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Blueberry anthocyanins in health promotion: A metabolic overview



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ARTICLE INFO

Article history:

Received 8 April 2013

Received in revised form

9 August 2013

Accepted 22 August 2013

Available online 21 September 2013

Keywords:

Anthocyanin

Bioavailability

Blueberry

Metabolic syndrome

Obesity

ABSTRACT

Diet has gained scientific community attention due to the crucial role in health maintenance, but also in disease treatment, and essential in disease prevention. Several food and food components, particularly phenolic rich foods, have been investigated as they present themselves as putative functional foods. In the past decades, obesity has reached epidemic proportions and consequently, metabolic syndrome (a set of disorders as impaired glucose tolerance, insulin resistance, abdominal obesity, dyslipidemia and high blood pressure, which increase the risk of cardiovascular disease and diabetes) incidence is increasing worldwide at an alarming rate and this phenolic rich foods, specially berries have been investigated to their potential beneficial effect in this disorders.

In the present work the chemistry of blueberries (BB) (fruits of some *Vaccinium* species) was summarised as well as the knowledge about bioavailability and biokinetic of anthocyanins from blueberries with particular emphasis on its implications in metabolic disorders.

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Abbreviations: acgal, acetylgalactose; acglu, acetylglucose; ACNs, anthocyanins; ara, arabinose; AT, adipose tissue; BB, blueberry/bilberry(ies); BJ, biotransformation of blueberry juice; Cy, cyanidin; C3G, cyanidin-3-glucoside; Dp, delphinidin; D3G, delphinidin-3-glucoside; gal, galactose; glu, glucose; HF, high-fat; IR, insulin resistance; LF, low-fat; Mv, malvidin; M3G, malvidin-3-glucose; NO, nitric oxide; Pg, pelargonidin; P3G, pelargonidin-3-glucoside; Pn, peonidin; PPAR γ , peroxisome proliferator-activated receptor gamma; Pt, petunidin; RNS, reactive nitrogen species; ROS, reactive oxygen species; SUG, sugar moiety

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<http://dx.doi.org/10.1016/j.jff.2013.08.015>

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1. Introduction

Diet has a crucial role in health, and consequently in disease treatment, but essential in disease prevention. Thus, knowledge concerning an identification of dietary components involved in disease prevention is a priority in actual science.

In this review we discuss the chemistry of blueberries (BB) (fruits of some *Vaccinium* species) and summarise the knowledge about bioavailability/biokinetic of anthocyanins from blueberries and its role on metabolic disorders.

The consumption of a diet high in fat but rich in polyphenol compounds may minimize the complications of metabolic disorders (DeFuria, Bennett, Strissel, Perfield, 2009; Lila et al., 2011; Meydani & Hasan, 2010; Prior et al., 2008, 2009, 2010; Tsuda, 2008; Tsuda, Horio, Uchida, Aoki, & Osawa, 2003; Valcheva-Kuzmanova et al., 2007). Since the polyphenolic compounds, including ACNs, have strong antioxidant properties, they can confer protection to pancreatic β -cells from glucose-induced oxidative stress (Al-Awwadi et al., 2005; Johansen, Harris, Rychly, & Ergul, 2005; Maritim, Sanders, & Watkins, 2003; Martineau et al., 2006). An excessive adipose tissue (AT) accumulation has metabolic consequences, as adipocyte dysfunction, strongly associated with the development of obesity and diabetes, involving insulin resistance (IR). However, a few studies suggest that AT is an important site of *Vaccinium* species actions to ameliorate obesity complications (DeFuria et al., 2009; Suzuki et al., 2011). ACNs were shown to regulate obesity and insulin sensitivity associated with adipocytokine secretion and PPAR γ activation in adipocytes (Tsuda, Horio, Uchida, Aoki, & Osawa, 2003; Tsuda et al., 2004).

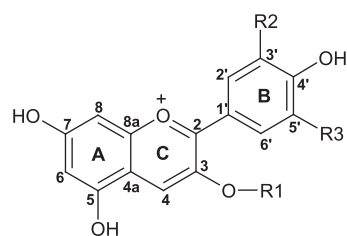
The food industry have demonstrated interest in BB, not only because of its known health-promoting properties, but also due to anthocyanin (ACN) unusual colors in acidic conditions (Faria et al., 2005; Faria et al., 2008) as well as some natural ACN derivative pigments (Bakker & Timberlake, 1997; Fulcrand, Benabdeljalil, Rigaud, Cheynier, & Moutounet, 1998; Mateus, Silva, Rivas-Gonzalo, Santos-Buelga, & de Freitas, 2003; Mateus, Silva, Vercauteren, & de Freitas, 2001) that present bluish and orange hues.

2. Blueberry chemistry

The content and profile of phenolic compounds in BB (namely, *Vaccinium angustifolium* (lowbush BB) and *Vaccinium corymbosum* (highbush BB)) has been studied by the scientific community. The phenolic compounds have been reported for their biological activities, such as: (i) anti-oxidant (Borges, Degeneve, Mullen, & Crozier, 2010; Faria et al., 2005; Kahkonen & Heinonen, 2003; Kalt et al., 2001; Mazza, Kay, Cottrell, & Holub, 2002; Prior & Wu, 2006; Tsuda, Horio, Kitoh, & Osawa, 1999; Tsuda, Ohshima, Kawakishi, & Osawa, 1996;

Tsuda et al., 1994; Tsuda et al., 2004; Wang et al., 1999); (ii) anti-inflammatory (DeFuria et al., 2009; Karlsen et al., 2010; Schreckinger, Wang, Yousef, Lila, & de Mejia, 2010; Wang et al., 1999); (iii) anti-proliferative (Faria et al., 2010; Li et al., 2009; Pacheco-Palencia, Mertens-Talcott, & Talcott, 2010); (iv) anti-obesity properties (Meydani & Hasan, 2010; Tsuda, Ueno, Kojo, Yoshikawa, & Osawa, 2005; Vuong et al., 2009), and (v) neuroprotective actions (Andres-Lacueva et al., 2005; Barros et al., 2006; Joseph et al., 2003).

Anthocyanins (ACNs) are water-soluble glycosides responsible for the coloration of many fruits, flowers and vegetables, among other, berries (BB, blackberry, chokeberry, elderberry, grape, raspberry, etc.), cherry, strawberry, purple corn, sweet potato and red onions (Borges et al., 2010; Fossen & Andersen, 2003; Kahkonen, Hopia, & Heinonen, 2001; Kalt et al., 2008). The six anthocyanidins commonly found in the edible parts of plants are cyanidin (50%), pelargonidin (12%), peonidin (12%), delphinidin (12%), petunidin (7%), and malvidin (7%) (Kong, Chia, Goh, Chia, & Brouillard, 2003). Their classification is made according to the number and position of hydroxyl and methoxyl groups on the flavan nucleus, i.e., depend on the chemical groups in R1 and R3 (Fig. 1). The most common ACNs found in BB are monoarabinosides, monoglucosides and monogalactosides of cyanidin (Cy), petunidin (Pt), peonidin (Pn), delphinidin (Dp) and malvidin (Mv) though several other phenolic compounds, and their glycosides, have been described (e.g. catechin, epicatechin, myricetin, kaempferol, quercetin, myricetrin and caffeic, *p*-coumaric and ferrulic acids) (Kader, Rovel, Girardin, & Metche, 1996; Riihinen, Jaakola, Kärenlampi, & Hohtola, 2008; Taruscio, Barney, & Exon, 2004). A recent work (Yousef et al., 2013) characterized anthocyanins content of different commercial blueberry cultivars and found that malvidin-3-O-galactoside, delphinidin-3-O-galactoside, malvidin-3-O-arabinoside, cyanidin-3-O-arabinoside and delphinidin-3-O-arabinoside constituted about 70% of total anthocyanins. Delphinidin and malvidin derivatives are described as the majority of anthocyanins found in blueberries (Rodriguez-Mateos, Cifuentes-Gomez, Tabatabaee, Lercas, & Spencer, 2012; Yousef et al., 2013). Acylated ACNs are also found in blueberries but they account as a small portion of total ACNs. Recently, (Vrhovsek, Masuero, Palmieri, & Mattivi, 2012) has further characterized the flavonol glycosides present in several BB cultivars having found not only a wider array of compounds than those previously described, but also compounds that appeared to be specific of a few of the cultivars (e.g. isorhamnetin-3-rhamnoside was only found in 4 of 15 genotypes tested) or genotypes contemplated in their work. A particular example of a genotype specific compound is quercetin-3-rutinoside, compound only found in one of the two genotypes tested for the Elliot cultivar. The differences found between the different papers dedicated to the characterization of the fruit may be a direct consequence of the dif-



Anthocyanin	R1	R2	R3
Cy 3-sug	glu, ara, gal, acglu	OH	H
Dp 3-sug	glu, ara, gal, acglu,	OH	OH
Pn 3-sug	gal	OCH ₃	H
Pt 3-sug	glu, ara, gal, acglu	OCH ₃	OH
Mv 3-sug	glu, ara, gal, acgal, acglu	OCH ₃	OCH ₃

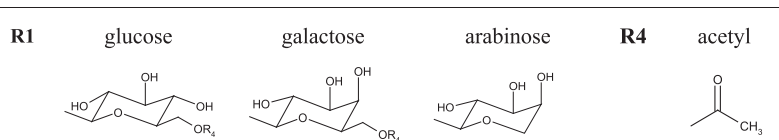


Fig. 1 – Representation of the main chemical structure of anthocyanins found in blueberries (Yousef et al., 2013). acglu – acetyl-glucose; acgal – acetyl-galactose; ara – arabinose; gal – galactose; glu – glucose; sug – sugar moiety.

ferent cultivars, stages of ripeness and environmental factors (Kader et al., 1996; Kalt et al., 2001; Perkins-Veazie, Collins, & Howard, 2008). ACNs content of BB are conditioned by environment variables (Connor, Luby, Hancock, Berkheimer, & Hanson, 2002) however, Kalt et al. showed that, regardless of the method, lowbush BB were consistently higher in ACNs, total phenolics, and antioxidant capacity when compared with highbush BB (Kalt et al., 2001). Nevertheless, this issue is controversial since recent studies showed similar values between lowbush and highbush cultivars (Rodriguez-Mateos et al., 2012).

3. Biokinetic of anthocyanins

To evaluate the bioavailability and potential health effects of phenolic compounds, in particular ACNs, it is necessary to evaluate its absorption, metabolism, distribution in the tissues and excretion as well as their biochemical actions and interaction of these compounds with other nutrients. However, to date the few *in vivo* studies, do not allow us to reach broad conclusions.

3.1. Absorption and bioavailability

Several authors argue that ACNs are absorbed but that its rate of absorption is influenced by their chemical structure (Mazza et al., 2002; Miyazawa, Nakagawa, Kudo, Muraishi, & Someya, 1999; Talavera et al., 2004; Wu, Cao, & Prior, 2002; Yi, Akoh, Fischer, & Krewer, 2006), by the dose ingested/administered (Kay, Mazza, & Holub, 2005), and by the matrix of the food source (Mazza et al., 2002; Miyazawa et al., 1999; Prior et al., 2008; Yang, Kao, Song, & Chun, 2010), one of the key factors affecting ACNs' stability (Aura et al., 2005).

The results from several studies on the absorption rate of total or individual ACNs either in human subjects or animals was estimated to be extremely low (Andlauer, Stumpf, Frank, & Furst, 2003; Cao, Muccitelli, Sanchez-Moreno, & Prior, 2001; Del Bo et al., 2010; Ichiyanagi, Shida, Rahman, Hatano, &

Konishi, 2006; Kalt et al., 2008; Mazza et al., 2002; Miyazawa et al., 1999; Passamonti, Vrhovsek, Vanzo, & Mattivi, 2003; Sakakibara et al., 2009; Suda et al., 2002; Talavera et al., 2004; Talavera et al., 2005; Yi et al., 2006).

To evaluate the absorption of ACNs, it is important to bear in mind a preceding step, i.e., the quantity of the food source or extract provided because the absorption of certain nutrients, xenobiotics and drugs may be influenced by the concomitant ingestion of other substances. Recently, it has been reported that sources rich in phenolic compounds modulate the intestinal uptake of organic cations and considering that most vitamins, nutrients and xenobiotics pass the intestinal barrier as organic cations (Faria, Mateus, De Freitas, & Calhau, 2006; Faria et al., 2008; Monteiro, Calhau, Martel, Guedes de Pinto, & Azevedo, 2005; Monteiro et al., 2005; Talavera et al., 2004), this became a good example of food-food interactions. On the other hand, ACNs' bioavailability differs between the various food sources, depending on the type of glycosides in the molecule. Numerous studies demonstrated that ACNs are absorbed mostly in their intact glycoside forms (Cao et al., 2001; Matsumoto et al., 2001; Mazza et al., 2002; Miyazawa et al., 1999; Talavera et al., 2004) in both rats and human subjects, nevertheless other studies show that they are held in their aglycone forms (Ichiyanagi et al., 2006; Talavera et al., 2005). A review study from Manach et al. refers that the aglycones can be absorbed more rapidly than the glycosidic forms (Manach, Williamson, Morand, Scalbert, & Remesy, 2005).

Table 1 summarized the more recent studies of ACNs' absorption in which the source or extract ingested/administered was derived from the *Vaccinium* species.

It is very interest to verify that the transport/absorption efficiency of ACNs varied with the type of anthocyanin. Besides the chemical structure, other influential factors were the dose ingested/administered and the time of exposure with clear differences depending on the organ/tissue studied. The transport/absorption efficiency can be explained with the presence of more free hydroxyl groups and less OCH₃ groups

Table 1 – Absorption, distribution in the tissues and excretion of phenolic compounds from *Vaccinium* species.

Diet/Extract	Subject	Tissue	Treatment duration	ACNs detection	Conc/percentage absorption	Time	References
Freeze-dried BB powder containing 1.20 g of total ACNs (42% of the total phenolics lowbush BB; <i>Vaccinium angustifolium</i>) + HF meal	Human	Blood serum		+	5.43–16.9 ng/ml	4 h	Mazza et al. (2002)
Highly purified bilberry (<i>V. myrtillus</i> L.) extract (final ACN concentration of 75.8 µmol/L)	Male Wistar rats	Intestinal perfusion	45 min	+	8.1–19.1%	–	Talavera et al. (2004)
Bilberry (<i>V. myrtillus</i> L.) extract administration with 400 mg/kg 153.2 mg/kg as ACNs)	Male Wistar SP rats	Blood plasma		+	1.2 µM	15 min	Ichiyanagi et al. (2006)
Highly purified ACN extracts (~90% purity) obtained from 3 cultivars of BB (Briteblue, Tifblue, Powderblue)	Human cell culture (Caco-2)	Intestinal cell monolayers		+	<1%	120 min	Yi et al. (2006)
Diets supplement with 0, 1, 2, or 4% w/w BB (<i>Vaccinium corymbosum</i> L. 'Jersey') (BB)	Pigs	Liver	4 weeks	+	1.30 pmol/g of FW		Kalt et al. (2008)
		Eyes	4 weeks	+	1.58 pmol/g of FW		
		Cortex	4 weeks	+	0.878 pmol/g of FW		
		Cerebellum	4 weeks	+	0.664 pmol/g of FW		
0,5% Bilberry extract	Male C57BL/6 mice	Plasma	2 weeks	+	153 nM		Sakakibara et al. (2009)
		Liver	2 weeks	+	173 pmol/g FW		
		Kidney	2 weeks	+	114 pmol/g FW		
		Testes	2 weeks	+	148.5 pmol/g FW		
		Lung	2 weeks	+	116 pmol/g FW		
		Spleen	2 weeks	–			
		Thymus	2 weeks	–			
		Heart	2 weeks	–			
		Muscle	2 weeks	–			
		Brain	2 weeks	–			
		White fat	2 weeks	–			
		Eye balls	2 weeks	–			
67.3 µmol ACNs of bilberry extract/100 mg extracts/kg body weight	Male C57BL/6 mice	Plasma		+	227 nM	60 min	
BB-enriched (<i>V. angustifolium</i>) diet 8% wild lowbush blueberry diet	Male Sprague-Dawley rats	Urine	4 weeks	+	886.8 ng	24 h	Del Bo et al. (2010)
		Urine	8 weeks	+	1989.6 ng	24 h	
		Feces	4 weeks	+	76.7 µg	24 h	
		Feces	8 weeks	+	55.4 µg	24 h	

which decrease the bioavailability of ACNs (Weiguang, Akoh, Fischer, & Krewer, 2006). Talavéra et al. (Talavera et al., 2004) demonstrated that aglycone structure had an impact on ACN intestinal absorption, caused by the presence of methoxylated groups that reduced intestinal absorption. Furthermore, a previous study from the same author (Talavera et al., 2003) showed that ACNs glycosides (more specifically, Dp) were absorbed in high proportions from the stomach because constitutes a favourable environment for their stability (Talavera et al., 2003). A recent *in vitro* study, that makes use of a stomach cell line, confirmed that ACN are absorbed and that M3G was the anthocyanin tested with higher transport efficiency (Fernandes, de Freitas, Reis, & Mateus, 2012). During the passage of ACNs through the gastrointestinal tract, ACNs are exposed to different pH environments and therefore might exist as different forms. This translates into a rate limiting step for understanding the ACN forms present in various regions of the body. Passamonti et al. suggests that bilitranslocase (an organic anion carrier) expressed in the gastric epithelium of rats could be involved in the absorption of ACNs in the stomach (Passamonti, Vrhovsek, & Mattivi, 2002). On the other hand the transport pathway of sugar moiety may be involved in ACNs' absorption. In human studies, Hollman et al. concluded that the sugar moiety is a major determinant of the absorption of dietary flavonoid glycosides in humans, i.e., absorption of aglycones is improved by conjugation with glucose, possibly by absorption through the sodium-dependent glucose transport system (SLGT1) (Hollman & Katan, 1999). Additionally, an *in vitro* study suggests the involvement of GLUT2 (a facilitative transporter of glucose) in ACNs intestinal uptake (Faria et al., 2009).

The presence of the cation group seems to influence their intestinal metabolism (Talavera et al., 2004). In addition, the electron deficiency of ACNs structure makes anthocyanidins highly reactive, and its stability is pH and temperature dependent. ACNs and other polyphenols also interact with nutrients in the gut lumen (Santos-Buelga & Scalbert, 2000; Scalbert, Morand, Manach, & Remesy, 2002) and form stable complexes with a non-heme dietary iron, which limit its absorption in the gut (Hurrell, Reddy, & Cook, 1999). Finally, the time of exposure to ACNs through diet or the incubation time in cell studies, seems to be a crucial factor for its bioavailability (Del Bo et al., 2010; Ichiyanagi et al., 2006; Kalt et al., 2008; Matsumoto et al., 2001; Mazza et al., 2002; Sakakibara et al., 2009; Talavera et al., 2004; Yi et al., 2006). Nevertheless, the bioavailability of ACNs is low as compared with other BB polyphenols (Manach et al., 2005; McGhie & Walton, 2007) but these low levels of ACNs can be bioavailable and well retained in tissues (Kalt et al., 2008).

3.2. Biotransformation

The glycosidic forms of ACNs may initiate their metabolic alteration before absorption due to bacterial digestion of the glycosidic linkage of ACNs by the gastrointestinal system (Tamura, Gold, Ferro-Luzzi, & Ames, 1980). Metabolites of ACNs in biological fluids occur mainly as methylated, glucuronated, and sulfo-conjugated forms (Cao et al., 2001; Felgines et al., 2003; Kay et al., 2005; Milbury, Cao, Prior, & Blumberg, 2002; Miyazawa et al., 1999; Mulleder, Murkovic, & Pfannhaus-

er, 2002; Nielsen, Dragsted, Ravin-Haren, Freese, & Rasmussen, 2003; Wu et al., 2002). Some studies have reported glucuronidated and methylated ACNs in the urine and blood of humans and animal models (Felgines et al., 2003; Kay, Mazza, Holub, & Wang, 2004; Kay et al., 2005; Tsuda et al., 1999). Nevertheless, the metabolites resulting from ingestion of diets containing ACNs are not completely characterized and there is the possibility of unidentified ACN metabolites contribute to the biologic reported effects of ACNs. However, the results of both urinary and serum analyses (after an oral dose equivalent to 120–230 g of whole berries (fresh weight)) indicate that parent compounds and their metabolites had similar kinetic profiles (Kay et al., 2005). The proportion of ACNs excreted in the urine is reported to be <1% of the total ingested dose (Cao et al., 2001; Kay et al., 2005; Milbury et al., 2002; Miyazawa et al., 1999; Mulleder et al., 2002; Nielsen et al., 2003; Wu et al., 2002). Relatively urinary excretion is commonly used to estimate the minimal absorption rate but, this parameter for quantitative assessment can lead to an underestimated absorption when phenolic compounds appear in blood circulation or are highly excreted in bile and urine (Felgines et al., 2003; Kay et al., 2004; Kay et al., 2005; Liang et al., 2012; Talavera et al., 2004; Wu et al., 2002).

Further studies involving ACNs' metabolites are necessary for the complete characterization of ACNs' biokinetics.

4. Metabolic syndrome

Metabolic syndrome is a set of disorders as impaired glucose tolerance, insulin resistance (IR), abdominal obesity, dyslipidemia and high blood pressure, which increase the risk of cardiovascular disease and diabetes. Oxidative stress is defined in general as excess formation and/or insufficient removal of highly reactive molecules such as reactive oxygen species (ROS) and reactive nitrogen species (RNS) (Maritim et al., 2003). Ameliorating oxidative stress through treatment with antioxidants might be an effective strategy for reducing some metabolic disorders. Over the past two decades, chemicals derived from plants and known as “phytochemicals” have gained the interest of public and scientific communities for their role in maintaining health and preventing disease (Meydani & Hasan, 2010). Notably, BB are fruits from the *Vaccinium* plant that have powerful antioxidants (Faria et al., 2005; Kahkonen & Heinonen, 2003; Kong et al., 2003; Mazza et al., 2002; Prior & Wu, 2006; Wang et al., 1999) that can improve the condition of many of the diseases caused by reactive species (Faria et al., 2010; Li et al., 2009; Pacheco-Palencia et al., 2010; Riso et al., 2012). For Borges et al. (Borges et al., 2010) the antioxidant capacity of BB is mainly attributed to ACNs (responsible for 84% of the antioxidant capacity) and not to vitamin C which is present in lower level.

The function of insulin is to maintain normal blood glucose levels either by suppression of glucose output from liver or by the stimulation of glucose uptake and its metabolism (Ross, Gulve, & Wang, 2004). However, an insufficient release of insulin or loss of insulin action at target tissues leads an elevated glucose levels in the blood, characteristic of diabetes (Jovanovic & Gondos, 1999). So when the insulin inadequately stimulates glucose transport in skeletal muscle and fat and

Table 2 – Effects of BB derivatives consumption on metabolic parameters in a mice model (C57BL/6J) subject to different diets. All the effects shown are in comparison with the respective diet control group. ↓ significantly decreased in comparison to diet control; ↑ significantly increased in comparison to diet control; - no significant changes; nd – not determined.

Source		10 % Freeze-dried whole BB powder Prior, et al., 2009; Prior, et al., 2008		Purified BB ACNs Prior, et al., 2009; Prior, et al., 2008	4% Freeze-dried whole BB powder DeFuria et al. (2009)	BB juice Prior, et al. (2010)		Purified BB ACNs (0.2mg/ml) Prior, et al. (2010)		11.5% Whole BB powder on dry weight basis Prior, et al. (2010)
Treatment duration		91 days		70 days	8 weeks	79 days		79 days		79 days
Energy density (from fat)		10%	45%	60%	60%	10%	45%	10%	45%	45%
Biomonitoring	Food intake	=	=	=	nd	=	=	=	=	=
	Energy intake	=	=	=	=	=	=	=	=	=
	Body weight	=	↑	=	=	=	=	=	=	=
	Tissue weight	=	↓	=	nd	nd	nd	nd	nd	nd
	Adipose tissue weight	=	↑	↓	=	=	=	=	↓	=
Biochemical and metabolic parameters	Cholesterol (serum)	=	=	=	nd	=	=	=	=	nd
	Triglycerides (serum)	↑	↑	↓	nd	=	=	=	=	=
	Triglycerides (hepatic)	=	=	=	nd	=	=	=	=	nd
	Liver total lipids	=	=	=	nd	nd	nd	nd	nd	nd
	Cholesterol (hepatic)	=	=	↓	nd	=	=	=	=	nd
	Leptin	=	=	↓	nd	=	↓	-	↓	↓
	Glucose	=	=	nd	↓	=	=	=	=	=
	Insulin	=	=	=	=	=	=	=	=	=
TNF-α	nd	nd	=	↓	nd	nd	nd	nd	nd	

inadequately suppresses hepatic glucose production a disorder arises (insulin resistance). Several studies report that consumption of fruits and vegetables, namely rich in phenol compounds, associated with a low-fat (LF) diet, may reduce the IR and reduce the incidence of type-2 diabetes (Al-Awwadi et al., 2005; Blakely et al., 2003; Chambers & Camire, 2003; Grace et al., 2009; Jayaprakasam, Vareed, Olson, & Nair, 2005; Johansen et al., 2005; Landraut et al., 2003; Martineau et al., 2006).

Stull et al. hypothesized effectiveness in increasing insulin action *in vivo*, in obese and insulin resistant (nondiabetic) human subjects, with a daily increase consumption of bioactives BB (Stull, Cash, Johnson, Champagne, & Cefalu, 2010). At the end of the study, insulin sensitivity was significantly improved in the BB-supplemented group, independent of any changes in inflammatory biomarkers or adiposity (Stull et al., 2010). Since the results show an increase in insulin sensitivity without changing the body weight, the authors suggest that the BB bioactives had a direct effect on increasing whole-body insulin action (Stull et al., 2010). However the group that developed this study argues that more cellular mechanistic studies are needed to clarify the specific cellular pathway involved in improving sensitivity to insulin when BB were consumed.

The hypoglycemic activity has been described by some studies (Grace et al., 2009; Jayaprakasam et al., 2005). However the mechanism of the hypoglycemic effect was not well understood. Jayaprakasam et al. (2005) developed a first study reporting that the ACNs and anthocyanidins act as insulin secretagogues and that the most potent among them was delphinidin-3-glucoside but, cyanidin-3-glucoside and pelargonidin-3-galactoside were also able to induce insulin secretion. However, the hypoglycemic activity of the anthocyanin-enriched fraction from lowbush BB was demonstrated in an acute mouse model type 2 diabetes when formulated with Labrasol (Grace et al., 2009). This indicates that the number of hydroxyl groups in B ring of ACNs played an important role on their ability to induce insulin secretion.

The protective effect for a diabetic pancreas (Grace et al., 2009; Jayaprakasam et al., 2005; Martineau et al., 2006) could be purely by their anti-oxidative properties. The fruit extract had no effect on glucose uptake (Jayaprakasam et al., 2005; Martineau et al., 2006) or insulin secretion (Martineau et al., 2006) *in vitro* and would not be expected to have any hypoglycemic effects *in vivo* in contrast to the ACN enriched (Grace et al., 2009). It is suggested that compounds may not act in those cell types used for the assays or the *in vivo* mechanism may be different (Grace et al., 2009).

The results of the studies summarized in Table 2 provide a biochemical basis for the use of freeze-dried BB powders, purified ACNs from BB, biotransformed BB juice, or ACNs as a functional food factor, which can have significant implications for prevention of metabolic disorders (as obesity, diabetes and/or dyslipidemia). Among these studies, there are also some controversial results. In the study by Tsuda et al. (2003), supplementation with cyanidin 3-O- β -D-glucoside (C3G) –rich purple corn color significantly suppressed mRNA levels of enzymes included in the fatty acids and triacylglycerides (TAGs) synthesis followed by the reduction of SREBP-1 mRNA level in white AT. Thus, concluding that supplementa-

tion significantly suppresses the development of obesity and ameliorates hyperglycemia induced by High-Fat (HF) diet in mice. Nevertheless, the development of obesity and dyslipidemia was prevented in mice fed with purified ACNs from BB, but diets containing whole berries or purple corn ACNs did not alter the development of obesity (Prior et al., 2009). HF diet along with BB attenuates IR and hyperglycemia in mice coincident with reductions in adipocyte death (DeFuria et al., 2009). Thus, DeFuria et al. hypothesize that the effects of BB on adipocyte physiology and bone marrow-derived inflammatory macrophages in AT gene expression may reflect the ability of BB ACNs to alter mitogen-activated protein kinase and nuclear factor- κ B stress signalling pathways, which regulate cell fate and inflammatory genes (DeFuria et al., 2009).

Prior et al. (2010) concluded that consumption of purified ACNs (0.2 mg/ml) in the drinking water (0.49 mg/mouse/day) improved β -cell function and, the rate of fat deposition was decreased, however, BB juice was not effective in preventing obesity. Interestingly enough, lower serum leptin concentrations were found in ACNs treatments, which retarded the development of obesity (Prior et al., 2010).

Vuong et al. (2009) showed beneficial effects of biotransformed BB juice (BJ) in prevention of diabetes development (Vuong et al., 2009). In this study, the authors observed that BJ decreases hyperglycemia in a diabetic animal model, at least in part by reversing adiponectin levels. In addition, it was observed that BJ also protects young pre-diabetic mice from developing obesity and diabetes (Vuong et al., 2009).

Indeed, more studies are needed to understand the mechanisms and the involved pathways in the beneficial effects of BJ in diabetes prevention as a novel complementary therapy.

In which concerns hyperlipidemia, the literature refers that berry fruits have hypolipidemic effect in animal models (Jankowski, Jankowska, & Niedworok, 2000; Valcheva-Kuzmanova et al., 2007), however, further mechanistic studies are required.

Obesity represents an abnormal accumulation of adipose tissue function leading to a metabolic dysfunction. The increased accumulation of fat tissue (hyperplasia or hypertrophy, local or ectopic) is associated with deleterious perturbations as excess fatty acid secretion, increased production of inflammatory cytokines, and abnormal adipocyte hormone signalling resulting in insulin resistance (Schuster, 2010).

According to Titta et al. (2010), orange juice anti-obesity effect on fat accumulation cannot be justified only by its ACN content. This work suggests that multiple components present in the orange juice might act synergistically to inhibit fat accumulation.

Animal studies using *Vaccinium ashei* cultivars, suggest that these cultivars have satiating activity because there was a reduce in food intake and, consequently a decrease in body weight gain (Molan, De, & Meagher, 2008).

Hyperglycemia and inflammation, involved components in the metabolic syndrome, increase the production of ROS (Demircan et al., 2008). It is believed that some chronic inflammatory diseases are associated with nitric oxide (NO) (Hopps, Noto, Caimi, & Averna, 2010; Schreckinger et al., 2010; J. Wang & Mazza, 2002). Wang and Mazza (2002) and Schreckinger et al. (2010) reported that ACNs had strong inhibitory effects on NO production.

In addition, Tsuda et al. have been developing studies suggesting that the pigments/ACNs may play an important role in the prevention of lipid peroxidation of cell membranes induced by active oxygen radicals in living systems as they become dietary antioxidants after ingestion (Tsuda et al., 1994; Tsuda et al., 1996; Tsuda et al., 1999).

Accordingly to Monteiro, de Castro, Calhau, and Azevedo (2006), the bigger the adipocyte, the more fragile it becomes to rupture when submitted to common physical forces. This indicates that adipocyte size is an important determinant of cell death.

Thus, results observed by Tsuda et al. demonstrated that dietary ACNs significantly normalized hypertrophy of the adipocytes in the epididymal white AT, with the advantage of ameliorating hyperglycemia induced by the HF diet in C57BL/6J mice (Tsuda et al., 2003).

Tsuda et al. demonstrated that ACNs enhance adipocytokines (adiponectin and leptin) secretion (Tsuda et al., 2003; Tsuda et al., 2004). Also, the lipolytic activity was significantly enhanced in adipocytes treated with C3G or Cy due to the elevation of the hormone sensitive lipase (HSL) gene expression (Tsuda et al., 2005).

Although the results of Tsuda et al. suggest that ACNs can regulate obesity and insulin sensitivity involving adiponectin and leptin secretion modulation (Tsuda et al., 2003).

The peroxisome proliferator-activated receptor gamma (PPAR γ) is a nuclear receptor mainly expressed in adipose tissue, responsible for the differentiation of adipocytes. Suzuki et al. determined PPAR γ and adiponectin mRNA levels in 3T3-L1 adipocytes treated with BB extracts and observed a down-regulation the mRNA levels of PPAR γ and similar effects on the expression of adiponectin (Suzuki et al., 2011). Their results showed that BB extracts are capable of inhibiting adipocyte differentiation of 3T3-L1 cells in a dose-dependent manner and to diminish lipid accumulation with concomitant down-regulation of PPAR γ . Additionally, all tested single ACNs (Pg, Cy, Dp, Pn, and Mv) also inhibit lipid accumulation in 3T3-L1 cells but Dp was most effective in down-regulating PPAR γ and sterol regulatory element-binding protein 1c (SREBP1c) mRNA levels (Suzuki et al., 2011). Although the reasons for differences in ACNs inhibition efficiency were not clarified by Suzuki et al., these may be due to the number of hydroxyl groups present on the B-ring of ACNs and their hydrophilic properties (Suzuki et al., 2011). The authors Suzuki et al. (2011) concluded that bilberry extracts inhibit adipocyte differentiation via insulin pathway and these effects are primarily mediated by the action of ACNs.

Despite the positive effect that ACNs and blueberry consumption have demonstrated, more studies are needed to understand the mechanisms of these beneficial effects in preventing obesity and metabolic syndrome disorders. Clinical trials involving BB consumption or BB extract will be recommended.

Acknowledgements

Financial support from Fundação para a Ciência e Tecnologia (FCT) – Fundo Social Europeu, Programa Operacional Potencial Humano da EU (POP), SFRH/BPD/75294/2010, SFRH/BD/

78367/2011 and SFRH/BD/90867/2012 is gratefully acknowledged. Supported by 13736 QREN - SI I&DT: MYRTILLUS – Mirtilos com inovação.

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