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Abstract

Recent experimental and clinical studies suggest that consumption of blueberry products has potential health benefits in ameliorating the development of obesity and its related comorbidities, including type 2 diabetes (T2D) and chronic inflammation. Blueberry fruits are enriched with numerous bioactive components such as vitamins, phenolic acid and anthocyanins which could contribute to these protective effects. Possible mechanisms by which blueberries exert their beneficial properties include counteracting oxidative stress, regulating glucose metabolism, improving lipid profile, and lowering inflammatory cytokine levels in animal models and preliminary human trials. This review focuses on the potential role of blueberries as a functional food in the prevention and treatment of obesity and its comorbidities. Although the current evidence is promising, further randomized controlled studies in the longer term are needed to evaluate the role of blueberries and blueberry extracts to support human health.

Keywords: Blueberry, Anthocyanins, Obesity, Type 2 diabetes, Inflammation, Animal studies, Human trials

Contents

1. Introduction

Obesity is a medical condition in which excess body fat has accumulated to the extent that it may have a negative effect on health, causing reduced life expectancy and/or increased health problems (Haslam & James, 2005). Obesity causes a dysfunction in the metabolic system via a number of mechanisms, including initiation of endothelial dysfunction, increasing free radical production, lipid peroxidation and production of inflammatory cytokines (Chen, Chen, Wang & Liang, 2015; Neale, Batterham & Tapsell, 2016). Obesity predisposes to various diseases, especially obstructive sleep apnoea, cardiovascular disease (CVD), type 2 diabetes (T2D) and certain cancers (Haslam & James, 2005). Obesity is caused by excessive energy intake coupled with a lack of physical activity, however the complex interplay between genetics and environmental factors means that obesity is difficult to treat.

Obesity increases the risk of developing T2D, a disease that is characterised by hyperglycaemia with an antecedent phase of insulin resistance (Musso, Gambino & Cassader, 2010; Zierath et al., 1996). Uncontrolled or poorly managed T2D can cause changes in the structure and function of major organs and tissues, including blood vessels, heart, nerves, eyes and kidneys which can lead to further serious and life threatening complications such as cardiac dysfunction, atherosclerosis, and nephropathy (Musso, Gambino & Cassader, 2010; Zierath et al., 1996). In the early stages of T2D (or prediabetes), the pancreatic β-cells respond to hyperglycaemia by secreting increased amounts of insulin to facilitate the cellular uptake of the excess plasma glucose. Over time, insulin dependent cells become desensitised to insulin, resulting in β-cell dysfunction, insulin resistance and chronic hyperglycaemia if left untreated (Hajiaghaalipour, Khalilpourfarshbafi & Arya, 2015). Furthermore, dyslipidemia and an increase in pro-inflammatory cytokines have been shown to be associated with insulin resistance (Guo et al., 2012). Oxidative stress is another factor that can cause β-cell dysfunction, impaired glucose tolerance, insulin resistance and eventually T2D (Evans,

Goldfine, Maddux & Grodsky, 2003). Many studies have demonstrated that dietary antioxidants are effective in neutralizing or trapping reactive oxygen species (ROS) and thus antioxidants may be useful anti-diabetic agents (Defuria et al., 2009; Laplaud, Lelubre & Chapman, 1997; Martineau et al., 2006; Poudyal, Panchal & Brown, 2010).

It is well known that obese and diabetic patients often present with dyslipidemia, characterized by elevated triglycerides (TG), low high density lipoprotein cholesterol (HDL-C) and predominance of small-dense low density lipoprotein (LDL) particles (Chan, Barrett & Watts, 2014). Dyslipidaemia in visceral obesity is principally the result of insulin resistance, which perturbs the kinetics of both apolipoprotein B- (apoB) and apolipoprotein A- (apoA) containing lipoproteins (Chan et al., 2002; Martinez-Fernandez, Laiglesia, Huerta, Martinez & Moreno-Aliaga, 2015). Effective management of dyslipidaemia in obesity and T2D therefore often requires lipid regulation.

Obesity is related to chronic inflammation due to an increased infiltration of inflammatory cells into tissues such as liver and adipose tissue (Jung & Choi, 2014). Excess body fat, especially central adiposity, is correlated with a concomitant and persistent increase in low grade inflammation, which results in increased pro-inflammatory adipokines, cytokines and chemokines such as monocyte chemoattractant protein-1 (MCP-1), interleukin (IL)-6, nuclear factor-kappa B (NF-κB) and tumour necrosis factor alpha (TNF-α), and reduced production of anti-inflammatory adipokines, including adiponectin (Joseph, Edirisinghe & Burton-Freeman, 2014; Matsuzawa, 2010).

Dietary and/or complementary strategies to alleviate the metabolic complications of obesity and its related metabolic conditions have aroused considerable interest and are now under exploration as alternatives to pharmaceutical interventions. This paper will review the possible health benefits of one such dietary component, blueberries and blueberry extracts, emphasizing emerging evidence for its potential to ameliorate the impacts of obesity, T2D and chronic

inflammation. Moreover, data collected from studies on bioactive compounds of blueberries, in particular phytochemical constituents are included. The mechanisms of action of blueberries, as well as mechanistic and signalling pathways involved in the effects of blueberries on obesity and its related chronic diseases are also discussed. Figure 1 shows the proposed effects of blueberries on obesity and its related comorbidities, as well as associated metabolic and molecular pathways.

2. Bioactive constituents in blueberries

Blueberries are perennial flowering plants with indigo-coloured berries from the family Ericaceae within the genus *Vaccinium* (Luby, Ballington, Draper, Pliska & Austin, 1999). Many species of blueberry come predominantly from North America, however they are now produced in almost all countries, including Australia, New Zealand and European countries. Depending on the growing season and harvesting time, several types of blueberries are commonly available, including highbush blueberry plants (*Vaccinium corymbosum* L.), the rabbiteye blueberry (*Vaccinium ashei* Reade), lowbush blueberry plants or wild blueberry (*Vaccinium angustifolium* Aiton), and bilberry (*Vaccinium myrtillus* L.) (Maatta-Riihinen, Kamal-Eldin, Mattila, Gonzalez-Paramas & Torronen, 2004; Michalska & Lysiak, 2015). Bilberry is a European wild blueberry that contains a higher content of anthocyanins (ACNs) than cultivated blueberry species (Chu, Cheung, Lau & Benzie, 2011). Blueberries are nutritious fruits as they are rich sources of carbohydrates, vitamins and minerals (Liu, et al., 2015b). Blueberries are also a good source of dietary fibres that constitutes 3%–3.5% of fruit weight (Michalska & Lysiak, 2015). In addition, blueberries have a high content of several phytochemicals, including ascorbic acid and phenolics. Many of the proposed beneficial health effects associated with blueberry consumption are linked to the bioactive properties of the phytochemical constituents. The predominant bioactive components contained in blueberries are ascorbic acid, flavonols (including kaempferol, quercetin and myricetin), hydroxycinnamic

148 acids (including caffeic acids, ferulic acids and coumaric acids), hydroxybenzoic acids
149 (including gallic acids and procatchuic acids), pterostilbene, resveratrol, and ACNs. The
150 potential benefits of blueberry for human health have received much attention in recent years
151 due to these bioactive components (Chen, Li & Xu, 2010; Koupy, Kotolova & Kucerova,
152 2015).

153 **2.1 Ascorbic acid**

154 Blueberries are rich in ascorbic acid, which is a water-soluble compound that fulfils several
155 roles in living systems, including enhancing immunity and reducing inflammation (Liu, et al.,
156 2015a; Nile & Park, 2014). Ascorbic acid is an antioxidant vitamin and is widely distributed in
157 various blueberry species and varieties. On average 100 g of blueberries provide 10 mg of
158 ascorbic acid, which is equal to one third of the daily recommended dietary intake (Capra,
159 2006; Prior et al., 1998), however varying amounts of ascorbic acid have been reported in
160 different species. The content of ascorbic acid in highbush blueberries (total eight species)
161 ranged from 5 to 15 mg/100 g of fresh fruit, compared with 16.4 mg/100 g in lowbush blueberry
162 (Prior et al., 1998). Fresh bilberry only contains small quantities of ascorbic acid (3 mg/100 g)
163 (Graff & Upton, 2001). Rabbiteye blueberries contain different amounts of ascorbic acid due
164 to the variety of species. Six species of rabbiteye blueberry were found to have a lower amount
165 of ascorbic acid (6 to 10 mg/100g) compared to the average content (Prior et al., 1998).
166 However, it has been reported that the concentration of ascorbic acid was high and up to 41
167 mg/100 g in fresh Ochlockonee fruit, belonging to the rabbiteye species, and 25 mg/100 g in
168 fresh highbush blueberry (Gündüz, Serçe & Hancock, 2015). There are also other contributors
169 to the potential variation in ascorbic acid in blueberries, such as cultivation, climate, weather
170 conditions and storage time. The concentration of ascorbic acid decreases when conditions
171 such as oxygen level and temperature are suboptimal during storage. Moreover, after storage

for 8-days at 20 °C the content of ascorbic acid in fresh fruit decreases by 27% (Kalt, Forney, Martin & Prior, 1999).

2.2 Phenolics

Phenolic compounds belong to a wide and heterogeneous group of chemical substances that possess one or more aromatic rings with a conjugated aromatic system and one or more hydroxyl groups. Phenolic compounds occur in free or conjugated forms with sugars, acids, and other biomolecules as water-soluble (phenolic acids, flavonoids and quinones) or water-insoluble compounds (condensed tannins) (Skrovankova, Sumczynski, Mlcek, Jurikova & Sochor, 2015). The total content of phenolic compounds in blueberries is highly variable, with variation upwards of 10-times higher or lower (e.g. ranges from 48 up to 304 mg/100 g of fresh fruit weight (up to 0.3%) (Ehlenfeldt & Prior, 2001; Moyer, Hummer, Finn, Frei & Wrolstad, 2002) depending on the cultivar (Taruscio, Barney & Exon, 2004), growing conditions and maturity (Castrejón, Eichholz, Rohn, Kroh & Huyskens-Keil, 2008), and its estimation may vary depending on the method of analysis (De Souza et al., 2014; Maatta-Riihinen, Kamal-Eldin, Mattila, Gonzalez-Paramas & Torronen, 2004). Phenolic compounds presented in blueberries contain stilbenoids, tannins [hydrolyzable tannins (gallotannins and ellagitannins) and condensed tannins (proanthocyanidins)], and flavonoids, including flavan-3-ols, ACNs, and their polymeric condensation products, flavanones, flavonols (i.e., kaempferol, quercetin, myricetin) and flavones (Borges, Degeneve, Mullen & Crozier, 2010; Seeram, 2008; Taruscio, Barney & Exon, 2004). High amounts of phenolics are found in blueberry and account for 50–80% of the total polyphenol content, which can reach a concentration of up to 3000 mg/kg fresh weight (Kuntz et al., 2015; Muller, Schantz & Richling, 2012).

Tannins are a unique group of phenolic metabolites with molecular weights between 500 and 30,000 Da, which are widely distributed in all berry species and specific berries may contain an abundance of a particular group of tannins (Ferreira, Gross, Kolodziej & Yoshida, 2005;

Serrano, Puupponen-Pimia, Dauer, Aura & Saura-Calixto, 2009). It has been suggested that tannins may have therapeutic potential in the treatment of diabetes, mainly through two ways; (i) they may lower glucose levels by delaying intestinal glucose absorption and an insulin-like effect on insulin-sensitive tissues, and (ii) they may delay the onset of insulin-dependent T2D by regulating the antioxidant environment of pancreatic β-cells (Serrano, Puupponen-Pimia, Dauer, Aura & Saura-Calixto, 2009). Previous studies showed that tannins were an effective inhibitor of intestinal α-glucosidase activity (Mcdougall et al., 2005; Toda, Kawabata & Kasai, 2001), and they also inhibited glucose uptake in intestinal cells (Song et al., 2002). Proanthocyanidins, known as condensed tannins, are the most widely represented products of plant secondary metabolism throughout nature, after lignins (Gu et al., 2003). Blueberries contain predominantly proanthocyanidins, compared with other berries, such as blackberries, black raspberries, red raspberries, and strawberries, which contain predominantly ellagitannins (Seeram, 2008). Therefore, the unique biological properties of blueberries may be associated with the specific chemical structures of tannins. The distinct biological effects of blueberries on neuronal function in different regions of the brain and behaviour in aging animals may be due to the effects of individual classes of tannins (Shukitt-Hale, Carey, Jenkins, Rabin & Joseph, 2007).

Flavonoids are a large heterogenic group of benzo-γ-pyron derivatives, which are abundantly present in food products and beverages derived from fruits and vegetables (Heo & Lee, 2004). Many physiological benefits of flavonoids have been attributed to their antioxidant and free radical scavenging properties to exert positive health effects on chronic disease, including cancer and neurodegenerative disorders (Lau, Bielinski & Joseph, 2007; Neto, 2007; Nile & Park, 2014). Blueberries have also been demonstrated to contain high levels of flavanoid compounds, ranking them among the foods showing the highest antioxidant activity (Barberis

et al., 2015; Borges, Degeneve, Mullen & Crozier, 2010; Moyer, Hummer, Finn, Frei & Wrolstad, 2002).

The predominant flavonoids in blueberries are quercetin glycosides (quercetin-3-galactoside, quercetin-3-glucoside, quercetin-3-rutinoside) and myricetin glycosides (myricetin-3-glucoside, myricetin-3-rhamnoside) (Skrovankova, Sumczynski, Mlcek, Jurikova & Sochor, 2015). Quercetin, one of the most frequently researched flavonoids, has shown antioxidative and anti-carcinogenic activities to protect against oxidative stress (Heo & Lee, 2004). The content of quercetin in blueberry and bilberry were 24 and 30 mg/kg fresh fruit, respectively, which were accounted to 50% and 60% of total flavonoids (Hakkinen, Karenlampi, Heinonen, Mykkanen & Torronen, 1999). Several *in vitro* studies indicated its efficacy in the prevention of different types of cancer induced by potent carcinogens, such as benzo(a)pyrene, azoxymethane, and N-nitrosodiethylamine (Kamaraj et al., 2007; Seufi, Ibrahim, Elmaghraby & Hafez, 2009; Volate, Davenport, Muga & Wargovich, 2005) and its anti-cancer capability has also been demonstrated in animal models (Caltagirone et al., 2000; Devipriya, Ganapathy & Shyamaladevi, 2006). Myricetin is a bioflavonoid abundant in berries and it was reported that the anti-diabetic effectiveness of myricetin is due to its anti-inflammatory activity (Fu et al., 2013; Wang et al., 2010; Wu, Zheng, Gong & Li, 2016). The content of total flavonoids in blueberries ranged from 2.5 to 387.48 mg/100 g fresh fruit (Hakkinen, Karenlampi, Heinonen, Mykkanen & Torronen, 1999; Sellappan, Akoh & Krewer, 2002), depending on the species and the method used (Borges, Degeneve, Mullen & Crozier, 2010; Buran et al., 2014; Taruscio, Barney & Exon, 2004). Taruscio et al (2004) reported the contents of flavonols extracted from eight blueberry species, including three species of highbush blueberry, three species of half-highbush blueberry and two species of bilberry. The HPLC analytical results showed that myricetin and quercetin were the principal flavonols in blueberries (Taruscio, Barney & Exon, 2004). Bilberry contained the highest level of quercetin (163.6 µg/g in frozen fruit) followed

by half-highbush blueberry (102.5 µg/g in frozen fruit) and highbush blueberry (86.4 µg/g in frozen fruit) (Taruscio, Barney & Exon, 2004). Bilberry also contained the highest content of myricetin (200 µg/g in frozen fruit) at the level of nearly 10 and 15-fold higher , compared to half-highbush blueberry (19.8 µg/g in frozen fruit) and highbush blueberry (12.9 µg/g in frozen fruit) (Taruscio, Barney & Exon, 2004).

Anthocyanins (ACNs), pigments that contribute to the intense colours in blueberry, have been shown to exhibit numerous bioactive properties, such as anti-inflammatory, antioxidant and anti-cancer activities (Faria et al., 2010; Vendrame, Daugherty, Kristo, Riso & Klimis-Zacas, 2013; Zepeda et al., 2012). The most common anthocyanidin aglycones are peonidins, pelargonidins, malvidins, delphinidins, cyanidins and petunidins (Li, Wang, Guo & Wang, 2011). These then combine with organic acids and sugars to generate various ACNs (Figure 2) (Rodriguez-Mateos, Heiss, Borges & Crozier, 2014). Muller et al (2012) found that malvidin and delphinidin are the main components and constitute almost 72% of all identified anthocyanins (Muller, Schantz & Richling, 2012). However, other studies reported less concentrations of malvidin (22%–33%) and delphinidin (27%–40%) in five genotypes of blueberries (Cho, Howard, Prior & Clark, 2004). There are up to 27 different ACNs found in blueberries (Prior et al., 1998). The content and type of ACNs depend on the species, fruit size, ripening stage, as well as on climatic, pre-harvest environmental conditions and storage (Muller, Schantz & Richling, 2012; Scibisz & Mitek, 2007). The concentration of ACNs is up to 800 mg/100 g fresh weight in highbush species and more than 1000 mg/100 g fresh fruit in lowbush species (Cho, Howard, Prior & Clark, 2004; Hosseinian & Beta, 2007). The high content of ACNs in different *Vaccinium* species is a main contributor to their antioxidant activity and is responsible for about 84% of total antioxidant capacity (Borges, Degeneve, Mullen & Crozier, 2010). Whereas ascorbic acid was only found to contribute to 10% of the antioxidant capacity despite being present in a significant amount (Barberis et al., 2015).

Although structural and categorical diversity can be noticed among bioactive constituents in blueberries, other factors influence this diversity including, but are not limited to, species and genetic makeup of blueberries, agricultural practices, growing condition, season of harvest, irrigation, and storage of the fruits (Castrejón, Eichholz, Rohn, Kroh & Huyskens-Keil, 2008; Scibisz & Mitek, 2007).

2.3 Phenolic acid

Phenolic acid, in general, describes phenols that possess one carboxylic acid functionality (Robbins, 2003). Phenolic acids account for approximately one-third of the dietary phenols present in plants (Zadernowski, Naczk & Nesterowicz, 2005). Researchers have become increasingly interested in phenolic acids and their derivatives due to their high nutritional and antioxidant properties in foods (Chalas et al., 2001; Zadernowski, Naczk & Nesterowicz, 2005). Clifford (1999) estimated that the average amount of phenolic acids consumed is between 25 mg and 1 g daily (Clifford, 1999). In blueberries, only a minor fraction of phenolic acid exists as free forms, with the majority of phenolic acid existing in conjugated forms, which are linked with esters, amides and glycosides (Robbins, 2003). Vanillic acid, hydroxycinnamic acids, ferulic acid, caffeic acid, chlorogenic acid, p-coumaric acid, gallic acid and salicylic acid are the principal phenolic acids in blueberry (Zadernowski, Naczk & Nesterowicz, 2005). Among them, chlorogenic acid is the most abundant in blueberry species (Kang, Thakali, Jensen & Wu, 2015), however its content was highly variable between species with highbush and lowbush blueberry varieties ranging from 34.3 to 113.8 mg/100 g fresh weight (Rodriguez-Mateos, Cifuentes-Gomez, Tabatabaee, Lecras & Spencer, 2012). This high concentration of chlorogenic acid present in blueberries is likely to contribute to the anti-inflammatory effects of blueberries (Santos, Almeida, Lopes & De Souza, 2006). A previous study showed that seven phenolic acid mixture including hydroxycinnamic acid, hippuric acid, 3-(3-hydroxyphenyl)propionic acid, 3-(4-hydroxyphenyl) propionic acid, hydroxyphenylacetic

acid, hydroxybenzoic acid and ferulic acid from blueberry inhibited lipopolysaccharide (LPS)-induced production of pro-inflammatory cytokine, IL-6 and TNF-α by the reduction of mitogen-activated protein kinase, Jun amino-terminal kinases (JNK), p38 and Erk1/2 phosphorylation in murine macrophage cell line RAW 264.7 (Xie et al., 2011).

3. Effects on body weight and fat mass

The anti-obesity effects of blueberries and blueberry extracts have been investigated in both clinical studies and also several animal models, such as Obese Zucker rats, KKAy mice, C57BL/6J mouse and Sprague-Dawley rats (Prior et al., 2010; Seymour et al., 2009; Seymour et al., 2011; Vuong et al., 2009). Tables 1 and 2 summarise the impacts of consumption of blueberries and blueberry extracts on obesity in animal models and human trials.

3.1 Whole fruit or juice

It has been reported that body weight, liver weight, and total fat weight were significantly reduced in Obese Zucker rats fed a low-fat diet (LFD) combined with 2% (w/w) whole highbush blueberry powder (Seymour et al., 2009; Seymour et al., 2011). These results are consistent with the study of Prior et al. (2010) who reported that supplementation with blueberry juice (0.2 mg/mL) prevented weight gain in C57BL/6J mice that were fed a HFD (45% of kcal from fat). Furthermore, Voung et al. (2009) showed that incorporating blueberry juice in drinking water significantly reduced weight gain in obese KKAy mice. These positive results possibly related to the improved glucose tolerance and enhanced insulin sensitivity seen in these animals (Vuong et al., 2009). Contrary to these results, blueberry supplementation did not affect the body weight of C57BL/6J mice fed a HFD (60% of energy) with 4% (w/w) whole blueberry powder for 8 weeks (Defuria et al., 2009) or of Sprague-Dawley rats supplemented with 10% freeze-dried whole blueberry for 3 weeks (Seymour et al., 2009). Another study found no significant differences in weight gain after the 12 weeks of feeding

C57BL/6J mice with 5% bilberry compared with mice fed a HFD (45% kcal fat) (Mykkanen et al., 2012). Conversely, Prior et al. (2008) demonstrated that diets supplemented with 10% whole blueberry powder increased adiposity and body weight in C57BL/6J mice fed a HFD. However, blueberry-fed mice in this study consumed approximately 12% more energy/day than the control HFD group, which may have contributed to these outcomes (Prior et al., 2008).

Most of the clinical studies have shown that dietary supplementation with whole blueberry or blueberry juice failed to reduce body weight and waist circumference (Basu et al., 2010; Qin et al., 2009; Stull, Cash, Johnson, Champagne & Cefalu, 2010). This has been demonstrated in a randomised controlled trial with 48 obese participants (4 males and 44 females) in which participants consumed a freeze-dried blueberry beverage (50 g freeze-dried blueberries equivalent to 350 g of fresh blueberries) or water for 8 weeks. There were no significant differences observed in waist circumference, body weight or dietary intakes between the treatment group and the control group (Basu et al., 2010). Similar results were also observed in non-diabetic obese participants who were supplemented with either 22.5 g blueberry powder or a placebo twice daily for 6 weeks in that there were no significant differences observed between the treatment and control groups in body weight, adiposity and energy or macronutrient consumption (Stull, Cash, Johnson, Champagne & Cefalu, 2010). Overall there is limited evidence to suggest that blueberry supplementation alone affects adiposity in obese or overweight individuals. Future studies are encouraged to focus on calorie restriction and longer intervention periods in conjunction with supplementation, however whether this will result in clinically significant improvements in weight loss compared to calorie restriction alone is uncertain.

3.2 Extracts of blueberries

Several studies have examined the effects of blueberry extracts, particularly ACNs from fresh blueberry fruit, juice and peel on control of body weight and have indicated that the anti-obesity

capability of blueberry extract is quite different to whole fruit or juice (Prior et al., 2010; Prior et al., 2009; Prior et al., 2008). Although the reasons for these disparities are not clear, one possible explanation is that there are different types and amounts of bioactive constituents contained in blueberry and its products, which might change the response to extracts from blueberry, compared with purification or single components. For instance, blueberry juice contains not only ACNs but also other components such as procyanidins, chlorogenic acid, and other water-soluble compounds including sugars (Prior et al., 2010). Another possibility is that there are some specific components, such as uronic acids, neutral sugars, noncellulosic sugars including xylose and arabinose, or other factors as an obstruction in whole blueberry to counteract the potential benefit of blueberry consumption (Vicente et al., 2007). Wu et al. (2013) showed that ACNs from blueberry juice decreased body weight up to 7.3% in dietary-induced models of obesity. Dietary-induced weight gain, perirenal adipose tissue and epididymal weights were significantly lowered in male Sprague-Dawley rats fed a HFD supplemented with blueberry peel extracts for 5 weeks compared to an equivalent control group. It has been reported that blueberry peel extracts may potentially affect obesity by a reduction of adipogenesis and inhibition of fat accumulation through the PI3K/Akt/GSK3β pathway in 3T3-L1 preadipocytes (Song et al., 2013).

Further studies are required to assess the effect of ACNs consumption at various doses to establish the specific concentration of ACNs required for ameliorating the development of obesity. According to a previous study conducted by Prior et al. (2010), the low concentration of ACNs (0.2 mg/mL) decreased retroperitoneal and epididymal fat (% body weight) by 31% and 25%, respectively in mice fed a LFD, and 26% and 29%, respectively in mice fed a HFD for 72 days. However, retroperitoneal and epididymal fat levels were not decreased in HFD-fed mice treated with higher concentration of ACNs (1.0 mg/mL) but were similar to, or slightly higher than the HFD mice without ACNs (Prior et al., 2010). ACNs intake was measured as

0.6 and 3.4 mg/day for each mouse fed a LFD, and 0.5 and 1.8 mg/day for each mouse fed a HFD, according to liquid intake with the low concentration (0.2 mg/mL) and high concentration (1.0 mg/mL) of ACNs (Prior et al., 2010). This indicated that low concentrations of ACNs are potentially more beneficial compared to higher doses; however the exact reasons for this observation are unknown. Conversely, another independent study has demonstrated that supplementation of a higher concentration (2.8 mg/day/mouse) of purified ACNs for 92 days significantly prevented the development of obesity, but 3.75 mg/day/mouse failed to prevent body weight gain in HFD induced obese mouse model (Prior et al., 2008). Thus it appears to be no clear dose dependent effect and further investigation is needed to define the effective dose of ACNs or blueberries for body weight control in cases of obesity.

4. Effect on glucose metabolism and insulin signalling

Animal models (Table 1) and clinical studies (Table 2) have demonstrated that supplementation or consumption of blueberry or blueberry bioactive compounds cause changes in glucose metabolism and improve insulin sensitivity.

4.1 Whole fruit or juice

Supplementation of 2% freeze-dried blueberry powder for 13 weeks in Obese Zucker rats have demonstrated significant reductions in glucose, fasting insulin and insulin resistance, as indicated by the Homeostasis Model Index of Insulin Resistance (HOMA-IR) (Seymour et al., 2009; Seymour et al., 2011). Likewise, Vuong et al. (2009) showed that fermented blueberry juice by the *Serratia vaccinii* bacterium significantly reduced blood glucose levels and maintained the glycaemia of pre-diabetic KKAy mice to a normal level. These results indicate that blueberry intake could reduce phenotypes of diabetes in obesity-prone rats by regulating glucose metabolism. Conversely, Prior et al. (2008) reported that long term supplementation with freeze-dried whole blueberry powder did not affect the results of a glucose tolerance test

that were administered to C57BL/6J obese mice. These inconsistent results mainly depend on the variation of animal models, the duration of the treatment, and the dose of bioactivity components in blueberry. Furthermore, clinical studies have also reported that blueberry supplementation did not show the impact on fasting serum glucose (Basu et al., 2010; Kolehmainen et al., 2012; Stull, Cash, Johnson, Champagne & Cefalu, 2010). Specifically, Basu et al. (2010) documented that a freeze-dried blueberry beverage (50 g freeze-dried blueberries equivalent to 350 g of fresh blueberries) for 8 weeks to 48 obese participants (4 males and 44 females) was not able to significantly change their serum glucose concentration. Also, glucose and insulin responses did not differ between the bilberry group (400 g fresh fruit) and the control group, when obese individuals consumed a diet rich in bilberries for 8 weeks (Kolehmainen et al., 2012). Likewise, no changes was observed in serum glucose during the intervention with 22.5 g blueberry bioactive twice daily for 6 weeks, although insulin sensitivity was improved significantly more in the blueberry group compared to the placebo group in participants who were obese, nondiabetic, and insulin resistant (Stull, Cash, Johnson, Champagne & Cefalu, 2010). *In vitro* studies have however consistently shown that blueberry improves glucose uptake. For instance, 6-h incubation of fermented blueberry juice with and without insulin enhanced glucose uptake into the adipocyte and muscle cells and increased the phosphorylation/activation of proteins in the insulin-independent pathway (i.e., AMP-activated protein kinase) but had no effect on phosphorylation of key proteins in the insulin-dependent pathway (i.e., AKT and ERK1/2) (Vuong, Martineau, Ramassamy, Matar & Haddad, 2007). These findings showed that the bioactive components in fermented blueberry improved glucose uptake into the cells via an insulin-independent mechanism. These positive cellular mechanistic studies provide evidence on the improvement of insulin sensitivity *in vitro*, however why the variation in the *in vivo* studies remains to be determined.

4.2 Extracts from blueberries

While the effects of blueberry juice on glucose tolerance *in vivo* is varied, supplementation with ACNs appear to have a more positive effect as it has been previously indicated that fasting serum glucose concentrations were decreased and oral glucose tolerance was increased in mice fed a HFD supplementation with ACNs compared to blueberry juice (Prior et al., 2010). This result is possibly attributed to other constituents in blueberry juice such as procyanidins, chlorogenic acid, and other water-soluble compounds including sugars, which are not present in ACNs. It is possible that this beneficial effect of ACNs on glucose tolerance may be due to a direct effect on the liver as blueberry ACNs (0.05–10 mg/mL) have been demonstrated to significantly reduce glucose production by 24–74% in H4IIE hepatocytes (Roopchand et al. 2013). In addition, diabetic C57BL/6J mice supplemented with 500 mg/kg body weight of a phenolic-rich fraction or an anthocyanin-rich fraction showed reductions in blood glucose levels by 33% and 51%, respectively. In these fractions, 287 mg/g ACNs was in a phenolic-rich fraction, while 595 mg/g ACNs (cyanidin-3-glucoside equivalents) was in an anthocyanin-rich fraction, which suggested that higher ACNs concentration in different fractions may contribute to more hypoglycaemic activity of the extracts (Grace et al., 2009).

Bilberry extract also reduces blood glucose level and enhances insulin sensitivity in diabetic KKAy mice (Sasaki et al., 2007). Furthermore, in the same study, the glucose transporter 4 (Glut4) was upregulated and retinol binding protein 4 (RBP4) was downregulated in the white adipose tissue in bilberry extract group (Sasaki et al., 2007). These results indicated that bilberry extract has a potent effect on glucose metabolism through the regulation of Glut4-RBP4 system. The beneficial effects of bilberry extracts are also supported in a human trial demonstrating that insulin and postprandial glycaemia was significantly reduced in diabetic volunteers supplemented a bilberry extract (containing 36 % (w/w) of ACNs which is equivalent to about 50 g of fresh bilberry) for 2 weeks, compared with the placebo group (a

polysaccharide drink and equivalent to 75 g of glucose) (Hoggard et al., 2013). A longer intervention (4 weeks) with the extracts (providing 50 mg 3,4-caffeoylquinic (chlorogenic) acid, and 50 mg myricetin) from blueberry leaf has also shown that fasting plasma glucose was reduced significantly in diabetic volunteers (Abidov, Ramazanov, Jimenez Del Rio & Chkhikvishvili, 2006). However, other clinical studies have indicated that there were no significant differences in fasting blood glucose between the treatment and the control groups after dietary supplementation with ACNs for 12 (Qin et al., 2009) or 24 weeks (Zhu et al., 2013).

There are up to 27 different ACNs present in blueberry, however, only several specific ACNs exhibit strong hypoglycaemic capacity (Roopchand, Kuhn, Rojo, Lila & Raskin, 2013). Grace et al. (2009) observed that in diabetic C57BL/6J mice treated with 300 mg/kg of the pure ACN delphinidin-3-O-glucoside (D3G) or malvidin-3-O-glucoside (M3G), M3G decreased blood glucose to a greater extent compared to D3G. It is likely that the metabolism and bioavailability affects the magnitude of bioactivity in different types of ACNs. Cyanidin-3-glucoside (C3G) is the predominant ACN in blueberries (Wang, Zhao, Wang, Huo & Ji, 2016). Several studies have shown that isolated C3G improved insulin sensitivity and hyperglycaemia in animal models of diabetes (Guo et al., 2012; Liu, Li, Zhang, Sun & Xia, 2014; Sasaki et al., 2007). There are several pathways involved in these effects, such as the modulation of Glut4-RBP4 system (Sasaki et al., 2007), the c-Jun N terminal kinase/forkhead box O1 signalling pathway (Guo, Guo, Jiang, Li & Ling, 2012) and adiponectin activating cAMP-PKA-eNOS signalling pathways (Liu, Li, Zhang, Sun & Xia, 2014).

In animal studies, following supplementation with blueberry extracts or pure ACNs (C3G), ACNs were detected in the liver, blood, kidney and ocular tissues with an intact form suggesting that ACNs and/or their metabolites can be distributed to various tissues via blood and are therefore expected to regulate metabolic changes in the body (Ichiyanagi, Shida,

Rahman, Hatano & Konishi, 2006; Mcghie, Ainge, Barnett, Cooney & Jensen, 2003; Takikawa, Inoue, Horio & Tsuda, 2010; Tsuda, Horio & Osawa, 1999). An *in vitro* study has also reported that glucose uptake was increased in C_2C_{12} cells treated with extracts from the root, leaf and stem of lowbush blueberry, and in 3T3-L1 cells only treated with extracts from root and stem of lowbush blueberry (Martineau et al., 2006). These results were consistent with an *in vivo* study that also demonstrated ACNs components in different fractions specifically contributed to improving hypoglycaemic activity in diabetic C57BL/6J mice (Grace et al., 2009). However, the fruit extract in lowbush blueberry did not show any effect on glucose-stimulated insulin secretion or glucose uptake in β TC-tet pancreatic β cells (Martineau et al., 2006). Since the ACNs composition extracted from the fruit are completely different, compared to those extracted from the leaf, root and stem, the hypoglycaemic compounds from the blueberry *in vitro* studies perhaps do not have the same effect *in vivo* due to the different mechanisms of action.

5. Effect on lipid metabolism

5.1 Whole fruit and fruit juice

Diets enriched with blueberries have been reported to improve dyslipidaemia (Seymour et al., 2009; Seymour et al., 2011; Vendrame, Daugherty, Kristo & Klimis-Zacas, 2014b; Wu et al., 2013). Plasma TG and total cholesterol (TC) concentrations were significantly reduced in Obese Zucker rats supplemented with 8% wild blueberry for 8 weeks (Vendrame, Daugherty, Kristo & Klimis-Zacas, 2014a) or 2% blueberry powder for 13 weeks in both LFD and HFD groups compared with the control groups (Seymour et al., 2009). These observations were also supported by a reduction in serum TC and low density lipoprotein cholesterol (LDL-C), as well as the levels of liver TG and TC following consumption of blueberry juice. although the contents of liver lipids and cholesterol were not changed in C57BL/6 mice (Wu et al., 2013).

The consumption of 1%, 2% and 4% blueberry-supplements for 8 weeks has significantly reduced the TC and LDL-C concentrations in pigs (Kalt et al., 2008).

The possible pathways involved in the anti-dyslipidaemic effect of blueberries include the regulation and expression of key enzymes such as lipoprotein lipase (LPL) (Wei et al., 2011), fatty acid synthase (Tsuda, Ueno, Kojo, Yoshikawa & Osawa, 2005) and ATP-binding cassette transporter 1 (ABCA1) (Xia et al., 2005) which are involved in TG and cholesterol metabolism. Furthermore, the expression of transcription factors such as sterol regulatory element-binding transcription factor (SREBP) and peroxisome proliferator-activated receptor (PPAR) in bioactive tissues could also explain the observed effects of blueberry consumption on lipid profiles (Cutler, Petersen & Anandh Babu, 2016; Vendrame, Daugherty, Kristo & Klimis-Zacas, 2014a). In a recent study, the expression of PPARα and PPARγ in Obese Zucker rats were increased in the abdominal adipose tissue (AAT), while that of total SREBP-1 was decreased in both the liver and the AAT of the rats following consumption of a diet enriched with 8% wild blueberry for 8 weeks (Vendrame, Daugherty, Kristo & Klimis-Zacas, 2014a). The activation of PPARα and PPARγ following blueberry consumption could partly explain such an effect on lipid accumulation in blood and bioactive tissues. The activation of PPARα is related to enhanced fatty acid uptake, conversion into acyl-CoA derivatives, and further catabolism (Pawlak, Lefebvre & Staels, 2015); moreover, the activation of PPARγ in adipose tissue is known to induce differentiation of preadipocytes and TG storage (Ferre, 2004). The down-regulation of the expression of SREBP-1 also helps to explain the reduction in TG and TC in the Obese Zucker rats supplemented with blueberry diet, since SREBP-1 isoforms promote the synthesis and accumulation of TG and cholesterol via the induction of multiple enzymes (Horton, Goldstein & Brown, 2002). Similar results were also observed by Seymour et al (2011) which showed blueberry intake increased PPARα and PPARγ activity in skeletal muscle in both HFD and LFD fed rats. In addition, the intake of blueberry significantly affected

mRNA of several genes related to fat storage and glucose uptake, such as PPARγ co-activator 1α, Acyl-CoA oxidase, fatty acid synthase, fatty acid-CoA ligase, Glut4 and insulin receptor substrate 1 in both skeletal muscle and retroperitoneal abdominal fat in HFD induced rats (Seymour et al., 2011). With regards to improving lipid profile, clinical studies of blueberry supplementation have not supported those of animal studies with freeze-dried wild blueberries showing no effect on TG, TC, HDL-C and LDL-C levels in obese subjects (Basu et al., 2010), in subjects with developing CVD risk (Riso et al., 2013), and in healthy middle-aged male subjects (Wang, Zhao, Wang, Huo & Ji, 2016).

5.2 Anthocyanins in blueberries

Mice that were fed a HFD and also had their drinking water supplemented with purified ACNs from blueberries, instead of whole blueberry, showed decreased serum TG and TC levels that were comparable with those of the lean control group (10% of kcal from fat) (Prior et al., 2009). This result indicated that sugars or other components in the whole fruits were possibly masking the benefits of ACNs and other components of blueberries. It should be noted that blueberry polyphenol was effective on serum TC level in C57BL/6 mice, which was 13.2% lower than in the control group (Roopchand, Kuhn, Rojo, Lila & Raskin, 2013). A human trial which investigated the effect of ACNs (from bilberry) supplementation on lipid profiles in dyslipidemic patients found that 160 mg of ACNs supplementation for 12 weeks increased cellular cholesterol efflux and HDL-C concentrations, as well as reduced the mass and activity of plasma cholesteryl ester transfer protein (CETP) and LDL-C concentrations, without affecting TC levels (Qin et al., 2009). Zhu et al. (2013) also found similar results, reporting that volunteers with hypercholesterolemia had greater reductions in LDL-C levels and greater increases in HDL-C after consuming 320 mg/day of purified ACNs for 24 weeks compared with controls. In an *in vitro* study, C3G reduced CETP activity in human HepG2 cells in a dose-dependent manner, suggesting that supplementation of ACNs may improve lipoproteins

by increasing HDL-C concentrations and decreasing serum LDL-C partially due to the inhibition of CETP target (Zhu et al., 2013). Other possible mechanisms by which blueberry ameliorates lipid profile are possibly related to the intact assimilation of blueberry bioactivity such as ACNs, which exhibited the antioxidant properties in serum and other tissues (Mazza, Kay, Cottrell & Holub, 2002; Mcghie, Ainge, Barnett, Cooney & Jensen, 2003). Studies have revealed that the high concentration of ACNs in wild blueberry is a major contributor to the antioxidant properties *in vitro*, instead of other antioxidant minerals, vitamins, or fibres (Prior et al., 1998). Moreover, the antioxidant properties of ACNs have been confirmed via other systems of oxidation such as that for the prevention of LDL oxidation *in vitro* (Laplaud, Lelubre & Chapman, 1997). It has been validated that ACNs can be absorbed intact in glycosylated and possibly acylated forms in male volunteers after the consumption of blueberries (Wu, Cao & Prior, 2002). Moreover, the presence of ACNs in the serum may be involved with a diet-induced increase in *ex vivo* serum antioxidant status (Mazza, Kay, Cottrell & Holub, 2002).

Taking all these data together, it can be concluded that blueberries and blueberry extracts may potentially improve dyslipidaemia by regulating TG, cholesterol and fatty acid metabolism through several signalling pathways. However, further studies are necessary to better clarify the mechanisms involved in these actions of bioactive components in blueberries.

6. Effect on inflammation and adipocytokine profile

Obesity is associated with systemic chronic inflammation, and this low-grade inflammation may play an important role in obesity associated insulin resistance, T2D, and other complications (Calder et al., 2011; Chen, Chen, Wang & Liang, 2015; Gabay, 2006; Giugliano, Ceriello & Esposito, 2006). A diet enriched in vegetables and fruits is inversely related to inflammatory stress, compared with meals that are energy dense which induce an acute

566 inflammatory status in both overweight and healthy adults (Calder et al., 2011; Manning et al.,
567 2008; Root et al., 2012; Vendrame, Daugherty, Kristo, Riso & Klimis-Zacas, 2013).
568 Blueberries contain various anthocyanins, phenolic acid and other bioactive components
569 recognized for their ability to provide and activate cellular antioxidant protection, scavenge
570 free radicals, inhibit inflammatory gene expression, and consequently protect against oxidant-
571 induced and inflammatory cell damage and cytotoxicity (Johnson, De Mejia, Fan, Lila &
572 Yousef, 2013; Kang, Thakali, Jensen & Wu, 2015; Nile & Park, 2014).

573 Dietary supplementation with 8% blueberries to Obese Zucker rats for 8 weeks has been
574 reported to decrease plasma concentrations of IL-6, TNF-α and CRP compared with the control
575 group (Vendrame, Daugherty, Kristo, Riso & Klimis-Zacas, 2013). Furthermore, in this study,
576 expression of TNF-α, IL-6 and NF-κB was down-regulated in both the AAT and the liver,
577 whereas CRP expression was down-regulated only in the liver (Vendrame, Daugherty, Kristo,
578 Riso & Klimis-Zacas, 2013). Similarly, supplementation with 4% whole blueberry powder
579 deceased IL-10 and TNF-α mRNA expression in adipose tissue inflammation of HFD fed
580 C57BL/6J mice, but no significant changes in other inflammatory biomarkers, such as nitric
581 oxide synthase (iNOS), IL-6 and MCP-1 (Defuria et al., 2009).

582 Bilberry consumption has also been demonstrated to attenuate pro-inflammatory responses
583 induced by HFD in C57BL/6J mice fed with a 5% or 10% (w/w) of whole bilberries for three
584 months, via reduction in MCP-1, IL-2, IL-1β, IL-6 and TNF-α (Mykkanen et al., 2014). In
585 particular, the levels of IL-15 and interferon gamma (IFN-γ) were increased in non-
586 supplemented HFD fed animals and reduced to non-detectable levels in animals that were
587 supplemented with bilberries (Mykkanen et al., 2014). In contrast, to the bilberry studies,
588 dietary supplementation with a blueberry pomace by-product failed to alter mRNA expression
589 of CD68 (an anti-inflammatory marker) and CRP in adipose tissue of Syrian Golden hamsters
590 compared to controls (Kim, Bartley, Rimando & Yokoyama, 2010). One explanation for the

591 inconsistency in these findings may be associated with different components among
592 blueberries, its fractions and its peel.

593 During the last few years a number of clinical trials have been carried out to assess the potential
594 anti-inflammatory function of blueberry supplementation in subjects who are obese and have
595 other disorders of metabolic syndrome (Table 2). Karlsen et al. (2010) reported that intake of
596 bilberry juice could regulate inflammatory mediators such as, IL-6, IL-15 and CRP in men and
597 women as well as improve the levels of plasma polyphenols. Furthermore, it was found that
598 the decrease of these inflammatory mediators were associated with NF-κB activation (Karlsen
599 et al., 2010). In a preclinical study, dietary supplementation with 400 g of bilberry for 8 weeks
600 decreased serum IL-6, IL-12, high sensitivity-CRP (hsCRP) and LPS concentrations in obese
601 individuals with low-grade inflammation (Kolehmainen et al., 2012). However, in another
602 study where 110 female volunteers consumed 100 g of fresh blueberry fruits for 33–35 days,
603 there were no differences observed in TNF-α between the baseline and treatment group at the
604 end of the intervention (Lehtonen et al., 2011). Similarly no alterations in plasma IL-6 and CRP
605 concentrations were observed in obese participants following consumption of freeze-dried
606 blueberries (50 g) for 8 weeks (Basu et al., 2010). Another study demonstrated that
607 consumption of blueberries (22.5 g) for 6 weeks did not affect the inflammatory biomarker
608 profile including TNF-α, hsCRP and MCP-1 in obese, nondiabetic, and insulin-resistant
609 volunteers (Stull, Cash, Johnson, Champagne & Cefalu, 2010). Perhaps the contradictions in
610 the observed impacts on inflammatory markers in these clinical studies may at least in part be
611 explained by the use of different species of berries [bilberry (Karlsen et al., 2010; Kolehmainen
612 et al., 2012) *vs.* blueberry (Basu et al., 2010; Lehtonen et al., 2011; Stull, Cash, Johnson,
613 Champagne & Cefalu, 2010)], the amount of berries consumed; type of serum samples used
614 for measuring inflammatory biomarkers [fasting serum (Karlsen et al., 2010; Kolehmainen et
615 al., 2012) *vs.* non-fasting serum (Stull, Cash, Johnson, Champagne & Cefalu, 2010)] or the

616 status of these individuals [overweight subjects with 25.6 ± 6.1 of BMI) (Karlsen et al., 2010)
617 *vs.* obese subjects with 36.8 ± 0.9 of BMI (Stull, Cash, Johnson, Champagne & Cefalu, 2010)
618 and 38.1 ± 1.5 of BMI (Basu et al., 2010)].

619 It has been reported that a purified ACN mixture exhibited higher anti-inflammatory activity
620 compared to single ACN or whole berries *in vitro* and *in vivo* (Zhu et al., 2013). In that study,
621 purified anthocyanin mixture (containing 17 ACN compounds from blueberries) produced a
622 stronger inhibitory effect on IL-6, IL-1β-induced CRP production in HepG2 cells and LPS-
623 induced vascular cell adhesion molecule-1 (VCAM-1) secretion in endothelial cells,
624 respectively, compared with the effects of single anthocyanin, D3G and C3G, which support
625 the observations in human subjects (Zhu et al., 2013). These studies suggest that the various
626 ACNs in blueberry may act synergistically to inhibit the inflammatory response. Hence,
627 consuming foods rich in different ACNs is likely to be more beneficial than consuming a single
628 ACN supplement.

629 Blueberry and its extracts have also demonstrated potential benefits on the regulation of
630 adipocytokines in animal and human studies. The concentration of adiponectin was higher in
631 C57BL/6J obese mice fed HFD and genetically diabetic db/db mice with C3G supplementation,
632 compared with mice only fed a HFD diet (Guo et al., 2012; Liu, Li, Zhang, Sun & Xia, 2014).
633 Similarly, wild blueberry consumption in Obese Zucker rats resulted in a significant increase
634 in circulating adiponectin level compared to the control group (+ 21.8%) (Vendrame,
635 Daugherty, Kristo, Riso & Klimis-Zacas, 2013). Adiponectin concentration, however has been
636 demonstrated not to differ from the control groups following supplementation of blueberry or
637 ACNs in several animal studies (Mykkanen et al., 2014; Roopchand, Kuhn, Rojo, Lila &
638 Raskin, 2013; Takikawa, Inoue, Horio & Tsuda, 2010; Vuong et al., 2009; Wu et al., 2013)
639 and human trials (Basu et al., 2010; Kolehmainen et al., 2012; Qin et al., 2009). Lehtonen et
640 al. (2011) demonstrated, however a decrease in adiponectin level after bilberry

supplementation in overweight and obese women for 33-35 days. Therefore the exact effect of consumption of blueberries on adiponectin level is unclear.

Leptin secretion has been demonstrated to be inhibited by diets enriched with blueberry, both in genetic models of obesity and dietary-induced obese animal models (Prior et al., 2010; Prior et al., 2009; Wu et al., 2013). However, no significant effect was observed on leptin levels in other animal studies (Mykkanen et al., 2014; Vuong et al., 2009), or indeed in a human trial (Kolehmainen et al., 2012).

Resistin is a hormone secreted from adipose tissue and it has been implicated in the modulation of insulin action, energy, glucose and lipid homeostasis and also has been linked to the onset of insulin resistance and obesity-associated diabetes (Abate et al., 2014). Mykkane et al. (2014) investigated the effect of blueberry supplementation (10% wild blueberry) in mice fed a HFD and indicated that serum resistin level was significantly reduced in the mice that were supplemented with blueberry for 12-14 weeks.

There are several potential mechanisms involved in the anti-inflammatory properties of blueberry. Firstly, antioxidants in blueberry, such as polyphenols and ACNs which exhibit the anti-inflammatory effect may be dependent on a reduction of pro-inflammatory cytokines and increase of anti-inflammatory mediators such as adiponectin (Guo et al., 2012). Secondly, oxidative stress, which leads to inflammation is reduced due to the strong antioxidant activity of blueberries and its extracts, which is subsequently involved in an increase of glutathione peroxidase 3 (a sensitive index of oxidative stress) gene expression (Lee et al., 2008). Thirdly, blueberry or its ACNs may be able to alter mitogen-activated protein kinase signalling, which modulate cell fate and inflammatory gene expression in various tissues and macrophages (Suganami et al., 2007). Finally the attenuation of NF-κB activation could be related to the antioxidant capacity of blueberries or its extracts, thereby providing a potential mechanism

with the observed anti-inflammatory effect of blueberry intake (Vendrame, Daugherty, Kristo, Riso & Klimis-Zacas, 2013).

7. Conclusion

This review focused on blueberries and their bioactive components that influence obesity and its related comorbidities, although it is necessary to indicate that there are still a large number of phytonutrients in blueberries under exploration at present, especially ACNs. A major question to be addressed is whether a single purified component or constituent in blueberries such as C3G or ACNs, or multiple constituents in this fruit produced synergic effects on human health. In addition, there is a need for determining the bioactive constituents of blueberry and their metabolites, which may accumulate in the target tissues and exert biological effects. Future studies could also focus on the interactions of nutrients and genes so we have a better understanding of the beneficial effects of blueberry at the molecular level, thus be able to develop effective intervention strategies and achieve better outcomes. According to the literature, the evidence suggests that several species of blueberries in the genus *Vaccinium* and their isolated compounds are potential contributors to the regulation of glucose, lipid metabolism and improvement of inflammation. A deep understanding of the potential roles of blueberries in controlling body weight, regulating blood glucose, and attenuating dyslipidaemia and related chronic inflammation will guide further rigorous investigations on the underlying mechanisms of their beneficial effects on health.

Conflict of Interest The authors declare that there is no conflict of interest.

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