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Review

Berry Anthocyanins in Rodent and Human Obesity and Diabetes: A Review of the Evidence

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Abstract: Obesity in America is a public health crisis that will continue to impact the country at an individual, social, and economic level unless we address the disease with dietary modifications to reduce or prevent its development. Nutritional interventions designed for obesity treatment are constantly evolving. Berries, which are a rich source of polyphenols, have been suggested as a potential bioactive component, as they have been reported to have anti-obesity effects. Therefore, this review will provide an overview of epidemiological studies to introduce the idea of berries for health promotion. Studies conducted in both rodents and humans are summarized. This review includes an overview of the physiological responses associated with berry consumption, including the effects on the composition of the gut microbiota in humans and rodents, which demonstrate how berry consumption may provide a protective effect against obesity and its related comorbidities. However, these findings have yet to be translated into feasible, long-term nutrition intervention in humans. Future research into different berries and their components will identify effective, accessible functional food options that can augment nutritional interventions.

Keywords: nutrition; obesity; type-2 diabetes; berries; anthocyanins

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1. Introduction

The most recent report from the CDC website indicates that adult obesity is at an all-time high: 42.4% in 2018 [1]. In addition, another one-third of American adults are overweight [2]. Relocating to western countries also causes obesity [3]. A palatable and affordable, calorically dense food supply, a workforce paired with automation requiring less physical exertion, and a physical environment prioritizing automobile transportation are components of the American obesogenic environment. Recent trends show Americans are sleeping less and working longer, irregular hours, which is deleterious to weight maintenance [4,5]. With the information age and 24/7 business hours, coupled with a disparity between inflation and pay rate, time-saving, cheap, and delicious ready to eat foods fuel the midnight oil as much as they expand the waistline. The average American now spends so much of their time in front of a screen that software developers have gone to the length of creating games that encourage people to go outside for a walk [6].

Lifestyle modification—more physical activity and fewer calories—remains the number one prescription to effectively treat obesity. Nothing in western medicine offers a feasible treatment for obesity beyond moderate physical activity and a balanced diet. We may one day possess an obesity treatment that is no less commonplace than iodized salt or folic acid fortified grains, but a breakthrough will be needed to reverse worsening trends. Meanwhile, nutritionists sift and winnow through food components that may potentiate the ability of food patterns to minimize or even alleviate adiposity, or dampen the effects of downstream comorbidities, primarily cardiovascular diseases and type-2 diabetes. This line of research is economical considering obesity's cost: an estimated $147 billion was spent on medical care related to obesity in 2008, primarily for pharmaceuticals to treat associated

diseases [7]. In addition, obesity dampens productivity and even perturbs recruitment into the armed forces [8]. It is imperative that nutritionists aid in the discovery for a "cure" for obesity, which is likely to include as much complexity as its etiology. Although obesity is grand in causes and conditions, it is not practical to review all facets in a nutrition review, instead we focus on one nutrient packed foodstuff that has caught the attention of many operating in obesity research. This review focuses on one heterogeneous class of fruits rich in phytonutrients and implicated in the reduction of obesity-related chronic diseases: berries.

The review will survey the recent nutrition research into berry interventions, obesity, and related comorbidities. Epidemiological studies will introduce the concept of berries as a health-promoting food, followed by primary research experiments describing the protective effects of berries and/or their extracts as they relate to obesity. Primary findings span rodent and controlled human trials, including a summary of studies on the burgeoning topic of the fecal microbiome. For a summary of mechanistic experiments and summaries of the chemical and phenolic compositions across various species of berries; the reader is referred to other reviews [9,10].

2. Epidemiological Studies with Fruits, Vegetables, and Berries

Several independent epidemiological studies highlight the protective effect of fruit and vegetable consumption against cardiovascular disease [11]. Further, a meta-analysis of sixteen prospective cohort studies reported a dose-response (4% reduction per serving) where one serving of fruits and vegetables per day had a significant protective effect against all-cause mortality, and added protection was negligible beyond five servings [12].

In addition to essential nutrients and fiber, fruits and vegetables provide a source of other phytonutrients that are an ever-growing area of nutritional sciences research, but whose health effects are less characterized relative to the classical view of nutrition and health. A diverse class of non-nutritive plant compounds are the polyphenols, appropriately named for their characteristic phenol moiety, which are described as health-promoting in epidemiological studies. An example of their bioactive effect is demonstrated with a reanalysis of the PREDIMED study, a prospective trial which demonstrated the cardioprotective (fewer cardiovascular events or deaths related to cardiovascular causes) effect of a Mediterranean style diet. The reanalysis estimated polyphenol intake by comparing food records to an online polyphenol database and discovered greater intake of polyphenols increased survival at six years of follow-up [13]. When parceled out into individual polyphenols, stilbenes, lignans, and isoflavones were the most protective against mortality [13].

Epidemiological studies set precedence for the contribution of berries towards the reduction of chronic disease and mortality. Two separate studies reexamined a prospective cohort study of middle-aged men residing in eastern Finland (the Kuopio Ischemic Heart Disease Risk Factor Study) to assess the effect of fruit, berries, and vegetables on both all-cause mortality and cardiovascular related death, or the development of type-2 diabetes [14,15].

The first study, in-line with those mentioned above, found a significant 35% and 57% reduction in the relative risk of all-cause mortality and CVD-related death, respectively, in the subjects in the highest quintile of fruit, berries, and vegetable intake compared to the lowest at 12 years of follow-up. Interestingly, men who died in the first five years of follow-up consumed 41% fewer fruits, berries, and vegetables compared to men who survived during the study [14]. Associations between individual nutrients and mortality identified vitamin C and folate as nutrients that explained the highest amount of the protective effect provided by fruit, berries, and vegetables. The study did not separate out fruits, berries, and vegetables to determine individual contributions to all-cause and CVD related mortality. Further, consumption of polyphenols and their contribution to the protective effect could not be estimated, likely because phenolic databases did not exist at the time of study.

The second Finnish study assessed risk of type-2 diabetes vs. quartiles of fruits, berries, and vegetables intake [15]. The observations included over 2300 men followed for

an average of nineteen years, in which 432 cases of type- 2 diabetes were noted. There was a non-significant hazard ratio of 0.76 for development of type-2 diabetes for the highest vs. lowest quartile using a model adjusted for type-2 diabetes risk factors [15]. However, when separated out into individual food groups, a significant 35% lower risk of type-2 diabetes in men with the highest berry intake was observed (greater than 60 g of berries per day) [15].

A Norwegian study of 547 elderly men (average age 70 years) assessed fruit and berry intake as it related to cardiovascular risk, measured by intima media thickness (IMT) of the carotid artery, an independent risk factor in atherosclerosis leading to cardiovascular disease [16]. The assessment of dietary intake on IMT was determined from the reanalysis of an earlier intervention study involving diet counseling or fish oil administration for three years [17]. When fruit and berry intake was divided into quartiles, the IMT of the highest quartile (greater than 255 g fruit and berries per day) was 5.5% less than the lowest group of intake (97 g or fewer of fruit and berries per day). Like the earlier study, berries were combined with intake of other fruits when estimating effects on IMT; intake of berry consumption alone was not a significant predictor of IMT in the multivariate regression model, possibly due to low overall intake in this population caused by seasonal variation (9.8 g per day in the highest quartile) [16].

A large, prospective Norwegian study followed over nine-thousand men for forty years—a combination of two separate samplings conducted in the late 1960s—where dietary habits of the men were assessed with a rudimentary food frequency questionnaire [18–20]. Hjartaker et al. reanalyzed the dataset to determine the association of berries, fruits, and vegetables (grouped together or partitioned) and all-cause mortality as well as cancer and CVD related deaths [20]. At the conclusion of the study the average time of follow-up was twenty years where 92% of respondents died. Like previously described studies, higher quartiles of the combined fruit, vegetable, and berry intake yielded a protective effect against mortality—with all-cause mortality, a benefit was noted with as little as the second quartile of intake. In this study, berries again demonstrated a protective effect against all-cause mortality, a marginal effect when assessing cancer-related morality, but no effect with CVD-related deaths [20]. This differs from the observation of CVD protective effect of fruits, berries, and vegetables mentioned in the Finnish study. The authors note that the questionnaire assessed frequency of consumption of various food items, but did not estimate servings, therefore their regression analysis could not adjust for energy intake [20].

While these epidemiological studies do not provide a definitive answer for the utility of berries in addressing the obesogenic state of America, what they do is provide evidence for its potential against chronic diseases related to or exacerbated by obesity. Moreover, the PREDIMED reanalysis demonstrates the utility of polyphenols in increasing survival in a population at-risk for cardiovascular related mortality. It is well documented that berries are a rich source of a variety of phenolic compounds, including flavonoids, stilbenes, lignans, tannins, and phenolic acids [9]. Yet, what may put berries front and center in obesity related research could be their high anthocyanin content, as seminal work from a rodent study described the anti-obesity effect of purple corn color (PCC), a rich source of one of the most frequently studied anthocyanins, cyanidin-3-O-β-glucoside (C3G) [21].

3. Anti-Obesity Effects in Rodent Studies with Berry or Anthocyanin Treatments

3.1. Purple Corn Color: Seminal Work on Anthocyanins and Obesity

The antioxidant and cardioprotective effects of the anthocyanin C3G are well described in vitro and in rodent models [22–28]. With heavy use of purple corn color as a food colorant in the Japanese food supply, an additive rich in C3G, coupled with the burgeoning challenge of obesity, it was tested for its applicability in a mouse model of obesity. C57BL/6J mice were fed a standard or high-fat diet (approximately 60% energy from fat) with or without supplementation of PCC (11 g per kg diet, or approximately 33–55 mg of PCC consumed per day) [21]. The reported density of C3G was 70 g per kg PCC, thus mice consumed approximately 2–4 mg of C3G per day. Four-week-old mice were fed one of four diet treatments for 12 weeks, and body weight and food intake were monitored [21]. Endpoints

included gene expression of lipogenic enzymes in the liver and white adipose tissue as well as adipokines in white adipose tissue, adipose tissue morphology and fat pad weights, and circulating glucose and insulin.

Findings were mostly positive for PCC administration vs. the diet-induced obese phenotype in genetically normal mice. The high-fat diet supplemented with PCC normalized the growth curve of mice despite similar food intake as the high-fat control treatment [21]. Histology of white adipose tissue showed a reduction in adipocyte size of the high-fat-PCC group, reduced fat pat weights, and mRNA expression of leptin (where circulating levels were also normalized) and tumor necrosis factor-alpha (TNF-α). Gene expression of lipogenic enzymes in the liver and white adipose tissue (fatty acid synthase and sterol regulatory element binding protein) were reduced with a high fat diet, but were lowered further by supplementation with PCC. The high-fat control diet increased gene expression of two other lipogenic enzymes (acyl-CoA synthase 1 and glycerol-3-phosphate acyltransferase) compared to the standard diet groups—characteristic of diet-induced obesity—an effect abrogated by the PCC treatment [21]. Taken together this study suggests multi-tissue, anti-lipogenic effects of PCC. The studied enzymes were also reduced in the normal weight animals fed PCC, suggesting that decreased enzyme expression of lipogenic enzymes is independent of hyperinsulinemia caused by a high-fat diet. The authors suggest modulation of the four enzymes via activation of AMP-activated protein kinase (AMPK), a molecular switch that signals consumption of stored energy substrates (glycolysis and β-oxidation) and inhibition of anabolic processes (glycogenesis and lipogenesis). The PCC treatment also prevented reduced insulin sensitivity and glucose intolerance [21]. In follow-up experiments the authors proposed mechanism of action [29] includes activation of AMPK in several tissues from a rodent study in diabetic mice treated with an anthocyanin-rich bilberry extract Figure 1 [30].

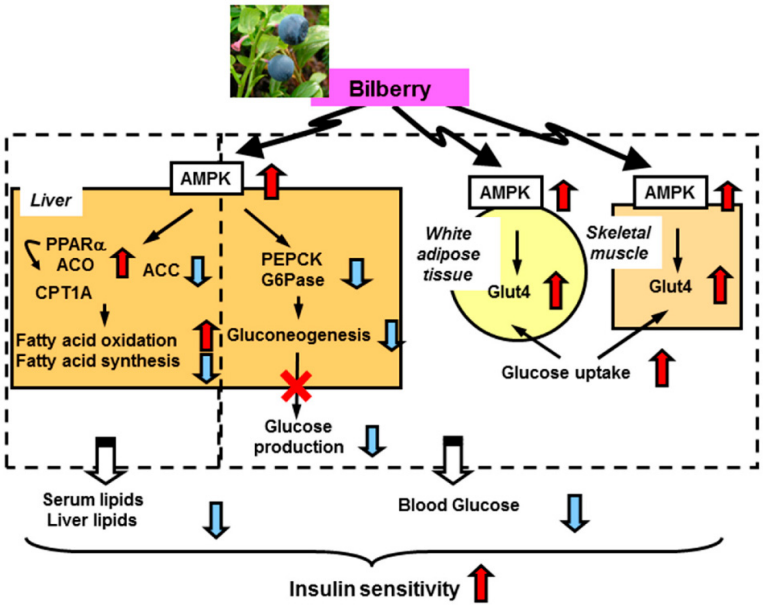

Figure 1. AMPK activation in several tissues by berry anthocyanins promotes anti-adipogenic and insulin sensitizing pathways. Figure reproduced from [29] under the terms of the Creative Commons license (https://creativecommons.org/licenses/by/4.0/) accessed on 1 February 2022. AMPK, AMP-activated protein kinase; ACO, acyl-CoA oxidase; PPAR, peroxisome proliferator-activated receptor; Glut4, glucose transporter type 4; CPT1A, carnitine palmitoyl transferase 1-alpha; G6Pase, Glucose 6-phosphatase; PEPCK, phosphoenol pyruvate carboxykinase.

3.2. Rodent Studies with Berry Powders or Extracts

These initial experiments provide the proof-of-concept for the potential of anthocyanin rich foods to prevent obesity. The findings stimulated a wave of experimental research in

a variety of models to investigate the bioactivity of purified anthocyanins, food extracts or whole food sources. Below, we focus on studies that used berries or berry extracts to further elucidate mechanisms that invoke bioactivity in the treatment or prevention of diet-induced obesity.

Prior et al. conducted a series of experiments using whole berries, juices, or berry anthocyanin extracts in drinking water to determine effects on high-fat diet induced obesity [31–33]. A preliminary experiment tested freeze-dried blueberry or strawberry powder incorporated into a standard or high-fat diet or delivered purified anthocyanins in drinking water, with high-fat diets ranging from 45% to 60% [33]. Animals were weanling (21d) C57BL/6J mice. Outcome variables included body weight and composition with MRI and tissue weights. The findings from the first report are in relative discord with the earlier findings by Tsuda et al.; the freeze-dried strawberry powder treatment offered no protection to the high-fat fed mice, and the blueberry treatment exacerbated the obese state as evidenced by a greater growth curve and higher body fat percentage [33]. By comparison, the results of the second experiment reproduced the PCC study, in that anthocyanin extracts placed in the drinking water protected the mice from weight gain when fed a high-fat (60% energy from fat) diet, but not to the extent that their weight was normalized to the low-fat control group [33]. As discussed in a later communication [34] the formulation of the diets in the first experiment challenge the findings of greater weight gain in the freeze-dried powder blueberry arm, as the mice in this group consumed 11% more energy relative to the high-fat control [33]. Further, in formulating the freeze-dried diets, the authors displaced corn starch for incorporation of the respective fruit powder, likely higher in simple sugars (not reported). The discrepancies in diet design may partially explain the significant increase in body weight and body fat with the blueberry powder treatment.

Prior et al. performed two subsequent experiments manipulating the mode and source of berry anthocyanins [31,32]. In one experiment they increased the concentration of purified anthocyanins in drinking water to include their original dose, 0.2 mg/mL, as well as a higher dose, 1.0 mg/mL, which added no observable benefit against weight gain that was again observed with the low-dose [32]. However, a novel finding of purified anthocyanins in drinking water was the reduction in body fat percent even when mice were fed the standard fat diet. They also included arms that were supplemented with blueberry juice in place of drinking water, which provided a nonsignificant reduction in cumulative weight gain and body fat as measured by fat pad weight compared to the control animals fed the high-fat (45%) diet [32].

A second and final follow-up experiment used a similar experimental design with high-fat (60%) diets supplemented with anthocyanins in the drinking water, juice, or freeze-dried berry powder, except that black raspberries were used as the source of anthocyanins instead of blueberries or strawberries, to determine if source of anthocyanins resulted in altered effects on body weight and fat percentage in their model [31]. Ultimately the results were similar to the previous experiments, where purified anthocyanins in the drinking water yielded more of a benefit, and the fruit juice and freeze-dried powders exacerbated obesity [31]. Taken together, the series of experiments with preparations of anthocyanins from three berry sources highlights two separate points: anthocyanins can protect mice from diet-induced obesity; but mode of delivery needs to be taken into consideration, as freeze-dried powders, and sometimes juice forms, can further complicate diet-induced obesity, perhaps due to concentration of sugar content or elimination of the fiber component. The authors note plausible differences in the magnitude of anti-obesity effects of anthocyanins derived from various berry sources as their anthocyanin profiles are drastically different (Table 2 of [33], and Table 1 of [32]), and structural variation along with level of glycosylation likely determine biological activity [9].

A study of freeze-dried blueberry powder in a rodent obesity model explored potential biomolecular mechanisms of action [34]. They used diabetic Zucker fatty rat, which due to a leptin receptor mutation, presents spontaneous obesity and models type-2 diabetes, insulin resistance, dyslipidemia, and chronic inflammation. Seven-week-old fatty rats

were fed a 45% high-fat diet supplemented with 2% blueberry powder or sugar control for 90 days [34].

After 90 days of treatment, body weights between control and blueberry fed groups did not differ, however visceral fat depots (retroperitoneal and epididymal) weighed significantly less in the blueberry fed group [34]. The study connects this finding to the molecular level by demonstrating greater peroxisome proliferator-activated receptor (PPAR)-α and PPAR-γ activities and gene expression in both adipose and skeletal muscle tissues. PGC1α, considered the master regulator of mitochondrial biogenesis, was also upregulated in these tissues with blueberry feeding, however changes in energy expenditure were not tested [34]. A key issue raised, thiazolidinediones are troublesome for their singular PPAR-γ activation, which, while remediating insulin resistance and dyslipidemia, are problematic in that they promote adipogenesis. Novel in this study is pan-PPAR agonism: the removal of lipids from the circulation and disposal (oxidation) in peripheral tissues [34]. The diabetic state of the blueberry fed group also improved, resulting in a 4% reduction in glucose area under the curve, 20% reduction in fasting insulin, improved Homeostatic Model Assessment for Insulin Resistance (HOMA-IR) score, and 5% lower fasting triglycerides—supported molecularly by an increase in glucose transporter-4 (GLUT4) and insulin receptor substrate-1 (IRS1) gene expression in both tissues [34]. Although outside of the scope of the "berries" theme of the current review, the research group performed similar experiments using anthocyanin rich tart cherry and discovered similar benefits in metabolic stress attributable to dual PPAR activation in several models [35,36].

A Swedish study performed a comprehensive assessment of the anti-obesity and anti-diabetic potential of eight different berry varieties (lingonberry, blackcurrant, bilberry, raspberry, acai, crowberry, prune, or blackberry) in a C57BL/6J rodent model of diet-induced obesity [37]. Six-week-old mice were fed either a high fat (45%) control diet or high fat diet supplemented with 20% freeze dried berries for thirteen weeks ad libitum. Dependent variables included body, organ, and fat pad weight, body composition, blood chemistry, and lipid analyses of liver and feces. Main study findings included a battery of improvements for three particular berries including lingonberry, blackcurrant, and bilberry [37]. Improved outcomes included reduced body weight and body fat percentage, fasting glucose, insulin and subsequent HOMA-IR score, liver weight and triglycerides (TGs), increased cecum weight and fecal TGs, and circulating plasminogen activator inhibitor-1 (PAI-1), a measure of systemic inflammation [37]. The most dramatic improvements were noted in the lingonberry group, whereas the acai berry exacerbated the increase in body weight and metabolic aberrations stemming from a high fat diet [37]. Quercetin in lingonberries may potentiate the effects seen with the berries containing predominantly anthocyanins; but only a qualitative analysis of flavonoid profiles for each berry was available [37]. The marked improvement in obesity phenotype attributable to berry treatment in this study is in stark contrast to the transient benefits observed in the above experiments [31–33]. This is despite a nearly identical design: similar modes of diet incorporation and ad lib feeding, study duration, and mouse model. A convenient difference is berry source for anthocyanins, but better control of diet formulation in this study may explain the disparate responses (see Table 2 of [37] vs. Table 1 of [33]). Both studies utilized the same diet manufacturer, but carbohydrate profiles are standardized across the different diets in the current report [37].

Another group performed a rodent feeding experiment with black elderberry extract (BEE) and a high-fat (60% fat) diet to test the berries effect on obesity [38]. To determine dose-response, BEE was fed to ten-week-old C57BL/6J mice at 0.25 and 1.25 percent of diet, spray-dried onto maltodextrin to maintain the macronutrient profile, which provided an estimated 20–200 mg of anthocyanins (as C3G-equivalents) per kg of bodyweight when fed for sixteen weeks. The authors considered these doses achievable by a normal human diet that incorporated 60 g of black elderberries daily [38]. There was no change in body weight or food intake, but a significant 13% reduction in liver weight by both BEE treatments [38]. Serum TGs were normalized by either BEE dose, while significant increases in cholesterol,

non-esterified fatty acids (NEFAs), and alanine aminotransferase attributable to high-fat feeding were unaltered by BEE treatments. However, serum monocyte chemoattractant protein-1, tumor necrosis factor alpha (TNFα), and fasting insulin concentrations, as well as HOMA-IR scores were significantly lowered by both doses of BEE compared to the high-fat control diet [38]. Liver and adipose tissue histology indicated no benefit of BEE treatments against macrophage infiltration or fibrosis of both tissues by the high-fat diet treatment. Hepatic cholesterol content and fatty acid synthase gene expression were decreased by the higher dose and both doses, respectively. In adipose tissue, PPARγ and lipoprotein lipase gene expression were significantly increased with the higher dose BEE treatment, which may help explain the reduction in circulating TGs. However, the higher dose treatment also had a greater staining for fibrosis and higher expression of transforming growth factor β compared to the other two high-fat diet treatments [38]. Thus, the BEE findings are mixed; low dose anthocyanin treatment—achievable levels in a normal human diet—showed protection against a high-fat diet via increased insulin sensitivity, moderate improvement in hepatosteatosis, and increased clearance of circulating TGs with improved function of adipose tissue, however the improvements may be at the cost of increased inflammation in the adipose tissue. A caveat of the study design is the exceptionally high fat diet (60%) used to induce obesity. Their berry doses were set at a level that were calculated to be achievable by humans, which is especially useful for translational purposes, however, it would have then also been more informative to use a diet with a fat content more in line with human consumption, which is 34% on average [39].

These studies using a variety of obese rodent models repeatedly describe a benefit in berries or purified C3G in preventing excess and ectopic body fat deposition. C3G is likely the favorite isolated anthocyanin for study due to its involvement in the seminal work by Tsuda et al. with PCC. However, as noted by the Heyman et al. study of several berries, various berry varieties contain a plethora of anthocyanins as well as other flavonoids. Specifically, the greatest berry effect in their hands was the lingonberry, which also provides several isoforms of quercetin. An example of emerging knowledge in less popular berries comes from a comprehensive study by Rojo et al. where maqui berry, which grows wild and abundant in Chile, had insulin-like properties [40]. Findings included positive effects in a type-2 diabetic (high-fat fed) mouse model as well as in both hepatic and muscle tissue which were attributed to the berries' most prominent (and less characterized) anthocyanin, delphinidin 3-sambubioside-5-glucoside [40]. Similarly, a separate study found insulin-sensitizing and anti-inflammatory effects of four wild berry varieties traditionally consumed by Native American populations [41]. While work should continue to describe C3G's capabilities against diet-induced obesity, holistic (whole berry) approaches of flavonoid delivery may uncover synergistic actions between flavonoids in alleviating the obese phenotype and provides the greatest translatability compared to forms that are not typically consumed by humans. The rodent studies discussed Section 3. are summarized in Table 1.

Table 1. Summary of findings related to anti-obesity effects of berry and anthocyanin treatments in rodents.

Intervention	Duration	Method	Species	Variables	Results	References
Purple corn color (PCC)	12 weeks	• Control • HFD (60% kcal from fat) • Control + PCC (11 g/kg diet) • HFD + PCC (11 g/kg diet)	4-wk old diet-induced obese C57BL/6J mice	• Lipogenic enzymes • Adipokines • Adipose tissue morphology • Fat pad weight • Plasma insulin • Plasma glucose • Liver enzymes	In HFD + PCC: • Reduction in adipocyte size, fat pad weight, leptin mRNA, TNF-α, • Reduction in fatty acid synthase and SRBP in HFD, with a more significant reduction in HFD + PCC • Prevented increase of acyl-CoA synthase 1 and glycerol-3-phosphate acyltransferase and reductions of insulin sensitivity and glucose tolerance	[21]
Blueberry juice or purified blueberry anthocyanins in drinking water	72 days	• LFD (10% kcal from fat) • HFD (45% kcal from fat) • LFD + blueberry juice • HFD + blueberry juice • LFD + anthocyanins • (0.2 or 1.0 g/mL) • HFD + anthocyanins • (0.2 or 1.0 g/mL)	25-d old C57BL/6J mice	• Total body weight (g) • Body fat (%) • Serum leptin • Fasting serum glucose • β-cell function	• NSD in body fat % in blueberry juice or 0.2 g/mL ACN compared to LFD control • Decreased retroperitoneal and epididymal adipose tissue weights in ACN groups • NSD between fasting blood glucose and β-cell function of HFD + 0.2 g/mL ACN and LFD control • Decreased serum leptin in HFD + 0.2 g/mL CAN compared to HFD control	[32]
Freeze-dried blueberry powder (BBP)	90 days	• Sugar control • HFD (45% kcal from fat) • + 2% BBP	7-w old diabetic Zucker fatty rat	• Weight change • Body composition and • Glucose tolerance modulation of (PPAR) α and γ activitiy and gene expression adipose and skeletal muscle tissue	• NSD in body weight between BBP and control • Significant decrease in weight of visceral fat depot in BBP group compared to control • Greater PPAR-α and PPAR-γ expression and activity in adipose and skeletal muscle of BBP group • 4% reduction in glucose area under the curve, 20% reduction in fasting insulin, improved HOMA-IR score, 5% lower fasting triglycerides in BBP group	[34]

Table 1. *Cont.*

Intervention	Duration	Method	Species	Variables	Results	References
Freeze-dried berry powders: • Lingonberry • Blackcurrant • Bilberry • Raspberry • Acai • Crowberry • Prune • Blackberry	13 weeks	• HDF control (45% kcal from fat) • HFD + 20% freeze-dried berries	6-wk old diet-induced obese C57BL/6J mice	• Body, organ, and fat pad weight, • Body composition • Blood chemistry • Lipid analyses of liver and feces	For lingonberry, blackcurrant, and bilberry: • Reduced body weight and body fat percentage, fasting glucose, insulin and HOMA-IR score, liver weight and TGs, increased cecum weight and fecal TGs, and circulating PAI-1 compared to HFD control	[37]
Black elderberry extract (BEE)	16 weeks	• HFD control (60% kcal from fat) • HFD + BEE (0.25% of diet) • HFD + BEE (1.25% of diet)	6-wk old C57BL/6J mice	• Body and organ weight • Blood chemistry • Lipogenic enzymes • Hepatic and adipose tissue morphology • Genetic markers of inflammation	• NSD in body weight or food intake • 13% reduction in liver weight in both BEE treatments • Serum TGs were normalized by either BEE dose • Significant decrease in serum monocyte chemoattractant protein-1, TNFα, fasting insulin concentrations, and HOMA-IR scores by both doses of BEE compared to the high-fat control diet • Significant increase in PPAR-γ and lipoprotein lipase gene expression in 1.25% BBE group • Greater staining for fibrosis and higher expression of transforming growth factor β in 1.25% BBE group	[38]

LFD, low-fat diet; HFD, high-fat diet; NSD, no significant difference; PPAR, peroxisome proliferator activated receptor; ACN, anthocyanin; TGs, triglycerides.

4. Berries and the Gut Microbiota

When considering mechanisms by which berry polyphenols affect human health, another area of research interest is the interaction between polyphenols and the gut microbiota. There is a well-established relationship between the gut microbiota and health, where alterations in the composition can have either positive or negative effects on host health [42]. Recently, prebiotics, compounds that selectively stimulate the growth and development of bacterial species in the colon, have received considerable attention, as they confer a health benefit [43]. Polyphenols are relevant because they are recognized as a class of prebiotics [44]. The subcategories of polyphenols are vast, and individual compounds under this broad classification cause varying effects. Developing a better understanding of how specific polyphenolic compounds impact the composition of the gut microbiota will improve human health. As previously mentioned, anthocyanins have been highlighted for their role in controlling and preventing metabolic diseases like obesity and type-2 diabetes. However, less is known about their interaction with the gut microbiota. Therefore, the aim of this section is to examine the current literature surrounding berry anthocyanins, notable changes in health status, and the effects of anthocyanins on the gut microbiota.

Although berry anthocyanins demonstrate their ability to improve health by ameliorating symptoms resulting from obesity and other metabolic disorders, the exact mechanisms are not entirely understood. However, one possible mechanism is through compositional changes in the gut microbiota from anthocyanin metabolites [45]. Indigenous bacteria in the colon, like *Bifidobacterium* spp. and *Lactobacillus* spp., metabolize anthocyanins into small metabolites that may promote the colonization of beneficial bacteria like *Lactobacillus* spp., *Bifidobacterium* spp., and *Akkermansia muciniphila* spp. [45–47]. In both humans and rodents, various berries have been studied to determine whether there is an effect on markers of metabolic health and/or the composition of the microbiota. Several interventions have shown promise for promoting positive changes in the composition of individual gut microbes. Regarding blueberries specifically, a crossover study involving healthy male volunteers with at least one risk factor for CVD found that supplementation with 375 mg of anthocyanins from a freeze-dried wild blueberry powder increased *bifidobacteria* in participant feces when compared to control [48]. Positive findings were also observed in an in vitro colonic fermentation system with isolated blueberry-polyphenol fractions [49]. The results found that when the colonic bacteria were fermented with an anthocyanin/flavonol glycoside fraction (200 mg/L), there was a significant reduction in *Escherichia/Shigella* (*Enterobacteriaceae*), which can cause disease in humans [50], when compared to the control [49]. Therefore, it seems that blueberry anthocyanins may promote favorable changes in the composition of the human gut microbiota. The key findings from these human studies are summarized in Table 2.

Table 2. Summary of results from human studies measuring the effect of berries on the gut microbiome.

Berry	Study Design	Methods	Variables	Results	References
Freeze-dried wild blueberry powder	• 18-week crossover study	• Healthy male volunteers with at least one risk factor for CVD supplemented with 375 mg of ACN or placebo control for 6 weeks. 6 week washout in between	• Bifidobacterial cell concentration in fecal samples	• Significant increase of B. longum subsp. Infantis when supplementing with blueberry powder	[48]
Isolated blueberry polyphenol fractions	• In vitro colonic fermentation system	• Fermentation of human colonic bacteria with anthocyanins/flavonol glycosides, proanthocyanidins, a sugar/acid fraction, and total polyphenols	• Fecal microbiota composition	• Significant reduction in Escherichia/Shigella (Enterobacteriaceae)	[49]

CVD, cardiovascular disease.

In rodents, blueberry anthocyanins cause changes in the gut microbiota that may provide health benefits to the host. A study found that diet-induced obese mice on a high-fat/high-sugar (HFHS) diet had increased insulin sensitivity after an oral glucose

test following a 12-week intervention with blueberry-derived anthocyanin-rich (ACT) and proanthocyanidin-rich (PCT) extracts, when compared to the HFHS control [51]. Using the microbiota from a mouse of each diet group, a fecal microbiota transplant (FMT) was then conducted in germ-free mice to determine whether the changes in insulin sensitivity were a specific result of changes in the microbiota. After mice were fed either chow or a HFHS diet for eight weeks post-FMT, another oral glucose test was conducted. The results found that mice that received an FMT from the mice fed the ACT and PCT extracts had an increase in insulin sensitivity when compared to the control. Thus, this demonstrates the gut microbiota's independent effect on metabolic health. Additionally, another notable finding from this study is that there was an increase in the proportion of *Muribaculum intestinale* in the stool of mice fed the PAC-HFHS diet. The *Muribaculaceae* family is underreported in the literature, however, it is associated with improvements in intestinal barrier function [51]. Furthermore, another study found that mice may be protected from diet-induced obesity if they possess a higher proportion of *Muribaculaceae* at baseline [52]. It was confirmed that the mice in the PCT group did not have a higher proportion of *Muribaculaceae* at baseline, however, it can ultimately be suggested that a PCT diet induces growth of the *Muribaculaceae* family and confers a protective effect against diet-induced obesity to the host [51]. Overall, it seems that blueberry anthocyanins can have positive effects on the composition of the gut microbiota that may benefit a rodent's metabolic health.

Anthocyanin effects have been observed in rodents when using other types of berries as well. Specifically, both red and black raspberries exhibit modulatory effects on the gut microbiota. When mice on a high-fat diet were supplemented with red raspberry polyphenolic extracts from either the whole fruit, seed, or pulp, there was a significant increase in the presence of *Roseburia* in the mice that received the pulp and seed extracts [53]. This is noteworthy because this species produces short-chain fatty acids and a loss of *Roseburia* has been associated with type-2 diabetes [54]. *Bifidobacterium* also increased, especially in the seed group, therefore another positive change in composition from red raspberry polyphenolic extracts. Like red raspberries, black raspberries (*Rubus occidentalis*) are studied for their effects on the gut microbiota. Mice were fed a high-fat diet (HFD) to induce obesity and were then administered *Rubus occidentalis* (RO) solutions of either 125 or 250 mg/kg concentration for 16 weeks [55]. Administration of RO in both concentrations ameliorated hyperglycemia induced by the HFD and caused compositional changes in the gut microbiota when compared to the control. Specifically, *Bacteroides*, *Butyricimonas*, and *Ruminococcus* were significantly increased in the 250 mg/kg group. An important characteristic of these bacteria is that they produce short-chain fatty acids (SCFA) [56,57]. The production of SCFAs, such as butyrate, propionate, and acetate, are associated with improved insulin sensitivity, protective effects against diet-induced obesity, reductions in lipid accumulation in adipose tissue, and improved glucose tolerance [58–60]. Therefore, the results of this study suggest that the bacterial production of SCFAs after RO supplementation may be an important component in the regulation of blood glucose [55]. Strawberries are another source of anthocyanins that alter the composition of the gut microbiome. When diabetic mice were given strawberry anthocyanins, in the form of a freeze-dried powder, there was an increase in the abundance of *Bifidobacterium* compared to the control [61]. Because a decrease in the abundance of *Bifidobacterium* has been associated with the development of type-2 diabetes, this is a potential mechanism by which berry anthocyanins may prevent the development of metabolic disease [62].

Finally, açai and black currant are other anthocyanin-rich berries that are studied for their anti-obesity effects and interactions with the gut microbiota. Interestingly, the results of two separate studies found that when mice were fed either a HFD or a HFD with one of the berries, the treatment groups had a significant increase in the abundance of *Akkermansia muciniphila* [63,64]. Furthermore, there were similar metabolic effects in that HFD-induced obesity, hyperlipemia, and hepatic steatosis were alleviated in both treatment groups. In mice that were given açai berries, there was also a reduction in fasting blood glucose levels compared to the HFD control [63]. A central finding from these studies is

that both treatments increased the abundance of *Akkermansia muciniphila*, which has been indirectly associated with obesity, diabetes, and other metabolic disorders [65]. Thus, the promotion of *Akkermansia muciniphila* from açai and black currant supplementation may be one possible mechanism for the observed protection against obesity and restored liver function. The reviewed studies of specific berries and their observed effects on the fecal microbiome are summarized in Figure 2 and Table 3.

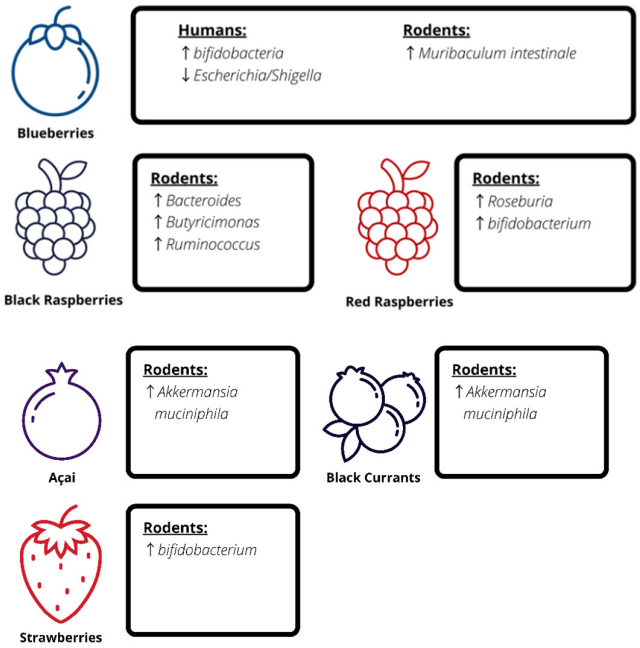

Figure 2. Summary of individual berries and the beneficial changes observed in the composition of the gut microbiota in humans and rodents. Up arrows indicate an increase in bacterial abundance, while down arrows indicate a decrease [48,49,51,53,55,61,63,64].

Within humans and rodents, the gut microbiota is a complex system that is not entirely understood. However, the composition of the gut microbiota may have positive and preventative effects on metabolic health and the risk of developing metabolic disease. While additional research is needed to further elucidate specific mechanisms of action for individual bacterial species, current findings show associations between the abundance of bacterial species and diseases like type-2 diabetes and obesity. Moreover, there is an association between the consumption of polyphenolic compounds, such as anthocyanins, and the presence of specific bacteria in the gut. As more studies are conducted, the information they provide will not only help to bridge the gap between the gut microbiota and human health but also increase the understanding of how nutrition plays a role in the treatment and prevention of metabolic disease.

Table 3. Summary of results from rodent studies measuring the effect of berries on the gut microbiome.

Intervention	Duration	Method	Species	Variables	Results	References
• Whole blueberry powder (BB) • Proanthocyanidin (PAC)-rich extract • Anthocyanidin (ANT)-rich extract	12-week diet intervention + 8-week FMT follow up	• Chow (control) • HF-HS diet • HF-HS + BB (160 mg/d) • HF-HS + PAC (1 mg/day) • HF-HS + ANT (17 mg/day) FMT group: • Chow • HFHS	8-wk old C57BL/6J male mice	• Body composition • Insulin sensitivity • Oral-glucose tolerance test • 16S rRNA gene-based gut microbial analysis	• FMT mice from ACT and PCT diets had higher insulin sensitivity than control • Significant increase in the proportion of Muribaculum intestinale in the stool of mice fed the PAC-HFHS diet	[51]
Red raspberry (RR) polyphenolic extract from: • Whole fruit • Seed • Pulp	16 weeks	• LFD (10% fat) • HFD (45% fat) • HFD + 0.4% (by weight) RR whole fruit extract • HFD + 0.1% RR seed extract • HFD + 0.3% by weight RR pulp extract	C57BL/6 male mice	• 16S rRNA gene sequencing	• Significant increase in the presence of Roseburia in the pulp and seed extract groups • Bifidobacterium also increased significantly in seed groups	[53]
Black raspberry extract (RO)	16 weeks	• HFD (45% kcal from fat) • HFD + 125 mg RO/kg/day • HFD + 250 mg RO/kg/day	C57BL/6N male diet-induced obese and hyperglycemic mice	• Body weight • Serum glucose level • IPGTT • Food intake • 16S rRNA gene sequencing	• NSD on body weight • Significant difference in IPGTT score and serum glucose for both 125 mg and 250 mg RO groups • Bacteroides, Butyricimonas, and Ruminococcus were significantly increased in the 250 mg/kg group	[55]
Freeze-dried strawberry	10 weeks	• Standard diet • Standard diet + 2.35% freeze-dried strawberry supplemented	6-week-old diabetic and non-diabetic C57BL/6J mice	• 16S rRNA gene sequencing	• Non-significant decrease in the abundance of Actinobacteria, Akkermansia • Significant increase in the abundance of Bifidobacterium and Bacteroides	[61]

Table 3. *Cont.*

Intervention	Duration	Method	Species	Variables	Results	References
Acai anthocyanin extract (AEA)	14 weeks	• LFD • HFD • HFD + 150 mg/kg AEA	4-week-old Male C57BL/6J mice	• 16S rRNA gene sequencing • Blood chemistry • Liver enzymes	• Decreased in serum TG, TC, NEFA, and LDL-C levels compared with the HFD + AEA group • Significantly reduced serum ALT and AST levels in HFD + AEA • Significantly lower fasting serum glucose and insulin levels in HFD + AEA • Decrease in the proportions of Firmicutes and Proteobacteria and increase in the abundance of Verrucomicrobia and Akkermansia muciniphila in the HFD + AEA group	[63]
Black currant (BC)	12 weeks	• LFD • HFD • HFD + 150 mg/kg BC	4-week-old Male SPF C57BL/6J	• 16S rRNA gene sequencing • Blood chemistry • Body weight • Histological analysis	• Significant reduction in body weight, serum TG, TC, NEFA, LDL-C levels, and adipocyte size of white adipose in BC group compared with the HFD group. • Significant increase in the composition of Akkermansia muciniphila and Mucispirillum in BC group	[64]

HFHS, high-fat, high-sucrose; FMT, fecal microbiota transplant; IPGTT, intraperitoneal glucose tolerance test; TG, triglyceride; TC, total cholesterol; NEFA, non-esterified fatty acids; LDL, low-density lipoprotein.

5. Berries and Anthocyanins vs. Obesity and Metabolic Aberrations: Human Studies

5.1. Meta-Analyses

A meta-analysis was conducted to determine if cardio-protective effects of berries exists based on information from controlled human trials [66]. Altogether, the study included twenty-two randomized controlled trails of over 1200 subjects, spanning eight different berries as phytochemical sources: elderberry, cranberry, bilberry, black currant, lingonberry, blueberry, whortleberry, and black raspberry. Subjects ranged from healthy to type-2 diabetic/metabolic syndrome, or presented with cardiovascular risk factors (excess weight, dyslipidemia, hypertension, and/or impaired glucose tolerance). The majority of studies used a parallel-arm design [66].

Key findings include significant reduction in low-density lipoprotein (LDL) cholesterol in groups consuming berries vs. control treatments (weighted mean difference of 0.21 mmol/L, or 8 mg/dL lower with berries) [66]. Similarly, berry treatment increased high-density lipoprotein (HDL) (WMD of 0.06 mmol/L, or 2.3 mg/dL higher with berries); however, this effect was marginal and the authors report that the required information size was not reached to definitively determine the effect of berry feeding. A secondary analysis reported significant improvements in systolic blood pressure, fasting blood glucose, hemoglobin A_1C (HbA_1C), body mass index (BMI), and TNFα. A subgroup analyses found greater effects (more benefit of berries) elicited by bilberry and whortleberry, with greater lengths of treatment (>8 weeks), and in subjects with greater cardiovascular risk [66]. Although risk of study bias was low, there was significant heterogeneity with the majority of dependent variables described, attributable to variability in research design, i.e., study populations, design and duration, and type of berry treatment as well as modality of delivery. Taken together, this meta-analysis reaffirms the benefits of berry consumption on cardiovascular health first described in epidemiological studies.

A second meta-analysis collected data from five prospective cohort studies to determine the effect of either berry or anthocyanin intake on the risk of developing type-2 diabetes [67]. Three of the prospective cohort studies were from the United States (the Nurse's Health Study I and II, and the Healthcare Practitioners Follow-Up study), and two were from Finland (the Finnish Mobile Clinic Health Examination Survey, and the Kuopio Ischemic Heart Disease Risk Factor Study) [15,68–70]. The meta-analysis included over 190,000 participants in which approximately 13,000 cases of type-2 diabetes were identified at follow-up. The American studies used food frequency questionnaires to determine berry intake while the Finnish studies used either in-person interviews or 4-day food records. The meta-analysis calculated the anthocyanin intake in the prospective studies using the USDA's flavonoid content database [71].

Relative risks were calculated for either berry or anthocyanin intakes to determine effect on type-2 diabetes development. Findings included a significant 15% reduction in risk when comparing highest vs. lowest intakes of berries, and a significant 18% reduction when comparing highest vs. lowest intake of anthocyanins [67]. Heterogeneity in the berry meta-analysis warranted a sub-analysis that described a protective effect of berries for females, but the same benefit was not clear in males. Further, older subjects (>50 years) and European cohorts demonstrated a greater benefit than younger or US counterparts, respectively, although all groups were reported to have a significant benefit from berry intake. Finally, the study demonstrated a significant dose response of either berry or anthocyanin intake in protection against type-2 diabetes: for every 17 g of berries or 7.5 mg of anthocyanins, there was an incremental 5% lower risk of developing type-2 diabetes (Figure 3 of [67]).

The compelling findings of the above meta-analyses are supported by other epidemiological studies that focus specifically on anthocyanin intake. Dietary anthocyanins are implicated in promoting weight maintenance, reducing heart attack, all-cause and CVD-related mortality, and reducing the risk of type-2 diabetes [70,72–74]. Moreover, cohort studies in type-2 diabetics report a superior cardiovascular risk factor profile in participants with a high average intake of polyphenols [75].

5.2. Berries and Human Obesity

The meta-analyses above highlight positive changes in biomarkers commonly affected in diet-induced obesity by berries or anthocyanins, from both experimental and observational research. The missing lynchpin connecting the rodent and tissue data described earlier to research on berry consumption in human data are clear and reproducible human studies demonstrating protective effects of berries against weight gain or a positive impact on weight loss. If the changes described in meta-analyses and epidemiological reports are an indication, there is likely influence by berries on how the body is storing and using fat.

A variety of clinical research on several kinds of high anthocyanin berries and their effects on clinical biomarkers influenced by obesity exist. Here we will review pertinent randomized, controlled, dietary research trails investigating anti-obesity (clinical complications related to obesity) effects of different berry modalities. Publications will be separated out based on how the berry anthocyanins were administered: anthocyanin rich extracts, freeze-dried powders, or as whole berries.

5.2.1. Human Studies with Anthocyanin Rich Extracts

A randomized, double-blind, placebo-controlled trial studied the effects of an anthocyanin extract in 120 community-dwelling, overweight subjects with dyslipidemia [76]. Subjects were fed 320 mg of a bilberry/black currant extract (majority of anthocyanins were glycosylated variants of cyanidin or delphinidin) or placebo control daily for 12 weeks. Findings include a significant 13.7% increase in HDL cholesterol, a 13.6% decrease in LDL cholesterol, as well as significant reductions in both concentration and activity of cholesterol ester transfer protein (CETP) in the group receiving anthocyanin capsules, where changes in both types of cholesterol had a significant association with changes in CETP [76]. These findings suggest a CETP-dependent effect on the positive changes in LDL and HDL cholesterol in dyslipidemic subjects consuming anthocyanins from berries, and that improvements translate to a 27% reduction in the risk heart disease [77].

In a separate study by the same group, a similar design was used: anthocyanin rich extract from bilberry and black currant was administered (320 mg per day) for 12 weeks to investigate the effect of anthocyanins on flow-mediated dilation (FMD) of the brachial artery—a marker of endothelial function, inflammation, and cyclic guanosine monophostphate (cGMP) (an indicator of nitric-oxide related vasodilation in endothelial cells) in 146 hypercholesterolemic, overweight men and women [78]. The study was randomized, double-blinded, and placebo controlled using a parallel-arm design. Results include a significant 12% increase in cGMP as well as improvement in FMD from 8% to 11% with anthocyanin administration while no changes were observed in the placebo-controlled group [78]. Vascular cell adhesion molecule-1 (VCAM-1), a marker of endothelial inflammation, also improved with anthocyanin treatment. Similar to the earlier study, the anthocyanin treatment caused improvements in HDL and LDL cholesterol. Interestingly, the researchers administered a nitric-oxide synthase inhibitor to a subgroup of subjects with or without a simultaneous infusion of purified anthocyanin and saw improvements in FMD by the anthocyanin infusion were blocked by the inhibitor, suggesting dependence on nitric oxide synthase activity in anthocyanin related improvements in FMD [78]. The improvements in endothelial function, HDL and LDL cholesterols, and inflammation by anthocyanins in this study suggest a reduction in atherosclerotic progression via anthocyanins in hypercholesterolemic subjects.

Two subsequent reports from the feeding study were produced, probing further into the anthocyanin extract's effects on inflammation and paraoxonase 1 (PON1) activity of HDL [79,80]. The anthocyanin extract significantly lowered circulating high sensitive c-reactive protein as well as IL-1β by 22% and 13%, respectively, providing further evidence of a systemic reduction in inflammation [80]. HDL-PON1 activity is responsible for protecting against oxidation of LDL and foam cells and delays the progression of atherosclerosis [81]. The anthocyanin extract elicited a significant 17% increase in PON1 activity in the hypercholesterolemic subjects as well as a 21% reduction in oxidation of HDL particles in a

dihydrorhodamine oxidation assay [79]. The proposed antioxidant effect of anthocyanins via PON1 on HDL particles allows for greater reverse cholesterol transport resulting in the observed decrease in LDL noted in the earlier work [76,79].

In an independent study, eight obese, type-2 diabetic males participated in an acute experiment consisting of a single 470 mg dose of bilberry extract (36% anthocyanins by weight) or placebo followed by a five-hour oral glucose tolerance test [82]. The study was double-blind, randomized, and crossed-over. Significant findings included a reduction in both glucose and insulin area under the curve, but no response in incretins (GIP and GLP-1) or inflammation via the measurement of monocyte chemoattractant protein-1 (MCP-1). The proposed bioactive effects of the extract are a combination of increased insulin sensitivity as well as delayed and/or blocked absorption of sugar in the gut via the inhibition of digestive enzymes and transporters [82]. We describe anthocyanin activity with glucose transporters elsewhere [10]. Their findings are supported by separate work from Liu et al. where forty-eight overweight, type-2 diabetics were fed a 320 mg dose of bilberry and black currant extract or placebo for 12 weeks [83]. Change in serum lipids were not different, like in the above hypercholesterolemia studies, but there was a 29% increase in serum adiponectin and 16% reduction in fasting serum insulin which demonstrate positive changes in glucose management in diabetics with chronic feeding of anthocyanin extracts [83].

Finally, whortleberry extract was studied for effects on blood lipids in a group of obese subjects with hyperlipidemia not treated with pharmacotherapy [84]. In a parallel-arm, double-blind design, 105 randomized subjects consumed 1050 mg of encapsulated whortleberry extract or placebo daily for two months to assess changes on serum lipids. The treatment corresponded to 7.4 mg of anthocyanins each day. Despite a lower dose of anthocyanins compared to the doses used by the two other studies described above, this study observed dramatic improvements on blood lipids: 28%, 19%, and 26% reductions in total cholesterol, TGs, and LDL, respectively, and a 38% increase in HDL [84]. These dramatic improvements in serum lipids despite lower dose of anthocyanins compared to the other studies using extracts warrant further elucidation of bioactives present in whortleberry.

Taken together, these controlled trials on anthocyanin enriched extracts demonstrate the potential utility of anthocyanins from berries against obesity's complications: dyslipidemia and impaired glucose tolerance. If these findings are confirmed it would provide an alternative or complementary measure to ameliorate the clinical disturbances associated with obesity, particularly if a patient doesn't tolerate pharmacotherapy, thus future work on extracts is warranted. Lack of diversity is a limitation of this research area; five of the seven projects described above come from the same research group [76,78–80,83], and the interventions only include three different berry sources for anthocyanin extraction. Future work warrants broader involvement by different research institutes as well as study of other high anthocyanin berries.

5.2.2. Human Studies with Freeze-Dried Powders

A double-blind, parallel arm study determined the effect of blueberry smoothies on insulin sensitivity in obese adults with insulin resistance [85]. Thirty-two subjects were fed a smoothie containing either 22.5 g of freeze-dried blueberry powder or a placebo twice daily for six weeks. Both groups also received nutritional counseling to accommodate the energy provided by either smoothie treatment. The 45 g of berry powder equated to two cups of blueberries, or over 600 mg of anthocyanins. Insulin sensitivity was assessed by a hyperinsulinemic-euglycemic clamp, where rate of glucose infusion required to maintain euglycemia was corrected by lean body mass. Subjects in the blueberry powder group had a greater improvement in percent increase in insulin sensitivity compared to placebo (22% vs. 5%, blueberries vs. placebo, respectively) [85]. Secondary analyses of body composition and markers of inflammation were unchanged.

The same group performed an additional study using a similar study design, i.e., a placebo controlled, double-blind, parallel arm trial with the same blueberry smoothie,

where they tested effects of blueberries on hypertension, insulin sensitivity, and endothelial function in subjects with metabolic syndrome (metS) [86]. The same dosing regimen was applied for six weeks in forty-four subjects. Major findings include no effect of the blueberry smoothie on 24-hour ambulatory blood pressure or insulin sensitivity. Changes in blood pressure due to blueberries could have been masked by antihypertensive medications, as their use was not excluded. A deviation of this study from the former was the use of an intravenous glucose tolerance test in place of the hyperinsulinemic-euglycemic clamp, which may have affected sensitivity to detect differences in insulin sensitivity. A positive finding from the study was an increase in endothelial function with the blueberry smoothie [86].

A similar study performed a randomized, placebo controlled trial with forty-eight subjects with metS where subjects consumed 50 g of blueberry powder reconstituted in 960 mL water or a water control for eight weeks [87]. The blueberry powder contained 742 mg of anthocyanins per 50 g dose. Key findings include significant reductions in systolic and diastolic blood pressure by 6% and 4%, respectively, in subjects in the blueberry treatment group [87]. No other differences were found on criteria of the metS. Oxidation of LDL particles was also significantly lower with blueberry treatment, but markers of inflammation were unchanged [87]. This study's findings on blood pressure are in direct contention with the earlier study. Despite the obvious difference with a slight increase in the dose as well as a few weeks longer duration in the present study, preparation of blueberry powder was different in that the former mixed the powder into a yogurt-based smoothie while the present dissolved the powder in water. Interestingly, the intervention was well tolerated in the former study while the present saw 27% attrition form their blueberry arm, notably due to gastric distress [87]. These differences in tolerability between the two studies may be an indicator of the potency of the different preparations of the blueberry powder. A useful measurement for similarly designed future studies could include plasma anthocyanins to note possible differences in absorption efficiency due to the food matrix.

A separate report supports antihypertensive effects of blueberries in at-risk populations [88]. Forty-eight postmenopausal, obese women with mild hypertension were randomized to receive 22 g of freeze-dried blueberries or an energy matched placebo for eight weeks in a double-blind, parallel arm study. Both treatments were reconstituted in water prior to consumption. Primary findings included significant reductions of systolic and diastolic blood pressures by 5% and 6%, respectively, as well as a reduction in arterial stiffness as measured by pulse-wave velocity in the blueberry treatment group where no changes were observed with the placebo. Further, nitic oxide levels in the serum were increased by 68% with the blueberry treatment. Like other work that delivered the powder in water, a 20% attrition rate was noted in the blueberry treatment with gastric distress cited as a main problem [88].

Another study enrolled sixteen obese, female subjects with metS to determine the effect of freeze-dried strawberry powder on indices of metS as well as inflammation [89]. The subjects had all indices of metS except for impaired fasting glucose. Subjects were fed 50 g of strawberry powder daily for four weeks. The powder was dissolved in water and provided 154 mg of anthocyanins each day. The study was not controlled or blinded. Key findings included significant reductions in total and LDL cholesterol as well as lipid peroxides by 5%, 6%, and 14%, respectively [89]. Body weight, blood pressure, blood glucose, and inflammation were unchanged by the strawberry powder. Compared to the study on blueberry powder, the strawberry powder intervention in this study was well tolerated, as none of the subjects dropped out due to gastric issues caused by treatment.

In a follow-up study of twenty-seven obese subjects with metS, a control group was included as a parallel arm and the duration of study was extended to eight weeks utilizing the same daily dose of strawberry powder [90]. The control group was assigned to consume the amount of water required to dissolve the strawberry powder (960 mL) thus the study was not blinded. This study confirmed the effects observed in the uncontrolled study, where

total and LDL cholesterols were significantly lowered by the strawberry powder treatment and to a greater extent than the previous work (10% and 11%, respectively) which could be explained by the longer study duration of eight weeks. An anti-atherosclerotic effect was also suggested with strawberry treatment by a reduction in circulating concentrations of VCAM-1.

A separate study focused on the acute effects of strawberry powder in twenty-five insulin resistant subjects with central obesity (waist circumference > 110 cm) [91]. Insulin resistance was defined as fasting insulin concentrations >13 µIU/mL or a HOMA-IR score greater than 1. In a randomized, crossover, single-blind design, subjects were given one of four doses of freeze-dried strawberry powder (0–40 g) incorporated into whole milk and provided up to 155 mg of anthocyanins. The treatments were given in combination with a high fat, high carbohydrate breakfast and postprandial changes on blood glucose, insulin, and lipids were assessed for six hours. The main finding from the study was a significant 12% reduction in six hour averaged postprandial insulin concentrations with the highest strawberry powder treatment (40 g) [91]. Further, a dose response effect was suggested by a statistical trend for lower insulin to glucose ratios with the 10 and 20 g doses of strawberry powder compared to the control group. These improvements in insulin sensitivity were observed while there were no differences between the postprandial glucose curves for all treatment groups [91]. Blood lipids and postprandial inflammation were not different between treatments. Postprandial plasma concentrations of pelargonidin (the major anthocyanin present in strawberries) and cyanidin conjugates confirmed the dose-response of the four treatments and changes in circulating pelargonidin were associated with changes in postprandial insulin with all strawberry powder treatments [91].

The noted improvements by strawberry powder on biomarkers in subjects with metS or insulin resistance are corroborated by a study with type-2 diabetic subjects [92]. A randomized, double-blind, parallel arm study administered 50 g of strawberry powder (providing 154 mg of anthocyanins) or placebo powder (both treatments dissolved in water) daily to thirty-six type-2 diabetic subjects for six weeks to assess changes on long-term glucose regulation and inflammation. The subjects consuming the strawberry powder experienced a 6.5% reduction in HbA_1C, where an increase was observed in the control arm. The group also saw a 20% reduction in high-sensitive c-reactive protein (CRP) and lipid peroxides [92]. The findings demonstrate a cardioprotective benefit of strawberry powder in type-2 diabetics by both improved regulation of glucose metabolism leading to lower protein glycosylation as well as reduced atherogenicity by lowering chronic inflammation and lipid oxidation.

These studies highlight the therapeutic effects of two common and accessible berries on clinical features associated with obesity and metS. Noted by Park et al., freeze-dried preparations are commonly studied as a surrogate delivery form for berries in order to ensure consistency of the anthocyanin content, and other bioactive components attributable to feeding the intact berry may be overlooked in these studies, namely, the positive health benefits associated with fiber [91]. Fortunately, the powder doses used in these studies are achievable from normal amounts of berry intake; between one and three servings (one to three cups) of berries contain an equivalent dose of anthocyanins.

5.2.3. Human Studies with Whole Berries

Twenty-three healthy subjects were recruited to assess changes in blood lipids, oxidative stress, and platelet function after consuming 500 g of fresh strawberries (equivalent to approximately 300 mg of anthocyanins) daily for thirty days [93]. The study was not placebo controlled. Sample collections included baseline, post thirty days, as well as after a fifteen-day washout to assess any long term changes by the intervention. Main findings include significant improvement of serum total cholesterol, LDL, and TGs, where concentrations were lowered by 9%, 14%, and 21%, respectively, after the thirty-day strawberry treatment. Lipid peroxides, oxidized DNA, and urinary isoprostanes—all makers of oxidative stress—were also significantly reduced after the strawberry regimen. Blood

platelet function was also improved, as the amount of activated platelets (central clustered morphology) was reduced by 31% after the thirty day treatment [93]. Interestingly, following the fifteen-day washout, all noted benefits due to the berry intervention disappeared. Because the study was not controlled a study effect on subjects (motivated to eat healthier due to participation in a health study) cannot be ruled out. However, the noted differences are worth pursuing in controlled trials.

Finally, a randomized, cross-over trial in eighty obese women fed one of four berry derived interventions: 100 g per day bilberries, 100 g per day sea buckthorn berries, or doses of either ethanol or oil extracts of sea buckthorn that are equivalent to a 100 g serving of sea buckthorn berries [94]. The aim was to determine effects of the two berries on biomarkers of metabolic disease. Each intervention and washout period were approximately thirty days. Changes in diet were monitored by food diaries. Key findings of the study include modest, significant reductions in weight (0.2 kg) and waist circumference (1.2 cm) with bilberries, where waist circumference was also reduced with sea buckthorn berries (1.1 cm) [94]. Inflammatory markers were improved by the berry treatments. Insulin and/or HbA_1C measures deteriorated during all four berry interventions, but the authors note significant deviations in the habitual diets of the subjects during both washout and intervention periods, which may explain these conflicting results [94]. Lack of a control group and the impracticality of controlling the basal diet of the subjects may have hindered the ability to accurately assess the health benefit caused by the berry interventions.

The two whole berry studies above demonstrate the ability to modulate pertinent complications of obesity that are commonly targeted by healthcare practitioners: blood lipids and body weight. However, due to limited study design [93] and difficult subject populations [94], protective effects of whole berries are inconclusive. Our group has reported both positive and mixed findings with highly controlled, short-term human feeding studies with blackberry and mixed-berry interventions in overweight and obese, but healthy subjects [95,96]. There is a dearth of information on the effects of whole berry treatments in obese subjects; more research is needed with better (placebo-controlled) study designs. One major challenge in clinical trials with food interventions is the influence of background diets on dependent variables of metabolic disease; this can only be resolved by controlling every aspect of what the test subjects consume—both food items as well as their quantities—unfortunately this is often impossible. The human studies discussed in Section 5. are summarized in Table 4.

Table 4. Summary of findings from berry and anthocyanin treatments in humans.

Study Type	Berry	Methods	Duration	Variables	Results	References
Randomized, double-blind placebo-control	Bilberry/black currant extract	Groups: • Overweight participants with dyslipidemia + placebo control • Overweight participants with dyslipidemia + 325 mg/day extract	12 weeks	• Serum lipids • CETP • LCAT	• Significant 13.7% increase in HDL, 13.6% decrease in LDL, reductions in CETP concentration and activity for treatment group	[76]
Randomized, double-blind placebo-control	Bilberry/black currant extract	• Overweight participants with dyslipidemia + placebo control • Overweight participants with dyslipidemia + 320 mg/day extract	12 weeks	• FMD of the brachial artery • Markers of endothelial function	• Significant 12% increase in cGMP in treatment group • Significant increase in FMD from 8% to 11% with berry treatment • Significant increase in VCAM-1 with berry treatment	[78]
Randomized, double-blind, placebo-control, crossover	Bilberry extract (36% anthocyanins by weight)	• Males with type-2 diabetes + placebo control • Males with type-2 diabetes + 470 mg/day dose of bilberry extract	<1 day	• Glucose response • GIP • GLP-1 • Inflammation	• Significant reduction in both glucose and insulin area under the curve in treatment group • No response in GIP, GLP-1, or MCP-1	[82]
Randomized, placebo-controlled	Bilberry/black currant extract	• Overweight individuals with type-2 diabetes + placebo • Overweight individuals with type-2 diabetes + 320 mg dose/day of bilberry and black currant extract	12 weeks	• Blood chemistry	• NSD in serum lipids between groups • 29% increase in serum adiponectin and 16% reduction in fasting serum insulin in treatment groups	[83]
Randomized, parallel-arm, double-blind, placebo-control	Whortleberry extract	• Obese individuals with untreated hyperlipidemia + placebo • Obese individuals with untreated hyperlipidemia + 1050 mg/day of encapsulated extract	2 months	• Serum lipids	**In treatment group:** • 28% reduction in total cholesterol • 19% reduction in TGs • 26% reduction in total LDL respectively • 38% increase in HDL	[84]

Table 4. *Cont.*

Study Type	Berry	Methods	Duration	Variables	Results	References
Randomized, parallel-arm, double-blind, placebo-control	Freeze-dried blueberry powder	• Obese individuals with insulin resistance + placebo • Obese individuals with insulin resistance + 22.5 g/day blueberry powder	6 weeks	• Insulin sensitivity	• 22% improvement in insulin sensitivity in blueberry treatment group • 5% improvement in insulin sensitivity in placebo group	[85]
Randomized, parallel-arm, double-blind, placebo-control	Freeze-dried blueberry powder	• Obese individuals with insulin resistance + placebo • Obese individuals with insulin resistance + 22.5 g/day blueberry powder	6 weeks	• Insulin sensitivity • Hypertension • Endothelial function	• Improvements in endothelial function with treatment group when compared to the control	[86]
Randomized, placebo-controlled	Blueberry powder	• Individuals with metabolic syndrome + placebo • Individuals with metabolic syndrome + 50 g/day of blueberry powder	8 weeks	• Blood chemistry • Blood pressure	• Significant reductions in systolic and diastolic blood pressure by 6% and 4%, respectively, in treatment group	[87]
Double-blind, placebo-controlled, parallel arm	Blueberry powder	• Obese, postmenopausal women with hypertension + placebo • Obese, postmenopausal women with hypertension + 22 g powder/day	8 weeks	• Blood pressure • Blood chemistry • Arterial stiffness	**In treatment group:** • Significant reductions in systolic and diastolic blood pressure by 5% and 6%, respectively • Decrease in arterial stiffness in treatment group • 68% increase in nitric oxide in treatment	[88]
Intervention study	Freeze-dried strawberry powder	• Female, obese participants with metabolic syndrome + 50 g/day of powder	4 weeks	• Blood lipids • Body weight • Blood pressure • Blood glucose • Inflammation	• 5%reduction in total cholesterol • 6% reduction in LDL • 14% reduction in lipid peroxides • No change in body weight, blood pressure, blood glucose, and inflammation	[89]

Table 4. *Cont.*

Study Type	Berry	Methods	Duration	Variables	Results	References
Randomized, parallel arm	Freeze-dried strawberry powder	Obese participants with metabolic syndrome + 50 g/day of powder	8 weeks	Blood lipids	• 10% reduction in total cholesterol • 11% reduction in LDL cholesterol	[90]
Randomized, single-blind, crossover	Strawberry powder	Insulin resistant subjects with central obesity + HF-HC breakfast + 1 of 4 doses of strawberry powder	6-hrs	**Postprandial changes on:** • Blood glucose • Insulin • Lipids	• Significant 12% reduction in six hour averaged postprandial insulin concentrations 40 g of powder • No significant difference in blood lipids and postprandial inflammation	[91]
Randomized, double-blind, parallel arm	Strawberry powder	• Participants with type-2 diabetes + placebo • Participants with type-2 diabetes + 50 g/day powder	6 weeks	• Glucose regulation • Inflammation	• 20% reduction in high-sensitive CRP in treatment group • 6.5% reduction in HbA1C in treatment group	[92]
Intervention study	Whole strawberries	Healthy participants consumed 500 g/day of strawberries	30 days	• Blood lipids • Oxidative stress • Platelet function	• 20% reduction in TGs • 14% reduction in LDLs • 9% reduction in total cholesterol • 31% reduction in activated platelets • Reduction in lipid peroxides, oxidized DNA, and urinary isoprostanes	[93]
Randomized, crossover trial	• Bilberries • Sea buckthorn • Ethanol sea buckthorn extract • Oil sea buckthorn extract	Obese women consumed one of the following: • 100 g/day bilberry • 100 g/day sea buckthorn • Extracts of oil or ethanol equating to 100 g/day of sea buckthorn	30 days/study + washout period	• Body weight • Waist circumference • Biomarkers of metabolic disease	• 0.2 kg reduction in weight with bilberry treatment • 1.2 cm reduction in waist circumference in bilberry treatment • 1.1 cm reduction in waist circumference with sea buckthorn	[94]

CETP, cholesterol ester transfer protein; FMD, flow-mediated dilation; VCAM-1, vascular cell adhesion molecule-1; GIP, gastric inhibitory polypeptide; GLP-1, glucagon-like peptide 1; MCP-1, monocyte chemoattractant protein-1; NSD, no significant difference; TGs, triglycerides; LDL, low-density lipoprotein; HDL, high-density lipoprotein.

6. Conclusions

Epidemiological data indicates a protective effect of fruit and vegetable consumption against mortality. Specifically, epidemiological reports indicate a protective effect of polyphenols. Further, anthocyanins have been implicated for their health promoting effects. The obesity epidemic continues to tax the American healthcare system, and an aging population means the comorbidities associated with the condition will worsen. This literature review has described rodent and human clinical trials investigating the effect of anthocyanins or berries—a rich and natural source of anthocyanins—against obesity and its secondary complications across several modes of research. Results from rodent studies using berry interventions suggest an anti-obesity effect. Human clinical trials of berry extracts and powders touch upon the potential protective effect of berry treatments on clinical parameters complicated by obesity, but with inconsistent findings. Interestingly, both rodent and human research points to synergistic and bidirectional effects of berries with the gut microbiota.

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