate-activated protein kinase (AMPK) in the skeletal muscle and liver of type 2 diabetic mice. This activation was accompanied by the up-regulation of glucose transporter 4 GLUT4 in skeletal muscle, providing more energy to muscle cells, and the down-regulation of gluconeogenesis -the breakdown and release of stored glucose in the liver- in type 2 diabetic mice [34, 35].

Study: EGCG maintained insulin sensitivity in rat muscle cells exposed to dexamethasone, a corticosteroid that induces muscle atrophy by inducing a glucose uptake deficiency, and dose-dependent increase in glucose uptake and GLUT4 translocation, increasing the number of glucose transporters in muscle cells, through activation of phosphoinositide 3-kinase (PI3K) signaling and subsequent AMPK activation [17]. Phosphoinositide 3-kinases (PI3Ks) are a family of enzymes involved in cellular functions such as cell growth, proliferation, differentiation, motility, survival and intracellular trafficking whereas AMPK or, 5' adenosine monophosphate-activated protein kinase, is an enzyme that plays a role in cellular energy homeostasis, largely to activate glucose and fatty acid uptake and oxidation when cellular energy is low [36-46]. Therefore, EGCG stimulates PI3K stimulating the regulation of cellular division whereas AMPK activation positively effects cellular homeostasis, such as glucose levels, cellular hydration and ATP production. Flavonoids maintain glucose blood levels in diabetic human and animal models by enhancing insulin sensitivity, inhibiting glucose uptake in enterocytes (intestinal absorptive cells) and enhancing glucose uptake in muscle cells [47].

Study: Suppressed liver glucose production, from the conversion of glycogen to glucose, appears to account for decreased glucose levels in EGCG and green tea feeding and *in vitro* studies [48-50]. In the rodent study [50] of EGCC supplementation of either 0.25%–1% for seven weeks, a

decrease of glucose levels in EGCC-treated db/db (non-insulin-dependent diabetic) mice was observed in a dose dependent manner, as compared with placebo-treated mice.

Study: The GK mRNA expression, an indicator of glucose utilization in the liver, was *increased* in the livers of mice supplemented with EGCC for seven weeks in a dose dependent manner and a *decrease* of PEPCK mRNA expression, an enzymatic process by which cells synthesize glucose, was observed in adipose tissue of EGCC supplemented mice. High-dose EGCC supplementation also *increased* acyl CoA oxidase-1, an enzyme that participates in 3 metabolic pathways: fatty acid metabolism, polyunsaturated fatty acid biosynthesis, and the PPAR signaling pathway. Furthermore, carnitine palmitoyl transferase-1, a mitochondrial enzyme that participates in fatty acid metabolism for the subsequent production of ATP, is also upregulated in both liver and adipose tissues [50]. Peroxisome proliferator-activated receptors (PPARs) are a group of nuclear receptor proteins that function as transcription factors regulating the expression of several genes involved in a specified metabolic pathway, for example, genes involved in fatty-acid metabolism [51]. Moreover, PPARs play essential roles in the regulation of cellular differentiation, development, and metabolism (carbohydrate, lipid, and protein) [52], of higher organisms.

Study: Studies utilizing a H4IIE hepatoma cells (human liver cancer cell-line), EGCC downregulated genes involved in gluconeogenesis and the synthesis of fatty acid, triacylglycerol and cholesterol, whereas genes involved in glycolysis, the breakdown of glucose, and glucose transport were increased [50].

Study: Supplementation of the soy isoflavones genistein and daidzein (0.02% in diet) improved glucose homeostasis in NOD (non-obese diabetic) mice with an increase in insulin/glucagon ratio and C-peptide level, a connecting peptide that joins insulins A-chain to its B-chain to form functional insulin, with preservation of insulin staining β-cells of the pancreas. All of these findings are indicators of nominal glucose homeostasis. In the liver, glucose-6-phosphatase (G_6Pase), PEPCK activities and oxidation of fatty acids were suppressed and lipogenesis, fatty acid synthesis subsequently delivered to cells for energy metabolism, was increased compared with the control group [53].

Study: In non-insulin-dependent-diabetic db/db mice, the citrus flavonoids hesperidin and naringin, were found to lower glucose levels through up-regulating hepatic GK. Hepatic GK acts as a glucose sensor, triggering shifts in metabolism or cell function in response to rising or falling levels of glucose, via the peroxisome proliferator-activated receptor gamma (PPAR-γ), a metabolic transcriptional activator that regulates fatty acid storage and glucose metabolism, and upregulating adipocyte GLUT4, an insulin-regulated glucose transporter found primarily in

adipose, skeletal and muscle tissues. Naringin also suppressed phosphoenolpyruvate carboxykinase (PEPCK) an enzyme involved in the metabolic pathway of gluconeogenesis, and glucose 6-phosphatase, an enzyme that hydrolyzes glucose 6-phosphate, resulting in the creation of a phosphate group and free glucose. This mechanism of citrus flavonoid is very similar to the mechanism of thiazolidinediones, a family of drugs used in the treatment of diabetes mellitus type 2 [54]. Ingesting fulvic acids reduces fasting blood glucose levels, PEPCK, G_6Pase and enhanced the levels of activated AMPK 5', an enzyme that plays a role in cellular energy homeostasis, largely to activate glucose and fatty acid uptake and oxidation when cellular energy is low [36-46]. The net effect of AMPK activation is stimulation of hepatic fatty acid oxidation, ketogenesis, stimulation of skeletal muscle fatty acid oxidation and glucose uptake, inhibition of cholesterol synthesis, lipogenesis, and triglyceride synthesis, inhibition of adipocyte lipogenesis, activation of adipocyte lipolysis, and modulation of insulin secretion by pancreatic β-cells [44]. See Figure 2.

AMPK is the Master Regulator of Cellular Energy

AMPK or 5' adenosine monophosphate-activated protein kinase is an enzyme that plays a role in cellular energy homeostasis, largely to activate glucose and fatty acid uptake and oxidation when cellular energy is low [36-46]. It is expressed in a number of tissues, including the liver, brain, and skeletal muscle. In response to binding AMP and ADP, activators of AMPK, results in the net stimulation of:

- Liver fatty acid oxidation
- Ketogenesis
- Stimulation of skeletal muscle fatty acid oxidation and glucose uptake
- Inhibition of cholesterol synthesis, lipogenesis, and triglyceride synthesis
- Inhibition of adipocyte lipogenesis, activation of adipocyte lipolysis
- Modulation of insulin secretion by pancreatic beta-cells

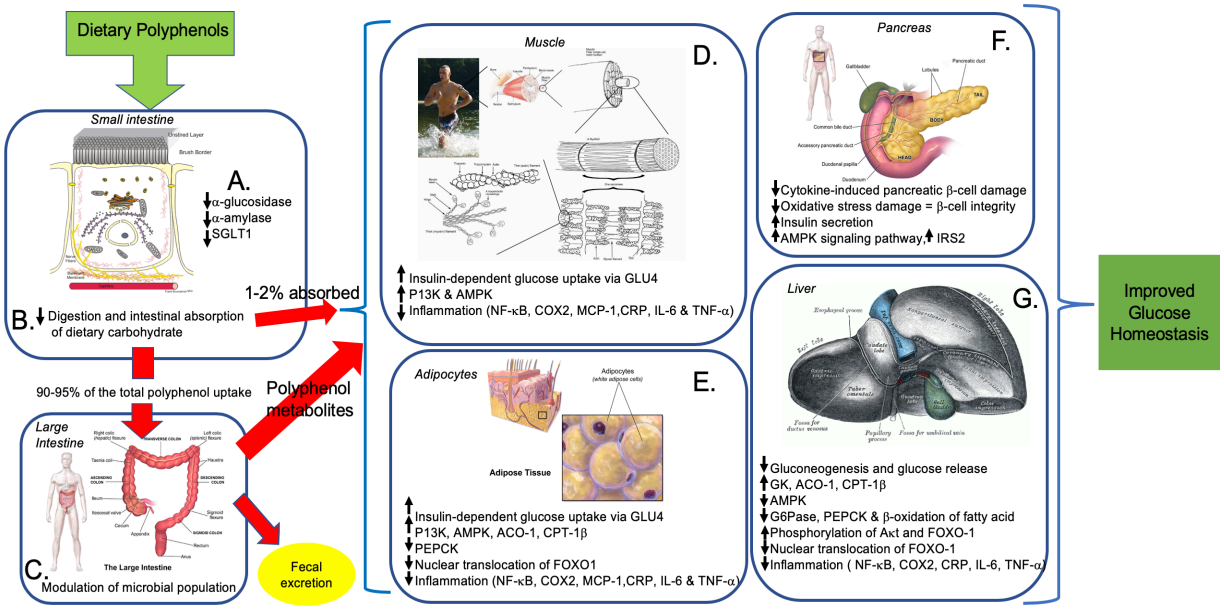

Figure 2. Summary of potential mechanisms linking dietary polyphenol metabolites to improved glucose homeostasis. ↑, increase; ↓, decrease of the designated gene expression. **A.&B.** A decrease of dietary carbohydrate digestion and absorption in the small intestine due to inhibition of α-glucosidase, α-amylase and the glucose transporter SGLT1, by polyphenols (e.g.1-2% of dietary carbohydrates absorbed). **C.** 90-95% of polyphenols pass to the large intestine where they are modified ("activated flavonoid metabolites") by the resident microflora and subsequently absorbed by the large intestine. **D.** Polyphenol metabolites increase muscle tissue insulin-dependent glucose uptake via the glucose transporter GLU4. P13K & AMPK expression are increased and are responsible for cellular functions such as muscle cell growth, proliferation, differentiation, motility, survival and intracellular trafficking. Proteins involved in muscle inflammation such as NF-κB, COX2, CRP, IL-6 and TNF-α are down regulated. **E.** In adipocytes (fat cells) flavonoids increased insulin dependent glucose levels via up-regulation of the glucose transporter GLU4. P13K, AMPK, ACO-1 and CTP-1β (inhibition of adipocyte lipogenesis, activation of adipocyte lipolysis) were upregulated in adipocytes, an indicator of activation of glucose and fatty acid uptake and oxidation when cellular energy is low (fat utilization). Nuclear translocation of FOXO1 was down regulated hence decreasing the formation of adipocytes (fat cells). Inflammation markers are also down regulated. **F.** Flavonoids decrease the expression of cytokine induced pancreatic β-cell damage; a decrease in oxidative stress damage and subsequent pancreatic β-cell integrity is maintained. Insulin secretion is increased along with the AMPK signaling pathway (modulation of insulin secretion by pancreatic β-cells) and IRS2 (a cytoplasmic signaling molecule that mediates effects of insulin). **G.** Dietary flavonoids decrease the release of glucose from the liver and decrease gluconeogenesis. GK (plays an important role in the regulation of carbohydrate metabolism by acting as a glucose sensor), ACO-1 (controls levels of iron inside the cells) and CPT-1β (plays a role in fatty-acid metabolism) are upregulated. AMPK is upregulated (stimulation of hepatic fatty acid oxidation). G_6Pase (plays a key role in the homeostatic regulation of glucose levels), PEPCK (plays a role in regulating the metabolic pathway of gluconeogenesis) and β-oxidation of fatty-acids are down-regulated. Phosphorylation of Akt (an important signaling molecule in the insulin signaling pathway) and FOXO1 (during stimulation by insulin, FOXO1 is excluded from the nucleus and is subsequently unable to prevent transcription of PPAR-γ thereby inhibiting adipogenesis (formation of fat cells). Phosphorylated FOX1 prevents the nuclear translocation of the transcription factor thus preventing lipogenesis). FOXO1 is a potential target for the genetic control of type 2 diabetes. The inflammation factors NF-κB, COX2, CRP, IL-6 and TNF-α are down regulated. Figure adapted from "Polyphenols and Glycemic Control" *Clifton et al., Nutrients, Vol. 8, Issue 7, 2016.*

Stimulation of skeletal muscle fatty acid oxidation and glucose uptake

During a single acute exercise bout, AMPK allows the contracting muscle cells to adapt to the energy challenges by increasing expression of hexokinase II, translocation of GLUT4, the insulin-regulated glucose transporter found primarily in adipose tissues, skeletal and cardiac muscle, to the plasma membrane, for glucose uptake, and by stimulating glycolysis [40, 42, 55-57]. If bouts of exercise continue through a long-term training regimen, AMPK and other signals will facilitate contracting muscle adaptations by escorting muscle cell activity to a metabolic transition

resulting in a fatty-acid oxidation approach to ATP generation as opposed to a glycolytic approach [36, 38, 44, 46, 55-66]. AMPK accomplishes this transition to the oxidative mode of metabolism by upregulating and activating oxidative enzymes such as hexokinase II [60, 65]. Hexokinase II phosphorylates glucose to glucose-6-phosphate that is a substrate for glycolysis generating ATP for energy utilization within muscle cells. AMP-activated kinase (AMPK) also regulates mitochondrial biogenesis by phosphorylating and activating PGC-1α, the master regulator of mitochondrial biogenesis, upon sensing an energy deficiency in muscle. Studies have demonstrated that reduced ATP/AMP ratios that occur during exercise, results in an energy depletion that results in AMPK activation. AMPK activation leads to activation of PGC-1α and Nuclear respiratory factor 1 (NKF). NKF functions as a transcriptional activator for the expression key metabolic genes regulating cellular mitochondrial DNA transcription and replication resulting in the stimulation mitochondrial biogenesis [37, 55, 56, 58, 65-67]. Mitochondrial biogenesis increases metabolic enzymes for glycolysis, oxidative phosphorylation and ultimately a greater mitochondrial metabolic capacity that results in increased exercise endurance and decreased recovery time [38, 44, 56-58, 60, 65].

Flavonoids as Mediators of AMPK Activity

The following studies, in animal models and humans, investigate the effects of dietary fulvic acids on body weight, metabolic syndrome, diabetes prevention and cardiovascular diseases. A common mechanism of action described in these studies are the decrease of absorption of lipids and proteins by dietary fulvic acids in the intestine, thus reducing calorie intake; and activating AMPK in the liver, skeletal muscle, and adipose tissues. Activated AMPK decreases gluconeogenesis and fatty acid synthesis and increases catabolism, leading to body weight reduction, lowered cholesterol and triglycerides in the blood and metabolic syndrome alleviation [46].

Study: Genistein is a is an isoflavone and a phytoestrogen found in several plants eaten by humans and food-producing animals that exerts a wide spectrum of biological activity. In this study, the impact of genistein on lipogenesis, the formation of fat cells, and lipolysis, the lysis of fat cells, was studied in isolated rat adipocytes [68]. Incubation of the cells with genistein clearly restricted glucose conversion to total lipids in the absence and presence of insulin. When lipogenesis was induced by acetate, genistein exerted a similar effect. Thus, the anti-lipogenetic action of genistein may be an effect not only of alteration in glucose transport (and subsequent conversion to total lipids) and metabolism, but this phytoestrogen can also restrict the fatty acids synthesis and/or their esterification. Incubation of adipocytes with estradiol (an estrogen steroid hormone) at the same concentrations also resulted in restriction of lipogenesis, but the effect was less marked. Genistein increased basal lipolysis in adipocytes. Genistein at the smallest

concentration (0.01 mM) increased epinephrine-stimulated lipolysis but failed to potentiate lipolysis induced by forskolin or dibutyryl-cAMP (lipolytic reagents). It can be concluded that genistein significantly affects both lipogenesis and lipolysis in isolated rat adipocytes. Thus it appears that in addition to regulating lipogenesis, AMPK, an enzyme that plays a role in cellular energy homeostasis, largely to activate glucose and fatty acid uptake and oxidation when cellular energy is low [36-46] also plays an important antilipolytic role by regulating hormone-sensitive-lipase, an enzyme that converts cholesterol esters to free cholesterol, in rat adipocytes [43, 68, 69].

Study: Rosmarinic acid, a rosemary extract polyphenol, increases skeletal muscle cell glucose uptake and activates AMPK. This study demonstrated a direct effect of rosmarinic acid (RA) to significantly increase glucose uptake in muscle cells to levels comparable to that of insulin and metformin (an insulin activator). The glucose uptake was partially, however, significantly inhibited in the presence of compound C, an inhibitor of AMPK, but was not affected by wortmannin, an inhibitor of phosphoinositide 3-kinase (PI3K), an enzyme involved in cellular functions such as cell growth, proliferation, differentiation, motility, survival and intracellular trafficking, indicating a mechanism of glucose uptake in muscle that is partially dependent on AMPK and independent of PI3K [64].

Study: Fenugreek (*Trigonella foenum-graecum* L.) is a well-known annual plant that is distributed worldwide and shown to possess hypoglycemic and hypercholesterolemia characteristics. Here, the effect of polyphenol stilbenes (fulvic acids) on adipogenesis and insulin resistance in 3T3-L1 adipocytes was investigated. Oil Red O staining, a dye used for staining of neutral triglycerides and lipids, and triglyceride assays showed that polyphenol stilbenes reduced lipid accumulation by suppressing the expression of adipocyte-specific proteins involved in the formation of adipocytes. In addition, polyphenol stilbenes improved the uptake of glucose by promoting the phosphorylation of protein kinase B (AKT), an enzyme involved in expressing the insulin receptor pathway by which insulin increases the uptake of glucose into fat and muscle cells and reduces the synthesis of glucose in the liver and hence is involved in maintaining glucose homeostasis. Moreover the stilbene polyphenols upregulated the expression of AMP-activated protein kinase (AMPK), an enzyme that plays a role in cellular energy homeostasis, largely to activate glucose and fatty acid uptake and oxidation when cellular energy is low [36-46]. In the present study, it was found that polyphenol stilbenes also had the ability to scavenge reactive oxygen species (ROS). Rhaponticin, one of the stilbenes from fenugreek, had the strongest effect on reducing lipid accumulation, among the three compounds *in vitro* [67].

Study: Prevalence of obesity has steadily increased over the past three decades both in the United States and worldwide. Recent studies have shown the role of dietary polyphenols in the

prevention of obesity and obesity-related chronic diseases. In this study the impact of commonly consumed polyphenols was evaluated, including green tea catechins and epigallocatechin gallates, resveratrol, and curcumin, on obesity and obesity-related-inflammation. Cellular studies demonstrated that these dietary polyphenols reduce viability of adipocytes and proliferation of preadipocytes, suppress adipocyte differentiation and triglyceride accumulation, stimulate lipolysis and fatty acid β-oxidation, and reduce inflammation. Concomitantly, the polyphenols modulate signaling pathways including the AMP-activated protein kinase and other enzymes that regulate adipogenesis, antioxidant and anti-inflammatory responses. Animal studies strongly suggest that commonly consumed polyphenols have a pronounced effect on obesity as shown by lower body weight, fat mass, and triglycerides through enhancing energy expenditure and fat utilization, and modulating glucose hemostasis [43].

Study: Tea, a popular beverage made from leaves of the plant *Camellia sinensis*, has been shown to reduce body weight, alleviate metabolic syndrome, and prevent diabetes and cardiovascular diseases in animal models and humans. Such beneficial effects have generally been observed in most human studies when the level of tea consumption was 3 to 4 cups (600–900 mg tea catechins) or more per day. Green tea is more effective than black tea. From a review of the literature, the investigators propose that the two major mechanisms are: 1) decreasing absorption of lipids and proteins by tea constituents in the intestine, thus reducing calorie intake; and 2) activating AMPK by tea polyphenols that are bioavailable in the liver, skeletal muscle, and adipose tissues. The activated AMPK would decrease gluconeogenesis and fatty acid synthesis and increase catabolism, leading to body weight reduction and Metabolic Syndrome alleviation [46].

Study: Nonalcoholic fatty liver disease (NAFLD), the most common chronic liver disease, is the leading cause of cirrhosis and has consistently been implicated in related metabolic disorders, such as dyslipidemia, an abnormal accumulation of lipids in the blood, and type 2 diabetes (T2D). However, the pathogenesis of NAFLD remains to be elucidated, and no established therapeutic regiments for treating NAFLD exist. Adenosine monophosphate (AMP)-activated protein kinase (AMPK), the main cellular energy sensor, has been implicated as a key regulator of hepatic lipid and glucose metabolism. Recently, emerging evidence indicates that many plant-derived natural products are capable of ameliorating NAFLD by targeting and activating AMPK. Significant advances have been made with respect to understanding the protective effects of plant-derived natural products against NAFLD. A variety of natural products, including alkaloids (berberine, demethyleneberberine, nicotine, caffeine, etc.), polyphenols (resveratrol, puerarin, curcumin, caffeic acid, etc.) and other compounds (β-caryophyllene, gastrodin, compound K, betulinic acid, etc.), have demonstrated promising results in preclinical studies. Mechanistic studies of these compounds have focused on their activation of AMPK and its downstream effectors involved in

lipid metabolism. The findings support the notion that plant-derived natural products capable of activating the AMPK signaling pathway are potential therapeutic agents for NAFLD [45].

Study: Beneficial effects of green tea (GT) polyphenols against obesity have been reported in the literature. However, until this moment the molecular mechanisms of how green tea can modulate obesity and regulates fat metabolism, particularly in adipose tissue, remain poorly understood. The aim of the study was to evaluate the role of GT extract in the adipose tissue of obese animals and its effect on weight gain, metabolism and function (de novo lipogenesis and lipolysis), and the involvement of AMP-activated protein kinase (AMPK). Male Wistar rats were treated with GT by gavage (12 weeks/5 days/week; 500 mg/kg of body weight), and obesity was induced by cafeteria diet (8 weeks). Obese rats treated with GT showed a significant reduction in indicators of obesity such as hyperlipidemia, fat synthesis, body weight, and fat depots (e.g. deposits) as compared to those treated with standard control diet. AMPK was induced in adipose tissue in rats that were treated with GT restored insulin sensitivity, increased mRNA expression of GLUT4, an insulin-regulated glucose transporter found primarily in adipose, skeletal and muscle tissues, reducing the concentrations of plasma and liver lipid content, and stimulated fatty acid oxidation in the same tissue. Importantly, repression of de novo lipogenesis in the adipose tissue, reduced lipid droplets in the liver, and the development of insulin resistance in diet-induced obese rats were accompanied by AMPK activation. Identified metabolic changes caused by GT intake induced AMPK activation and modulate the expression of genes involved in metabolism, particularly in adipose tissue, thus offering a therapeutic strategy to combat insulin resistance, dyslipidemia, and obesity [40].

Study: Ginger exerts protective effects on obesity and its complications. The objectives of the study were to identify bioactive compounds that inhibit adipogenesis and lipid accumulation *in vitro*, elucidate the anti-obesity effect of gingerenone A (GA) in diet-induced obesity (DIO) mice, and investigate whether GA affects adipose tissue inflammation (ATI). Oil red O staining, a dye used for staining of neutral triglycerides and lipids, showed that GA had the most potent inhibitory effect on adipogenesis and lipid accumulation in 3T3-L1 cells among ginger components tested at a single concentration (40 μM). Consistent with *in vitro* data, GA attenuates DIO by reducing fat mass in mice. This was accompanied by a modulation of fatty acid metabolism *via* activation of AMP-activated protein kinase (AMPK) *in vitro* and *in vivo*. Additionally, GA suppressed ATI by inhibiting macrophage recruitment and downregulating pro-inflammatory cytokines. These results suggest that GA may be used as a potential therapeutic candidate for the treatment of obesity and its complications by suppressing adipose expansion and inflammation [41].

Study: Catechins are abundant in green tea and induce a variety of biologic actions, including anti-cancer, anti-obesity, and anti-diabetes effects, and their clinical application has been widely investigated. To clarify the underlying molecular mechanisms of these actions, we examined the effect of catechins on AMP activated protein kinase (AMPK) in cultured cells and in mice. In Hepa 1-6, L6, and 3T3-L1 cells (liver cells), epigallocatechin gallate (EGCG) induced increases in AMPK activity. Analysis of the molecular specificity of eight naturally occurring fulvic acids revealed that flavanols with a gallocatechin moiety or a galloyl residue act as AMPK activators. In addition, phosphorylation of LKB1, which is a tumor-suppressor protein and a major AMPK-activating protein, was increased by the flavanol treatment. EGCG-induced phosphorylation of AMPK was suppressed by treatment with catalase, an enzyme involved in deactivating reactive oxygen species (ROS), suggesting that ROS are involved in EGCG-induced activation of the AMPK activation pathway. Oral administration of EGCG (200 mg/kg body weight) to laboratory mice induced an increase in AMPK activity in the liver. EGCG administration also increased oxygen consumption and fat oxidation, as determined by indirect calorimetry. These findings suggest that multiple effects of the flavanols, including anti-obesity and anti-cancer effects, are mediated, at least in part, by the activation of AMPK in various tissues, and that these effects vary according to the flavanol structure [70].

Summary

In vitro and *in vivo* studies have shown that dietary polyphenolic compounds improved glucose homeostasis through potential multiple mechanisms of action in the intestine, liver, muscle adipocytes and pancreatic β-cells, as well as through prebiotic effects in the digestive tract. Overall, most epidemiological studies showed that dietary polyphenols were associated with a lower risk of type 2 diabetes. Fulvic acids may be promising candidates for diabetes prevention and management. There have been limited clinical studies of dietary polyphenols in relation to glucose and insulin homeostasis with promising results, however, more clinical studies are required before irrefutable conclusions can be made for fulvic acids and their role in maintaining glucose homeostasis. However, scientific evidence is accumulating supporting the notion that dietary flavonoids act in concert throughout the body, fine tuning gene expression in various organs through metabolic changes and enzymatic activity modulation which provide optimal physiological and metabolic balance, including glucose homeostasis, thus providing critical tools required towards maintaining overall optimal health and well-being.

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