

## Potential of rooibos, its major C-glucosyl flavonoids, and Z-2-( $\beta$ -D-glucopyranosyloxy)-3-phenylpropenoic acid in prevention of metabolic syndrome

Christo J. F. Muller , Christiaan J. Malherbe , Nireschni Chellan , Kazumi Yagasaki , Yutaka Miura & Elizabeth Joubert

To cite this article: Christo J. F. Muller , Christiaan J. Malherbe , Nireschni Chellan , Kazumi Yagasaki , Yutaka Miura & Elizabeth Joubert (2016): Potential of rooibos, its major C-glucosyl flavonoids, and Z-2-( $\beta$ -D-glucopyranosyloxy)-3-phenylpropenoic acid in prevention of metabolic syndrome, Critical Reviews in Food Science and Nutrition, DOI: [10.1080/10408398.2016.1157568](https://doi.org/10.1080/10408398.2016.1157568)

To link to this article: <http://dx.doi.org/10.1080/10408398.2016.1157568>



Accepted author version posted online: 15 Jun 2016.  
Published online: 15 Jun 2016.



Submit your article to this journal [↗](#)



Article views: 133



View related articles [↗](#)









View Crossmark data [↗](#)



Citing articles: 5 View citing articles [↗](#)



## Potential of rooibos, its major C-glucosyl flavonoids, and Z-2-( $\beta$ -D-glucopyranosyloxy)-3-phenylpropenoic acid in prevention of metabolic syndrome

Christo J. F. Muller <sup>a</sup>, Christiaan J. Malherbe <sup>b</sup>, Nireshni Chellan <sup>a</sup>, Kazumi Yagasaki <sup>c,d</sup>, Yutaka Miura <sup>c</sup>, and Elizabeth Joubert <sup>b,e</sup>

<sup>a</sup>Biomedical Research and Innovation Platform, South African Medical Research Council, Tygerberg, South Africa; <sup>b</sup>Post-Harvest and Wine Technology Division, Agricultural Research Council (ARC), Infruitec-Nietvoorbij, Stellenbosch, South Africa; <sup>c</sup>Division of Applied Biological Chemistry, Institute of Agriculture, Tokyo University of Agriculture and Technology, Fuchu, Tokyo, Japan; <sup>d</sup>Center for Bioscience Research and Education, Utsunomiya University, Utsunomiya, Tochigi, Japan; <sup>e</sup>Department of Food Science, Stellenbosch University, Private Bag X1, Matieland (Stellenbosch), South Africa

### ABSTRACT

Risk factors of type 2 diabetes mellitus (T2D) and cardiovascular disease (CVD) cluster together and are termed the metabolic syndrome. Key factors driving the metabolic syndrome are inflammation, oxidative stress, insulin resistance (IR), and obesity. IR is defined as the impairment of insulin to achieve its physiological effects, resulting in glucose and lipid metabolic dysfunction in tissues such as muscle, fat, kidney, liver, and pancreatic  $\beta$ -cells. The potential of rooibos extract and its major C-glucosyl flavonoids, in particular aspalathin, a C-glucoside dihydrochalcone, as well as the phenolic precursor, Z-2-( $\beta$ -D-glucopyranosyloxy)-3-phenylpropenoic acid, to prevent the metabolic syndrome, will be highlighted. The mechanisms whereby these phenolic compounds elicit positive effects on inflammation, cellular oxidative stress and transcription factors that regulate the expression of genes involved in glucose and lipid metabolism will be discussed in terms of their potential in ameliorating features of the metabolic syndrome and the development of serious metabolic disease. An overview of the phenolic composition of rooibos and the changes during processing will provide relevant background on this herbal tea, while a discussion of the bioavailability of the major rooibos C-glucosyl flavonoids will give insight into a key aspect of the bioefficacy of rooibos.

### KEYWORDS

Dihydrochalcone; aspalathin; flavones; orientin; isoorientin; diabetes; obesity; bioavailability

### Introduction

In recent years, great emphasis has been placed on phytochemicals, including those present in dietary sources, to play a role in strategies aimed at prevention and protection against risk factors associated with obesity and diabetes (Wolfram et al., 2006; Pinent et al., 2008; Thielecke and Boschmann, 2009). Polyphenols, in particular flavonoids, are increasingly under scientific scrutiny for their potential beneficial effects on obesity and diabetes related conditions (Malviya et al., 2010; Yun, 2010). Pharmacologically, the beneficial effects are related to their ability to act as antioxidants (Fraga et al., 2010) and to modulate cell signaling cascades (Williams et al., 2004) and gene expression (Kuo, 2002; Dong et al., 2010), underscoring their potential preventative and protective roles against cardiovascular complications and diabetes. Specific effects relating to the metabolic syndrome include protection of vulnerable cells such as pancreatic  $\beta$ -cells against increased oxidative stress and inflammation associated with insulin resistance and obesity, and the regulation of genes and proteins involving the glucose and lipid metabolic pathways (Mueller and Jungbauer, 2009; Yun, 2010).

With mounting evidence of the antidiabetic properties of rooibos extracts, the current review focuses on their potential to prevent the metabolic syndrome. A summary of the use of rooibos as herbal tea and food ingredient extract, its phenolic

composition and the changes during processing will provide relevant background on the product. A discussion of the metabolic syndrome from a molecular and biochemical perspective will provide context for the beneficial in vitro and in vivo effects observed for rooibos and its phenolic compounds. The absorption and metabolism of the major C-glucosyl flavonoids of rooibos, and in particular aspalathin, a C-glucosyl dihydrochalcone, will be briefly discussed to give insight into a key aspect of the bioefficacy of rooibos. The bioavailability of O-glycosyl flavonoids, present in rooibos, has been the topic of many papers and will not be covered here.

### Rooibos

#### Use as herbal tea and food ingredient extract

Use of rooibos herbal tea, produced from the endemic South African legume, *Aspalathus linearis* (Burm.f.) Dahlg., predates 1900. Anecdotal evidence collected in the late 1960s, indicating that rooibos alleviates infantile colic, placed the spotlight on rooibos as a healthy beverage (Joubert et al., 2008). Reports of its antioxidant activity, combined with market hype surrounding the role of antioxidants as “anti-ageing” agents, further promoted its image as a healthy drink during the past two decades. Its caffeine-free status, which contributed to the popularity of

rooibos by modern consumers (Joubert and de Beer, 2011), was not always considered to be an advantage. A 1917 report on rooibos by the Imperial Institute of London concluded “It seems doubtful whether this material would be acceptable in the United Kingdom as a substitute for ordinary tea, as it contains no caffeine or other alkaloids and would subsequently not have the stimulating effect of tea.” Today the United Kingdom is one of the top importers of rooibos, and together with Germany, the Netherlands, Japan, and the USA, represents more than 80% of the export market (data supplied by South African Rooibos Council, 2013).

The traditional product is comprised of the “fermented” (oxidized) leaves and stems of *Aspalathus linearis* (Joubert and de Beer, 2011). Prior to commercialization of an “unfermented” variant, also known as green rooibos or “unfermented” rooibos, the name “rooibos” referred to the fermented product. Recent protection of the name “Rooibos” in South Africa (Anon., 2013) and the subsequent recognition of its status as a geographical indication (GI) in the European Union (Anon., 2014), not only offers ownership of this particular name to South Africa, but it will ensure that the term will be applicable only to rooibos products. Other common names used by international markets are red bush (translation of rooibos), red rooibos, and red tea. The latter name can lead to confusion with other herbal teas such as one prepared from the leaves of the hibiscus flower. Although a misnomer, the terms “unfermented” and “fermented” are widely used in the context of rooibos. In this paper “green” and “fermented” will be used to make a clear distinction between the two types of rooibos, as “fermentation” not only affects the sensory properties of this herbal tea, but also composition and bioactivity. Extracts produced from rooibos are used mainly as food ingredient in a variety of products, including ready-to-drink iced teas, yoghurt, “instant cappuccino” (Joubert and de Beer, 2011) and

recently also bread. Tinctures and food supplements containing rooibos extract are also on the market.

### Phenolic composition and changes during processing

Aspalathin is the major flavonoid present in unprocessed rooibos plant material and a characteristic chemical marker for *Aspalathus linearis*. Its levels can vary extensively, depending on various factors, including leaf-to-stem ratio. The aspalathin content of separated stems and leaves showed a large difference, i.e., 0.6 and 13.48% (on a dry weight (DW) basis), respectively (Manley et al., 2006; Joubert et al., 2013). Most samples of a large set of dried shoots contained between 2 and 6% aspalathin (Manley et al., 2006). Other major compounds that accumulate in the leaves at levels higher than 1% DW in the leaves are nothofagin, another C-glucosyl dihydrochalcone, and the flavone analogues of aspalathin, isoorientin and orientin, with isoorientin more abundant than orientin (Joubert et al., 2013). These compounds also represent the major flavonoid constituents of extracts and infusions prepared from green rooibos (Beelders et al., 2012b; Muller et al., 2012). The flavone analogues of nothofagin, vitexin, and isovitexin are present at substantially lower levels than orientin and isoorientin (Table 1). Other major compounds are several quercetin O-glycosides, i.e., quercetin-3-O-robinobioside, rutin, hyperoside, and isoquercitrin, of which quercetin-3-O-robinobioside and rutin are present in the highest quantities (Muller et al., 2012). Together the quercetin O-glycosides could comprise as much as 1.34% of the extract.

Another compound of interest present in rooibos is the enolic phenyl pyruvic acid glucoside (PPAG; Z-2-(β-D-glucopyranosyloxy)-3-phenylpropenoic acid; Table 1), a precursor in the biosynthesis of flavonoids (Marais et al., 1996). Although per definition not phenolic due to the absence of a hydroxyl moiety on the phenyl ring, its occurrence in rooibos extracts and

**Table 1.** Flavonoid and phenyl pyruvic acid glucoside (PPAG) content of green and fermented rooibos extracts and infusions.

Compound	Extract (g/100 g) <sup>a</sup>		Infusion (mg/L) <sup>b</sup>	
	Green <sup>c</sup>	Fermented	Green <sup>f</sup>	Fermented (n = 114) <sup>g</sup>
<i>C-Glucosyl dihydrochalcones</i>				
Aspalathin	11.949	0.59 (nd – 2.79) <sup>e</sup>	157.71 (78.32 – 250.75)	5.84 (nd <sup>h</sup> – 15.66)
Nothofagin	1.397	0.03 (nd – 0.18) <sup>e</sup>	18.90 (6.37 – 33.30)	0.95 (nd – 2.76)
<i>C-Glucosyl flavones</i>				
Orientin	1.057	1.01 (0.69 – 1.16) <sup>e</sup>	9.99 (4.31 – 18.32)	10.84 (10.33 – 14.31)
Isoorientin	1.432	1.07 (0.34 – 1.41) <sup>e</sup>	15.38 (6.61 – 29.48)	15.03 (7.40 – 20.47)
Vitexin	0.150	0.069 <sup>d</sup>	2.13 (0.83 – 4.00)	2.33 (1.30 – 3.32)
Isovitexin	0.176	0.152 <sup>d</sup>	2.80 (1.15 – 5.43)	2.40 (1.35 – 3.29)
<i>O-Glycosyl flavonols</i>				
Quercetin-3-O-rutinoside (rutin)	0.358	0.185 <sup>d</sup>	5.45 (2.84 – 8.49)	1.65 (nd – 5.71)
Quercetin-3-O-robinobioside	0.696	0.446 <sup>d</sup>	10.72 (3.99 – 18.86)	8.34 (0.89 – 18.41)
Quercetin-3-O-galactoside (hyperoside)	0.124	0.087 <sup>d</sup>	1.95 (0.42 – 4.23)	2.22 (nd – 6.79)
Quercetin-3-O-glucoside (isoquercitrin)	0.159	0.063 <sup>d</sup>	2.71 (0.82 – 5.10)	1.08 (nd – 5.79)
<i>Phenylpropenoic acid glucoside</i>				
PPAG	0.266	0.57 (0.09 – 0.81) <sup>e</sup>	5.37 (2.22 – 10.05)	6.91 (2.72 – 14.81)

<sup>a</sup>Extraction – extracted plant material with water at 1:10 solid:solvent ratio for >90°C/30 min

<sup>b</sup>Infusion – infused 2.5 g plant material in 200 mL freshly boiled water for 5 min equalling “cup-of-tea” strength

<sup>c</sup>Muller et al. (2012) (n = 3)

<sup>d</sup>Mazibuko et al. (2013) (n = 1)

<sup>e</sup>Joubert et al. (2013) (n = 18)

<sup>f</sup>Beelders et al. (2012b) (n = 10); data were converted to mg/L taking into account soluble solids content of infusions

<sup>g</sup>Joubert et al. (2012) (n = 114)

<sup>h</sup>Not detected

infusions deserves attention due to recent demonstration of antidiabetic properties for this compound (Muller et al., 2013; Mathijs et al., 2014). Leaves of green rooibos contain from “undetectable” levels of PPAG to 1.11% DW (Joubert et al., 2013). Hot water extracts (Muller et al., 2013) and the soluble solids of hot water infusions (Beelders et al., 2012b), prepared from green rooibos, contain up to 0.44% PPAG, making it one of the major constituents.

Comprehensive analysis of a hot water infusion of green rooibos demonstrated the presence of many minor compounds, previously also identified in fermented rooibos (Beelders et al., 2012b). Compounds identified included a C-5'-hexosyl derivative of aspalathin (tentative identification), dihydro-orientin, dihydro-isorientin (*R* and *S* configuration), chrysoeriol, luteolin, luteolin-7-*O*-glucoside, carlinoside, neocarlinoside, isocarlinoside, vicenin-2, patuletin-7-*O*-glucoside, phenolic acids, lignans, and coumarins. The presence of many unidentified compounds was demonstrated, using two-dimensional high-performance liquid chromatography (2-D HPLC) (Beelders et al., 2012a). Occurrence of dihydro-orientin and dihydro-isorientin in green rooibos (Beelders et al., 2012b) suggests their natural presence in the plant material or formation due to oxidative changes during preparation of the infusion. These compounds are intermediate oxidation products of aspalathin in the formation of orientin and isorientin (Marais et al., 2000; Krafczyk and Glomb, 2008).

Little structural information is available on the polymeric fraction of rooibos, which elutes as a poorly defined “hump” on 1-D and 2-D HPLC chromatograms (Beelders et al., 2012a). It contains the presence of an irregular procyanidin type heteropolymer, consisting of (+)-catechin and (–)-epicatechin chain extending units and (+)-catechin terminal unit (Marais et al., 1998).

Oxidative changes to the phenolic composition of rooibos form an essential part of the traditional “fermentation” process, employed to produce fermented rooibos (Joubert, 1996). This product has a red-brown leaf and infusion color. Its hot water infusion at a “cup-of-tea” equivalent strength has a slightly sweet taste, subtle astringency, and flavor with predominant honey, woody, and herbal-floral notes (Koch et al., 2013). While the changes during fermentation are desirable from a sensory perspective, the aspalathin content of the plant material is rapidly reduced to less than 10% of that originally present in the plant material (Joubert, 1996). It is converted to orientin and isorientin via flavanone intermediates (Koeppen and Roux, 1965; Marais et al., 2000; Krafczyk and Glomb, 2008). Follow-up studies provided further insight into the chemical conversion of aspalathin to dimers and high molecular weight, brown end products. Nothofagin also oxidises to form brown products, but at a much slower rate (Krafczyk et al., 2009a; Heinrich et al., 2012).

Given the susceptibility of aspalathin and nothofagin to oxidation, it is not surprising that extracts and infusions prepared from fermented rooibos have relatively low aspalathin and nothofagin contents (Table 1). An extract and infusion prepared from fermented rooibos, demonstrated to have bioactivity (Mazibuko et al., 2013; Sanderson et al., 2014), contained less aspalathin than orientin and isorientin. Analysis of a large number of hot water extracts confirmed the relative quantities

for these compounds (Joubert and de Beer, 2012; Joubert et al., 2013). Similarly, infusions at “cup-of-tea” strength had higher levels of the flavones than the dihydrochalcones (Joubert et al., 2012) (Table 1).

### Bioavailability of rooibos C-glucosyl flavonoid

Bioefficacy of bioactive food compounds depends on their bioavailability (absorption, distribution, metabolism, and excretion) as they need to reach the site of action (Manach et al., 2005). Many factors limit or enhance absorption of polyphenols in the digestive tract, such as the interaction with other dietary ingredients (Rein et al., 2012). Bioefficacy of flavonoids in in-vivo studies could, therefore, be expected to depend on the manner of their consumption, i.e., as part of a complex mixture such as a plant extract or a purified compound mixed into the feed, drinking water or through orogastric gavage of experimental animals. The various factors affecting bioavailability lead to disparity in efficacy results between in vitro and in vivo studies.

Considering Lipinski’s Rule of Five (Lipinski et al., 1997) and other physicochemical parameters such as the number of rotatable bonds and polar surface area (Veber et al., 2002), poor absorption of rooibos C-glucosyl flavonoids is to be expected. Absorption of aspalathin in a Caco-2 monolayer cell model improved when present in green rooibos extract as opposed to the pure compound (Huang et al., 2008), indicating that other plant components present in the extract may assist in its transport across the membrane. Courts and Williamson (2013) noted in their review on C-glycosyl flavonoids that Caco-2 model transport studies carried out in their laboratory showed passive diffusion of aspalathin across the intestinal epithelial monolayer without evidence of deglycosylation. Conjugation of aspalathin and nothofagin in the liver were demonstrated when treated with microsomal and cytosolic subcellular liver fractions (Van der Merwe et al., 2010). Two glucuronidated metabolites and one sulfated metabolite were observed for aspalathin. Based on the disappearance of radical scavenging ability of conjugated aspalathin when tested in an on-line HPLC antioxidant system, the catechol group on the B-ring was identified as the likely site of conjugation. Nothofagin, lacking the catechol group, also formed two glucuronidated metabolites, but the extent of conversion was less and no sulfated conjugate was formed.

In vivo studies on the oral bioavailability of aspalathin showed evidence of phase II metabolites in blood circulation. Its oral bioavailability was first evaluated in vivo, using the pig as model (Kreuz et al., 2008). No aspalathin or metabolites could be detected in the plasma of pigs fed an aspalathin-enriched, green rooibos extract (equaling 157–167 mg aspalathin/kg body weight/day) for 11 days. Several metabolites were found in the urine, demonstrating that deglycosylation of aspalathin is not a prerequisite for its absorption. Aspalathin conjugated with a methyl group or glucuronic acid or both was present in the urine. The aglycones of aspalathin and dihydro-(iso)orientin were also present in the urine. This was attributed to the liberation of the aglycones by colonic microflora. When the pigs received a three times higher, but single dose of the same aspalathin-enriched green rooibos extract, a trace amount

of aspalathin was detected in the plasma (Kreuz et al., 2008). Unpublished results from a preliminary study of the bioavailability of aspalathin in Vervet monkeys by our group showed that the plasma from a male contained very low concentrations of methylated aspalathin and dimethylated aspalathin. The monkeys received a single dose of aspalathin-enriched green rooibos extract, mixed with a bolus of food to deliver 25 mg aspalathin/kg body weight.

Three human studies on the bioavailability of rooibos phenolics have been done to date. Urine of subjects who consumed a single serving of 300 mL of green rooibos (91.2 mg aspalathin) excreted 0.74% of the total aspalathin consumed over a 12-h period in the form of 3-*O*-methylaspalathin and 3-*O*-methylaspalathin glucuronide, with the first metabolite predominant. The mean maximum concentration for both compounds in the urine occurred within 2 h after ingestion of the beverage, equaling 162  $\mu\text{g}$  of 3-*O*-methylaspalathin and 87  $\mu\text{g}$  of 3-*O*-methylaspalathin glucuronide (Courts and Williamson, 2009). In another human study, eight metabolites were detected in the urine after ingestion of rooibos (Stalmach et al., 2009). In this case, 500 mL of green and fermented rooibos beverages were consumed on different occasions by the same subjects. The results confirmed the findings of previous studies, showing *O*-linked conjugation of aspalathin with methyl and glucuronide groups. Additionally, sulfate conjugates were also detected. The main metabolite excreted following ingestion of green rooibos was an *O*-methyl-aspalathin-*O*-glucuronide, while eriodictyl-*O*-sulfate was the main metabolite after ingestion of fermented rooibos. Higher levels of the eriodictyol glucosides were present in the fermented rooibos beverage. In spite of relatively high levels of nothofagin in the green rooibos beverage no derivatives or metabolites of nothofagin were detected in the urine of the subjects. In this case overall metabolite levels excreted over a 2-h collection period accounted for 0.09 and 0.22% of the flavonoids in the fermented and green rooibos beverages, respectively.

Urinary excretion of aspalathin metabolites occurred mainly within 5 h of consumption of the beverages, while excretion of eriodictyol-*O*-sulfate occurred mainly during the 5–12 h urine collection period. These results indicated different sites of absorption, i.e., the small and large intestines, respectively. No flavonoid metabolites were detected in the plasma. Courts and Williamson (2013) pointed out that these results are at odds with common observation of circulating aglycone flavonoid metabolites in human plasma following ingestion of *O*-glycosyl flavonoids. Both Kreuz et al. (2008) and Courts and Williamson (2013) noted that strong affinity of the compounds for plasma carrier proteins such as serum albumin may be the reason for undetectable levels in plasma. In the most recent human study, the presence of aspalathin was demonstrated in the plasma after the subjects drank 500 mL of green rooibos infusion, containing 287 mg aspalathin (Breiter et al., 2011). Other *C*-glucosyl compounds found in the plasma were orientin, isoorientin, vitexin, isovitexin, and (*S*)-eriodictyol-8-*C*-glucoside. Its (*R/S*)-6-*C*-isomers were also detected in the plasma of some of the subjects. Although nothofagin was present in a higher quantity in the infusion than orientin and isoorientin, it was not detected in the plasma. Interestingly, when an isolated fraction of green rooibos (reconstituted in water) was consumed by the same subjects, the amounts of flavonoids detected appeared to be generally lower than when the green rooibos infusion was consumed, despite comparable intake of total flavonoids, suggesting that the composition of the beverage played a role in the absorption of the flavonoids. Recovery of aspalathin in the plasma after consumption of the green rooibos infusion was 0.2%. Breiter et al. (2011) postulated that matrix and synergetic effects may be responsible for this disparity. Table 2 summarizes the major bioavailability results of aspalathin from animal and human studies.

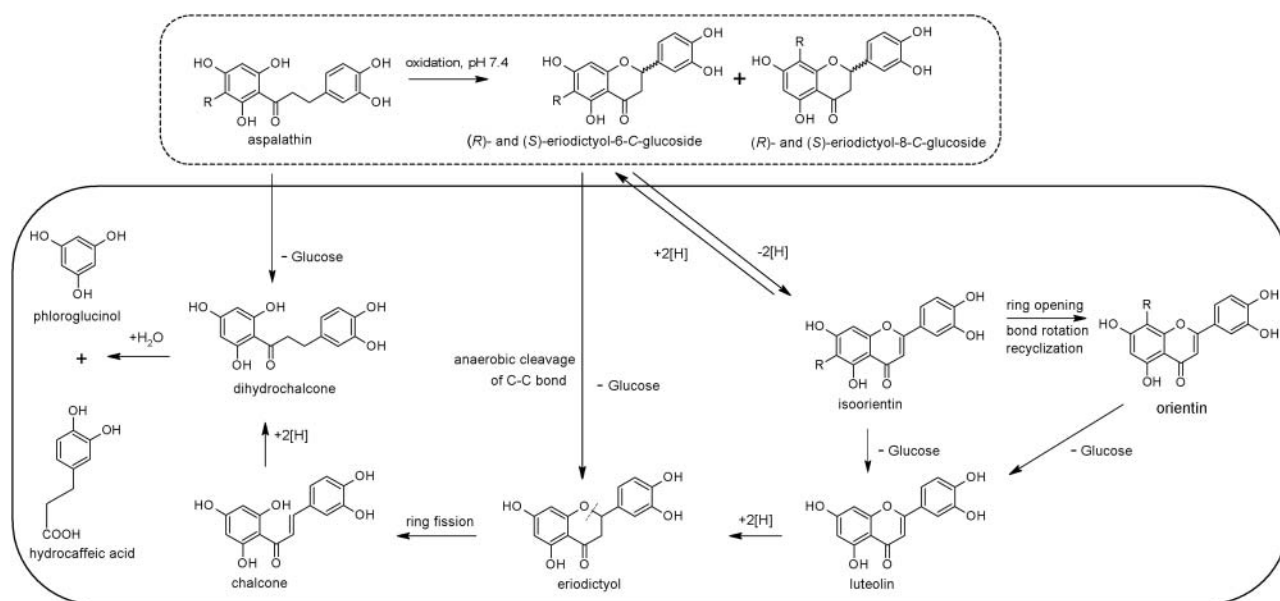
An absorption, tissue distribution, metabolism and excretion (ADME) study performed on rats using a crude extract of bamboo, provides some insight into the bioavailability of

**Table 2.** Presence of aspalathin and metabolites in plasma and urine of animals and humans after consumption of rooibos extract and infusions.

Model	Aspalathin dose	Dosage form	Plasma	Urine	Excretion in urine	Reference
Pig	157–167 mg/kg BW	Aspalathin-enriched green rooibos extract (16.3%), mixed with feed	nd	Aspalathin; aspalathin- <i>O</i> -GlcA; Me- <i>O</i> -aspalathin; Me- <i>O</i> -aspalathin- <i>O</i> -GlcA; aspalathin aglycone- <i>O</i> -GlcA		(Kreuz et al., 2008)
Vervet monkey	25 mg/kg BW	Aspalathin-enriched green rooibos extract (18.4%) mixed with bolus	Me- <i>O</i> -aspalathin; di-Me- <i>O</i> -aspalathin	Me- <i>O</i> -aspalathin; di-Me- <i>O</i> -aspalathin		Unpublished
Human	91 mg/subject	300 mL of green rooibos infusion	nd	Me-3- <i>O</i> -aspalathin; Me-3- <i>O</i> -aspalathin- <i>O</i> -GlcA	Max. conc. reached < 2 h after ingestion; 0.74% excreted during 0–24 h	(Courts and Williamson, 2009)
Human	41 mg/subject	500 mL green rooibos “ready-to-drink” beverage	nd	Aspalathin- <i>O</i> -GlcA (2x); Me- <i>O</i> -aspalathin- <i>O</i> -GlcA (3x); Me- <i>O</i> -aspalathin- <i>O</i> -sulfate; aspalathin- <i>O</i> -sulfate	Most excreted < 5 h after ingestion; 0.22% excreted during 0–24 h	(Stalmach et al., 2009)
Human	3.6 mg/subject	500 mL fermented rooibos “ready-to-drink” beverage	nd	Me- <i>O</i> -aspalathin- <i>O</i> -GlcA (3x); Me- <i>O</i> -aspalathin- <i>O</i> -sulfate; aspalathin- <i>O</i> -sulfate	0.09% excreted during 0–24 h	(Stalmach et al., 2009)
Human	287 mg/subject	Green rooibos beverage	Aspalathin	Aspalathin; Aspalathin- <i>O</i> -GlcA; Me- <i>O</i> -aspalathin; Me- <i>O</i> -aspalathin- <i>O</i> -GlcA (3x); Me- <i>O</i> -aspalathin- <i>O</i> -sulfate; aspalathin- <i>O</i> -sulfate; aspalathin aglycone- <i>O</i> -GlcA	0.2% excreted during 0–24 h	(Breiter et al., 2011)

Abbreviation: BW, body weight; GLcA – Glucuronic acid; Me- methyl





**Figure 1.** Schematic representation of the proposed metabolic breakdown pathway of aspalathin with R representing a  $\beta$ -D-glucopyranosyl moiety [Compiled from Braune and Blaut (2011, 2012); Braune et al. (2001); Hattori et al. (1988); Krafczyk and Glomb (2008); Schoefer et al. (2003); Zhang et al. (2007)].

orientin, isoorientin, vitexin and isovitexin. These C-glucosyl flavones also showed poor gastrointestinal absorption as none could be detected in the blood and urine of the rats, with more than 50% of the compounds eliminated from the gastrointestinal tract in the original form within 12 h (Zhang et al., 2007). The C-glucosyl flavones were also not detected in the brain, liver, kidney and thigh muscle of the rats. Another rat study confirmed that no orientin was detected in the plasma after oral administration of the pure compound, while intravenous injection resulted in its rapid distribution to tissue and elimination from plasma within 90 min (Li et al., 2008).

Given the poor intestinal absorption of these C-glucosyl compounds, catabolism by gut microflora could play an important role in their bioactivity. Anaerobic incubation of isoorientin with an isolate of human intestinal bacteria showed its conversion through hydrogenation of the 2,3-double bond on the C-ring to form the flavanone, eriodictyol 6-C-glucoside, followed by cleavage of the C-glucosyl bond to form the aglycone, eriodictyol. Subsequently, ring fission of the flavanone formed 3-(3,4-dihydroxyphenyl)-propionic acid (hydrocaffeic acid) and phloroglucinol. Direct conversion of isoorientin to luteolin via direct cleavage of the C-glucosyl bond was identified as a minor metabolic process (Hattori et al., 1988). A later study, investigating the metabolic fate of orientin, isoorientin, vitexin, and isovitexin in rats, proposed the same metabolic pathways (Zhang et al., 2007). End products of vitexin and isovitexin were apigenin, phloroglucinol, and phloretic acid.

Studies on human gut microbiota, able to deglycosylate various C-glycosyl compounds, have led to the identification of several bacteria (Sangul et al., 2005; Braune and Blaut, 2011, 2012; Nakamura et al., 2011; Kim et al., 2014). *Eubacterium cellulosolvens*, an anaerobic cellulolytic bacterium, isolated from mice, rabbits, sheep, and cow, can cleave isoorientin and isovitexin to form their aglycones, luteolin, and apigenin, respectively, while their C6 analogues, orientin, and vitexin, are not degraded (Braune and Blaut, 2012), indicating that the position of the

sugar moiety is important. However, the position of the sugar did not affect the human bacterial strain CG19-1 as it was able to remove the sugar moiety of these C-glucosyl flavones and degrade their aglycones, luteolin, and apigenin, to hydrocaffeic acid and phloretic acid, respectively (Braune and Blaut, 2011). *Eubacterium ramalus* and *Clostridium orbiscindens*, strict anaerobic bacteria isolated from human feces, are able to catalyze the degradation of luteolin and eriodictyol to hydrocaffeic acid and phloroglucinol, with the latter compound further degraded to acetate and butyrate (Braune et al., 2001; Schoefer et al., 2003).

Given the ready conversion of aspalathin to its flavanone and flavone analogues at neutral pH (Krafczyk and Glomb, 2008) it would encounter in the small intestine, it can be postulated that its microbial biotransformation by human intestinal bacteria will follow the same pathway outlined here for its oxidation products (Fig. 1), thus leading ultimately to the formation of hydrocaffeic acid and organic acids in the colon.

### Metabolic disease and relevance of plant based therapies

Noncommunicable diseases (NCDs), driven by the obesity and type 2 diabetes (T2D) epidemics, are the foremost cause of death globally, leading to more deaths each year than all other diseases combined (WHO, 2012). The escalation in the incidence of diabetes has been grossly underestimated as the 347 million adult diabetics in 2008 already exceeded the 285 million estimated for 2010 (Shaw et al., 2009; Danaei et al., 2011). Deaths attributable to cardiovascular disease (CVD) are almost double for diabetics compared to the general population (Peters et al., 2014). Under-resourced low- and middle-income countries (LMIC) are worst affected with over 80% of cardiovascular and type 2 diabetes related deaths occurring in LMIC. These deaths are projected to increase globally, but the projected rates of increase are higher in LMIC than high-income countries (HIC) (20 vs. 15% between 2010 and 2020). These

often avoidable deaths occur at a younger age in LMIC than HIC (29% of deaths occur before the age of 60 in LMIC compared to 13% in HIC) (Peer et al., 2012; WHO, 2012).

In view of the long-term inefficacy, side-effects and cost of modern oral antidiabetic agents, plant-based therapies for the treatment and prevention of T2D are gaining considerable prominence (Malviya et al., 2010). Therefore, drug discovery strategies based on natural products and traditional medicines are re-emerging as attractive options to discover new molecular entities, which remain largely untapped within the chemical diversity of the plant kingdom (Hays et al., 2008; Potterat and Hamburger, 2013). There is also a movement towards alternatives in the form of rationally designed, carefully standardized, synergistic traditional herbal formulations and botanical drug products, supported by robust scientific evidence (Patwardhan and Mashelkar, 2009). The use of conventional drugs in combination with natural products, specifically herbal medicines or supplements, as adjunctive therapies to treat chronic disease such as diabetes, is also gaining popularity world-wide (Dennis et al., 2009; Bradley et al., 2011; Hasan et al., 2011; Mansukhani et al., 2014).

The multifactorial nature and complex pathophysiology of metabolic diseases such as obesity and diabetes present major hurdles for effective treatment of the underlying causative pathologies. Current therapeutic approaches, due to their narrow pharmaceutical spectra, tend to target specific mechanisms, while a broader approach such as that observed for complex mixtures and phenolic compounds affect a broader range of therapeutic targets which could be more effective to address these metabolic diseases (Tiwari and Rao, 2002).

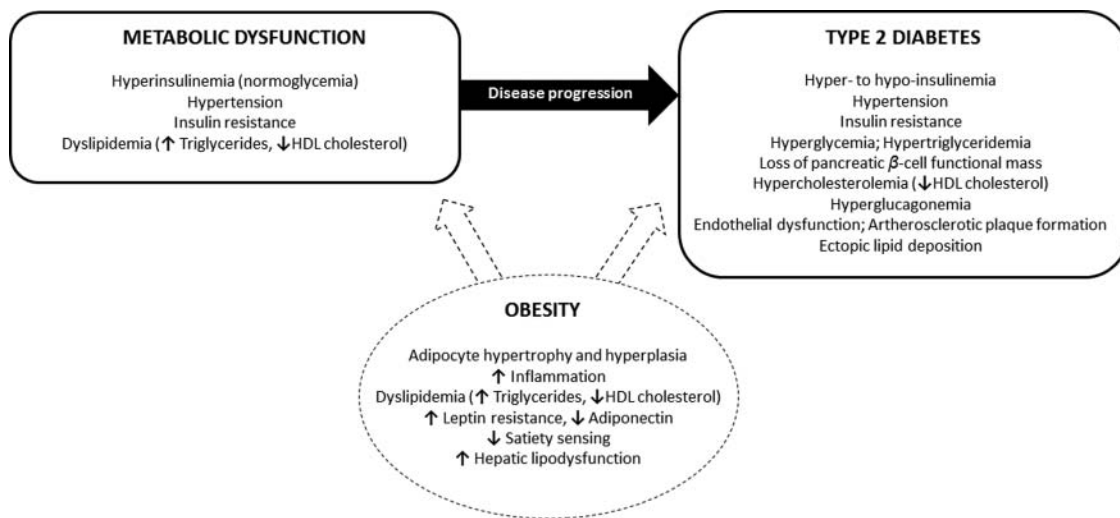
### Metabolic syndrome—a molecular and biochemical perspective

The metabolic syndrome is defined as a cluster of pathophysiological features such as insulin resistance, obesity, dyslipidaemia,

hypertension, impaired glucose tolerance and chronic inflammation (Fig. 2). Together these features are regarded as major contributing risk factors to serious disease including diabetes mellitus and related comorbidities such as cardiovascular and neural degenerative diseases (Miranda et al., 2005). Epidemiologically the metabolic syndrome is considered to be the major underlying cause of the global epidemic of obesity, diabetes, and cardiovascular disease (Zimmet et al., 2005).

### Insulin resistance and hyperinsulinemia

Key causal factors underlying the metabolic syndrome are insulin resistance (IR) and associated hyperinsulinemia (Reaven, 2005). IR is defined as the impairment of insulin to achieve its physiological effects, including the stimulation of glucose uptake and inhibition of hepatic glucose output. IR differentially affects tissues such as muscle, fat, kidney, and liver (Reaven, 2005). To compensate for IR and to maintain euglycemia, the pancreatic  $\beta$ -cells produce more insulin, but this is at the expense of the negative physiologic effects of hyperinsulinemia. IR-induced hyperinsulinemia acts, for example, on the liver leading to hepatic steatosis and hepatic overproduction of triglyceride-rich particles that results in an atherogenic lipoprotein profile characterized by hypertriglyceridemia, low high density lipoprotein, and increased small dense low density lipoprotein (LDL) (Reaven, 2005). In addition, hyperinsulinemia is associated with vascular smooth hypertrophy, enhanced sympathetic activity, and in the kidney increased sodium retention resulting in increased blood pressure, providing a link between IR and CVD (Semplicini et al., 1994; Reaven, 2005). In T2D the link to CVD is further exacerbated by elevated postprandial free fatty acids (FFA) that are strongly associated with endothelial dysfunction (Heine and Dekker, 2002). This, together with an atherogenic lipoprotein phenotype and high blood pressure contributes to the two- to fourfold increase in CVD mortality



**Figure 2.** Schematic illustration of the close relationship between metabolic dysfunction, metabolic syndrome and development of type 2 diabetes. Common to all three are glucose intolerance, lipid dysfunction, inflammation and oxidative stress. [Compiled from Kahn et al. (2014); Kahn et al. (2006)]. Abbreviation: HDL, high density lipoprotein.

for men and women with T2D (Heine and Dekker, 2002; Grundy et al., 2004).

### Obesity and inflammation

The incidence of obesity is increasing rapidly worldwide with more than 1.9 billion adults being overweight and at least 600 million of them clinically obese (WHO, 2015). The incidence of obesity in South Africa ranks third highest in the world (Ng et al., 2014). In obese IR individuals, hyperplastic fat tissue trigger adipose tissue inflammation by releasing proinflammatory cytokines like interleukin-6 (IL-6), tumor necrosis factor alpha (TNF- $\alpha$ ), interleukin-1beta (IL-1 $\beta$ ), and several chemokines including chemokine (C-C motif) ligand 2 (CCL2), chemokine (C-C motif) ligand 5 (CCL5), and chemokine (C-X-C motif) ligand 1 (CXCL5) (Yao et al., 2014). Increased levels of circulating lipid-reactive oxygen species (ROS) are produced that attenuate insulin signaling and perpetuate the metabolic syndrome (Sirikul et al., 2006; Silveira et al., 2008). Obesity associated chronic systemic inflammation causes endothelium dysfunction. The increased permeability of endothelium results in the subendothelial deposition of LDL. This initiates localized inflammatory responses in the vascular wall and triggers plaque development and CVD (Yao et al., 2014). The proinflammatory cytokine TNF- $\alpha$ , produced by the adipocytes, directly affects adipocyte function by downregulating the activity of lipoprotein lipase and acyl CoA synthase, enzymes responsible for the breakdown of triglyceride-rich lipoproteins and the synthesis of triglycerides. These functions are essential for the normal function of adipocytes and the maintenance of normolipidemia (Memon et al., 1998). In obese individuals PPAR- $\gamma$ , a key transcription factor involved in the regulation of adipogenesis and adiposity, is also suppressed by TNF- $\alpha$  (Rosen and MacDougald, 2006). Hyperadiposity or excessive accumulation of adipose mass can be the result of an increase in the number (hyperplasia) and/or an increase in the size (hypertrophy) of adipocytes (fat cells). Hypertrophic obesity has previously been shown to be more closely associated with IR than hyperplastic obesity (Gustafson et al., 2009).

In obese individuals the limited capacity of adipose tissue to properly store fat is over-run with resultant hyperlipidemia which leads to increased ectopic lipid storage in tissues such as the liver and skeletal muscle (Snel et al., 2012). Increased levels of circulating fatty acids exacerbate IR, causing hyperinsulinemia. Elevated insulin levels, together with increased endoplasmic reticulum (ER) stress and inflammatory cytokines that activate I $\kappa$ B kinase/nuclear factor- $\kappa$ B (IKK/NF- $\kappa$ B) signaling, overstimulate de novo lipogenesis in muscle and liver via the transcription factor sterol regulatory element-binding protein 1c (SREBP-1c) (Dowman et al., 2010; Ferré and Foufelle, 2010). In obese IR individuals, increased lipogenesis with decreased fatty oxidation accounts for the accumulation of intrahepatocellular triglycerides and lipid metabolites, causing nonalcoholic hepatic steatosis (Dowman et al., 2010). In skeletal muscle the accumulation of long chain FA acyl-CoA and its by-products of oxidation, diacylglycerol (DAG) and ceramides, promote the development and progression of insulin resistance by interfering with the phosphorylation of

proteins involved in the insulin signaling pathway, including insulin receptor substrate-1/2 (IRS-1/2), phosphatidylinositol-3-kinase (PI3-kinase) and protein kinase C (PKC) (Silveira et al., 2008). The increase of intramyocellular FFA metabolites and associated lipotoxic mitochondrial dysfunction cause impaired fat oxidation and a decrease in metabolic flux through the tricarboxylic acid cycle (Petersen et al., 2004). In IR individuals decreased mitochondrial fat oxidation and increased FFA influx into skeletal muscle attenuate peripheral glucose uptake, leading to postprandial hyperglycemia (Abdul-Ghani and DeFronzo, 2010).

### $\beta$ -cell dysfunction

Normal pancreatic  $\beta$ -cell function is regulated by complex interactions between neural factors, blood glucose levels and appropriate hormonal interactions (Ruiz and Haller, 2006). An increase in blood glucose is the main initiator of insulin release from  $\beta$ -cells. Persistent elevation of blood glucose (hyperglycemia), however, results in loss of  $\beta$ -cell differentiation capacity,  $\beta$ -cell dysfunction, and increased  $\beta$ -cell apoptosis (Blume et al., 1995; Butler et al., 2003).  $\beta$ -cell dysfunction is caused by glucose-induced  $\beta$ -cell overstimulation and oxidative stress related to the increased demand for and synthesis of insulin (Bensellam et al., 2012). Maintaining normoglycemia is thus essential for normal pancreatic  $\beta$ -cell function and for the maintenance of functional  $\beta$ -cell mass (Bensellam et al., 2012). The role of protein and lipid glycation (advanced glycation end products) (AGEs) as a major initiator of glucotoxic deterioration of  $\beta$ -cell function and survival has been established. The accumulation of ROS in  $\beta$ -cells can easily saturate the antioxidative systems in these cells, since they are known to have low levels of free-radical quenching enzymes such as glutathione peroxidase, catalase, and superoxide dismutase (Gehrmann et al., 2010). In addition, ROS activate pathways that initiate inflammation, including PKC and NF- $\kappa$ B, which further exacerbate  $\beta$ -cell dysfunction (Goldin et al., 2006; Bensellam et al., 2012). ER stress is also initiated under glucose (and lipid) overload, and is reported to activate several signaling pathways, such as the inflammatory c-Jun N-terminal kinases (JNK) and oxidative response pathways (Montane et al., 2014). With oxidative stress and inflammation playing such putative roles in  $\beta$ -cell dysfunction and failure, it is thus plausible that antioxidants or anti-inflammatory compounds could improve or rescue  $\beta$ -cell function in T2D conditions. In fact, rooibos antioxidants, such as aspalathin and luteolin, have been reported to protect  $\beta$ -cells under diabetic conditions both in vivo and in vitro. Luteolin was demonstrated to protect RIN-m5F  $\beta$ -cells against IL-1 $\beta$  and interferon gamma IFN $\gamma$ -mediated cytotoxicity and to suppress nitric oxide production by suppressing inducible nitric oxide synthase (iNOS) messenger RNA and protein expression and inflammation via inhibition of NF- $\kappa$ B activation (Kim et al., 2007). Aspalathin has been shown to stimulate insulin secretion in RIN-5F pancreatic  $\beta$ -cells (Kawano et al., 2009) and protect these cells against AGEs (Son et al., 2013). PPAG, lacking the structural features required for potent free radical scavenging ability, was also shown to protect mouse  $\beta$ -cells against ER stress induced apoptosis by increasing the



expression of the antiapoptotic protein B-cell lymphoma 2 (BCL-2) (Mathijs et al., 2014).

### Health benefits of rooibos

Investigations into the health promoting properties of rooibos and aspalathin have escalated during recent years. Several of these studies specifically focused on their potential antidiabetic and antiobesity properties. Table 3 summarizes findings of relevant studies on rooibos extracts and infusions. Rooibos derived compounds have been described to have several cellular effects on gene and protein expression, particularly those associated with glucose and lipid metabolism, as well as oxidative stress and inflammation (Fig. 3). In Table 4 relevant activities of aspalathin, nothofagin, their C-glucosyl flavone derivatives, the aglycone of orientin and isoorientin, luteolin, as well as those of PPAG are summarized. The antioxidant activity of rooibos flavonoids, in particular in comparison to aspalathin, has been covered in other papers (Von Gadow et al., 1997; Joubert et al., 2004; Krafczyk et al., 2009b; Snijman et al., 2009) and will not be discussed here.

### Antidiabetic properties

Inhibition of  $\alpha$ -glucosidase enzymes, which are present on the brush border of the small intestine and hydrolyse disaccharides and oligosaccharides into monosaccharides, has become a control strategy of postprandial elevation of blood glucose levels. Use of  $\alpha$ -glucosidase inhibitors (AGIs) is proposed as a first-line therapy after diet failure in T2D patients, especially in elderly patients (Scheen, 2003). A systematic literature review and meta-analysis of randomized controlled trials of at least 12 weeks duration comparing  $\alpha$ -glucosidase inhibitor monotherapy in T2D patients led to the conclusion that AGIs such as acarbose have a significant effect on glycemic control and insulin levels (Van de Laar et al., 2005). Another systematic literature review and meta-analysis showed that acarbose reduces the incidence of T2D in patients with impaired glucose tolerance (IGT) (Van de Laar et al., 2006). Adverse gastrointestinal effects, especially flatulence, may limit their long-term use (Scheen, 2003). Many phytochemicals, including flavonoids, have been assessed for their  $\alpha$ -glucosidase inhibitory activity, showing in many instances promising results (Kumar et al., 2011). Synergistic effects between acarbose and polyphenols suggest benefits in terms of dose reduction of the drug (Boath et al., 2012) and thus alleviation of side effects.

Muller et al. (2012) demonstrated inhibition of yeast  $\alpha$ -glucosidase by green rooibos extracts, containing high levels of aspalathin. In the same study, an on-line HPLC-DAD-biochemical detection method was employed to demonstrate inhibitory activity for aspalathin. The extract with the highest aspalathin content and inhibitory activity was subsequently tested in vivo in streptozotocin (STZ)-induced diabetic rats. Acute oral administration of the extract to these diabetic rats induced a sustained reduction in plasma glucose over a 6-h period. The extract tested at 25 mg/kg body weight showed a glucose lowering effect comparable to that of the drug, metformin, the most commonly prescribed hypoglycemic drug for T2D. Apart from aspalathin, the extract contained relatively

high levels of other flavonoids with proven in vitro  $\alpha$ -glucosidase inhibitory activity. The flavonol O-glycosides, rutin and isoquercitrin, inhibit both rat intestinal and yeast  $\alpha$ -glucosidase (Jo et al., 2009; Li et al., 2009b). A number of studies showed  $\alpha$ -glucosidase inhibitory activity for orientin, isoorientin, vitexin and isovitexin, as well as their aglycones, luteolin, and apigenin (Kim et al., 2000; Li et al., 2009a; Yao et al., 2011; Choo et al., 2012; Ha et al., 2012; Chen et al., 2013). IC<sub>50</sub> values for the flavones and acarbose obtained in different studies vary due to differences in experimental conditions, in particular the origin of the enzyme (Oki et al., 1999) and whether the enzyme is in a membrane-bound state or not (Oki et al., 2000). Results should, therefore, be interpreted with caution. Luteolin, for example, showed higher inhibition of yeast  $\alpha$ -glucosidase than acarbose (Kim et al., 2000), yet a dose of more than 200 mg/kg was required to elicit a postprandial reduction in blood glucose levels of Sprague-Dawley rats administered 2 g/kg of sucrose, while acarbose administered at 3 mg/kg resulted in a significant reduction of blood glucose levels (Matsui et al., 2002). The lack of activity of luteolin was attributed to the membrane-bound state of the enzyme in vivo (Matsui et al., 2002). Treatment of sugar-loaded STZ-induced diabetic mice required 20 mg/kg isovitexin and 50 mg/kg vitexin to reduce blood glucose levels significantly. Their effective human equivalent dose, based on the body surface normalisation method (Reagan-Shaw et al., 2008), equals 3.3 and 8.1 mg/kg body weight, respectively. Much higher levels, i.e., 100 and 200 mg/kg of isovitexin and vitexin, respectively, were required to have a similar effect on blood glucose levels than acarbose at 5 mg/kg (Choo et al., 2012). In an in vitro study, using yeast  $\alpha$ -glucosidase, vitexin was more effective than isovitexin and both substantially more effective than acarbose (Li et al., 2009a). The latter study provided some insight into structural features of flavones affecting inhibitory activity. Apart from the 5,7,4'-trihydroxyflavone structure that is crucial for activity, the C3'-OH group of the B-ring increased the  $\alpha$ -glucosidase inhibitory activity, while glycosylation at the C6 or C8 position of the A-ring reduced the inhibitory effect. Orientin was less active than isoorientin, and vitexin less active than isovitexin, indicating that the position of the glucose moiety is important. Xiao et al. (2012), reviewing  $\alpha$ -glucosidase inhibition by various polyphenols, concluded that the C2 = C3 double bond of flavonoids is also an important structural feature contributing to enhanced activity compared to compounds with a saturated bond.

Kawano et al. (2009) demonstrated that aspalathin increases glucose uptake in muscle cells and insulin secretion from pancreatic  $\beta$ -cells. In addition to muscle cells, the activity of aspalathin on glucose uptake and utilization was also demonstrated in liver and fat cells (Mazibuko et al., 2015). Beltrán-Debón et al. (2011) demonstrated a hypolipidemic effect for rooibos in LDLr<sup>-/-</sup> mice fed a high fat diet, but this effect was stringently dependent on diet type. At a molecular level aspalathin resensitized insulin signaling suppressed by palmitate via protein kinase B (PKB, also known as Akt) and activated the insulin-independent AMPK pathway, culminating in increased glucose uptake via GLUT4 (Mazibuko et al., 2015). Son et al. (2013), using L6 myotubes, reported that aspalathin increased glucose uptake by increasing AMPK phosphorylation and GLUT4 translocation to the membrane. They also demonstrated that

**Table 3.** Summary of studies related to the antioxidant, antibiosty, antidiabetic, insulin resistance, anti-inflammatory, and antihypertensive activities of rooibos infusions and extracts.

Bioactivity	Plant material type; Infusion/ extract <sup>a</sup>	Model	Effective concentration/dose	Outcome	Reference
Anti-inflammatory	Fermented <sup>b</sup> ; aq. extract	In vitro OVA stimulated and anti-CD3 antigen treated isolated mouse splenocytes	3.25 g rooibos/200 mL water, boiled for 15 min; effective concentration 10 – 1000 µg extract/mL	Increased IL-2 secretion, inhibited apoptosis and suppressed IL-4 secretion in OVA stimulated splenocytes; increased IL-2 secretion in anti-CD3 treated splenocytes	(Kunishiro et al., 2001)
Anti-inflammatory	Fermented <sup>b</sup> ; aq. extract	In vivo OVA antigen stimulation and cyclosporin-induced immunosuppression in Wistar rats and OVA antigen stimulation in BALB/c mice	3.25 g rooibos/1000 mL water, boiled for 15 min; extract administered as sole drinking fluid ad libitum; consumption ca. 30 mL/rat/day for one week prior and two weeks after inoculation; BALB/c mouse intake ca. 4 mL/mouse/day starting three weeks before inoculation	Largely restored reduction of cyclosporin A-induced OVA antibody production (IgM) in rat serum; marginally increased IL-2 secreting splenocytes in the BALB/c mice	(Kunishiro et al., 2001)
Anti-inflammatory	Fermented <sup>b</sup> ; aq. extract	Whole blood culture assay; unstimulated, endotoxin stimulated and PHA stimulated	25 g rooibos/ 1,000 mL, steeped in boiling water; effective concentration 0.5 mg extract/mL	Increased secretion of IL-6, IL-10, and IFN-γ in unstimulated cells; increased IL-6, decreased IL-10 and no effect on TNF-α secretion in stimulated cells	(Hendricks and Pool, 2010)
Anti-inflammatory	Fermented <sup>b</sup> ; DMSO extract	LPS-stimulated macrophages (in vitro)	100 mg rooibos/1 mL DMSO, extracted for 24 h at room temperature; effective concentration 0.5 mg extract/mL	Reduced secretion of IL-6 and IL-10; increased expression of COX-2; no effect on TNF-α secretion and iNOS expression	(Mueller et al., 2010)
Anti-inflammatory	Unfermented; MeOH extract	Transiently transfected COS-1 cells with 0.5 µg DNA (baboon CYP17A1, CYP21 and CYP11B1) (in vitro)	30 g CHL-defatted rooibos/ 300 mL MeOH for 8 h; effective concentration 4.3 mg extract/mL	Decreased total steroid output 4-fold and reduced aldosterone and cortisol precursors	(Schloms et al., 2012)
Cardiovascular protection	Fermented <sup>b</sup> ; aq. extract	Ex vivo aorta, atria and trachea smooth muscle contraction; in vivo blood pressure in Sprague-Dawley rats	150 g rooibos/1000 mL, boiled for 10 min and steeped for 20 min at room temperature; tested 10, 30, 100 mg extract/kg BW i.v. diluted in saline	Dose-dependently reduced mean arterial blood pressure of male and female Sprague-Dawley rats	(Khan and Gilani, 2006)
Cardiovascular protection	Fermented <sup>b</sup> ; PBS infusion	In vitro endothelial cell model from human umbilical veins (HUVEC)	1 g rooibos/20 mL PBS, infused for 10 min; used at 1:200 and 1:400 dilution	No inhibition of ACE in HUVEC after 10 min incubation; dose-dependent increase in NO production in HUVEC after 24 h incubation	(Persson et al., 2006)
Cardiovascular protection	Fermented <sup>b</sup> ; aq. infusion	Single oral dose to healthy humans	10 g rooibos/400 mL freshly boiled water, infused for 10 min (single dose)	Inhibited ACE after 30 and 60 min and ACE II genotype activity at 60 min; no effect on NO concentration	(Persson et al., 2010)
Cardiovascular protection	Fermented; PBS infusion	Human serum ACE inhibition with the ACE inhibitor enalaprilat (positive control)	1 g rooibos/20 mL PBS, infused for 10 min; used at 1:4 dilution	Rooibos showed mixed type inhibition of ACE with similar enzyme kinetic mechanism as enalaprilat.	(Persson, 2012)
Cardiovascular protection	Fermented; aq. infusion	Oral administration to humans with cardiovascular risk factors	1 tea bag/200 mL freshly boiled water, infused for 5 min; 6 cups/day over six weeks; participants could add milk and/or sugar	Decreased CD, TBARS, LDL cholesterol and triacylglycerols levels; increased GSH level, GSH/GSSG ratio and HDL cholesterol	(Marnewick et al., 2011)
Cardiovascular protection	Fermented; aq. extract	Ex vivo STZ-induced diabetic Wistar rat cardiomyocytes	Rooibos:water (m/v) in 1:10 at 93°C/ 30 min. Effective concentration 10 µg extract/mL	Protected diabetic cardiomyocytes against exogenous and ischemic oxidative stress	(Dludla et al., 2014)
Antidiabetic	Fermented; aq. extract and alkaline extract (1% NaOH)	In vivo STZ-induced diabetic Wistar rat model	2.5 g rooibos/1000 mL water, boiled for 10 min, then steeped for 20 min at room temperature. Water extract fed ad libitum plus daily gavage of 300 mg alkaline rooibos extract/kg BW	No improvement of diabetic status of STZ-induced rats; both extracts decreased plasma creatinine, AGES and MDA levels in plasma and lens; water extract also decreased MDA in the liver	(Uličná et al., 2006)

(Continued on next page)

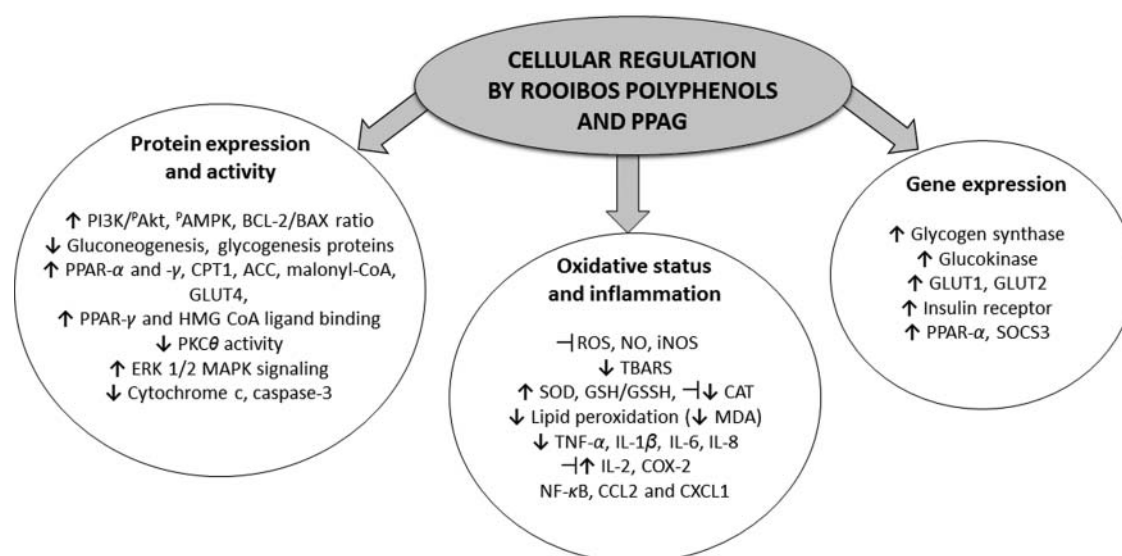
Table 3. (Continued).

Bioactivity	Plant material type; Infusion/ extract <sup>a</sup>	Model	Effective concentration/dose	Outcome	Reference
Antidiabetic	Fermented; aq. infusion	Single oral dose containing sugar with a high fat meal to healthy humans	2 g rooibos/100 mL freshly boiled water, infused for 5 min.; effective dose 500 mL infusion with 52.5 g sucrose added	Reduced plasma glucose, insulin, total cholesterol, LDL cholesterol, triglyceride, hsCRP, CD and TBARS levels; increased plasma antioxidant capacity and total glutathione level	(Francisco, 2010)
Antidiabetic	Green; aspalathin-enriched extract	C2C12 and Chang cells (in vitro) and Wistar rats (in vivo)	Rooibos extracted with 80% EtOH-water; in vitro glucose uptake effective concentration 5 µg extract/mL; α-glucosidase IC <sub>50</sub> 2.2 µg extract/mL; in vivo 25 – 30 mg extract/kg BW	Increased glucose uptake and reduced hyperglycemia; inhibited α-glucosidase (yeast origin)	(Muller et al., 2012)
Antidiabetic	Fermented and green; aspalathin-enriched extract	Insulin resistant C2C12 muscle cells (in vitro)	Fermented rooibos extract (Dludla et al., 2014); green rooibos extract (Muller et al., 2012); effective concentration 10 µg extract/mL	Reversed palmitate-induced insulin resistance	(Mazibuko et al., 2013)
Antidiabetic	Green; extract	In vitro L6 myotubes, RIN-5F pancreatic β-cells and in vivo obese diabetic KK-A <sup>y</sup> mice	Cold water extract (commercial); effective concentrations for L6 cells 350 µg/mL and RIN-5F pancreatic β-cells 50 µg/mL; diabetic mice, received 0.3% (initial three weeks) and 0.6% (subsequent two weeks) extract was mixed with food for five weeks	Promoted glucose uptake in L6 myotubes via phosphorylation of AMPK and Akt; protected RIN-5F pancreatic β-cells by reducing AGE-induced ROS levels. Suppressed increase in fasting blood glucose levels	(Kamakura et al., 2015)
Antiobesity	Fermented <sup>b</sup> ; aq. extract	Hyperlipidemic male LDLR –/– mice	Extract (10 g rooibos/L) as drinking fluid	Reduced serum cholesterol, triglyceride and free fatty acid levels; prevented dietary-induced hepatic steatosis	(Beltrán-Debón et al., 2011)
Antiobesity	Fermented <sup>b</sup> ; aq. extract	3T3-L1 mouse adipocytes (in vitro)	100 – 600 µg extract/mL	Concentration-dependent inhibition of triglyceride accumulation	(Beltrán-Debón et al., 2011)
Antiobesity	Fermented <sup>b</sup> ; DMSO extract	Polar Screen PPAR-γ ligand-binding competitive assay	IC <sub>50</sub> = 6.4 ± 0.9 µg extract/mL	Moderate ligand-binding activity	(Mueller and Jungbauer, 2009)
Antiobesity	Fermented <sup>b</sup> ; DMSO extract	Lantha Screen TR-FRET PPAR-γ coactivator assay	IC <sub>50</sub> = 3.6 ± 0.6 µg extract/mL	Strong antagonist of coactivator recruitment to PPAR-γ	(Mueller and Jungbauer, 2009)
Antiobesity	Fermented; aq. Infusion	3T3-L1 adipocytes (in vitro)	10 – 100 µg extract/mL	Suppressed adipogenesis	(Sanderson et al., 2014)

<sup>a</sup>Extract prepared by heating in water for fixed time or with organic solvent; infusion prepared by steeping in freshly boiled water or water/buffer at room temperature.

<sup>b</sup>Fermentation state of plant material used is not specified, but fermented tea is assumed as this is the most common type.

Abbreviations: aq, aqueous; ACE, angiotensin-converting enzyme; AGES, advanced glycation end products; Akt, protein kinase B; ALT, alanine aminotransferase; AMPK, 5' adenosine monophosphate-activated protein kinase; AST, aspartate aminotransferase; BW, body weight; CD, conjugated dienes; COX-2, cyclooxygenase-2; DMSO, dimethylsulphoxide; EtOH, ethanol; GSH, reduced glutathione; GSSG, oxidised glutathione; HDL, high-density lipoprotein; hsCRP, high sensitive C-reactive proteins; IC<sub>50</sub>, half maximal inhibitory concentration; IFN-γ, interferon gamma; IL-2, IL-4, IL-6, IL-10, interleukin-2, -4, -6, -10; IgM, immunoglobulin M; iNOS, inducible nitric oxide synthase; i.v. intravenous; LC<sub>50</sub>, lethal concentration needed to kill 50% of cells; LDL, low-density lipoprotein; LPS, lipopolysaccharide; MDA, malondialdehyde; MeOH, methanol; NO, nitric oxide; OVA, ovalbumin; PPAR-γ, peroxisome proliferator-activated receptor gamma; ROS, reactive oxygen species; STZ, streptozotocin; T2D, type 2 diabetes; TBARS, thiobarbituric acid reactive substances; TNF-α, tumor necrosis factor alpha.



**Figure 3.** The most common cellular effects of the rooibos C-glucosyl dihydrochalcones, and other related compounds on gene and protein expression, particularly those affecting glucose and lipid metabolism, oxidative stress, inflammation and cell death. [Compiled from Choi et al. (2014a); Choi et al. (2014b); Dłudla et al. (2014); Fidan et al. (2009); Francisco (2010); Fu et al. (2006); Hendricks and Pool (2010); Kamakura et al. (2015); Kim et al. (2007); Kim et al. (2010); Kunishiro et al. (2001); Lee et al. (2014); Marnewick et al. (2011); Mathijs et al. (2014); Mazibuko et al. (2015); Mazibuko et al. (2013); Mueller et al. (2010); Mueller and Jungbauer (2009); Muller et al. (2013); Ulicná et al. (2006)]. Abbreviations: ACC: acetyl-CoA carboxylase; AMPK: 5' AMP-activated protein kinase; BAX: BCL-2-like protein 4; BCL-2: antiapoptotic B-cell lymphoma 2; CAT: catalase; CCL2: chemokine (C-C motif) ligand 2; COX-2: cyclo-oxygenase 2; CPT1: carnitine palmitoyl-transferase; CXCL1: chemokine (C-X-C motif) ligand 1; ERK 1/2: extracellular signal-regulated kinases 1 and 2; GLUT-1/2/4: glucose transporter 1/2/4; GSH/GSSG: reduced/oxidized glutathione; HMG CoA: 3-hydroxy-3-methylglutaryl-coenzyme A; IL-1 $\beta$ : interleukin 1 beta; IL-2/6/8: interleukin 2/6/8; iNOS: Inducible nitric oxide synthase; MAPK: mitogen-activated protein kinases; MDA: malondialdehyde; NF- $\kappa$ B: nuclear factor kappa-B; NO: nitric oxide; <sup>p</sup>: phosphorylation; PI3K/Akt: phosphatidylinositol 3'-kinase-Akt; PKC $\theta$ : protein kinase C theta; PPAR- $\alpha$ / $\gamma$ : peroxisome proliferator-activated receptor alpha/gamma; ROS: reactive oxygen species; SOCS3: suppressor of cytokine signaling 3; SOD: superoxide dismutase; TBARS: thiobarbituric acid reactive substances; TNF- $\alpha$ : tumor necrosis factor alpha.

aspalathin protected against ROS generated by AGEs in RIN-5F rat insulinoma cells. While Kamakura et al. (2015) and Son et al. (2013) reported that both aspalathin and an aspalathin-rich green rooibos extract (GRE) could protect  $\beta$ -cells from ROS-induced dysfunctions or cell death through their antioxidant activities, this extract showed a stronger effect than aspalathin alone. For example, GRE, at the equivalent aspalathin concentration as used for testing of the pure compound, decreased intracellular peroxide levels twice as effective as aspalathin itself. This could possibly be due to additive or synergistic effects of the various polyphenols present in the extract. In fact, Muller et al. (2012) reported synergistic effects between aspalathin and rutin on blood glucose levels. Based on these results, we performed transcriptomic analyses of pancreatic  $\beta$ -cells treated with aspalathin or GRE to obtain some insights into molecular mechanisms for synergy among compounds in GRE (unpublished results). Rat insulinoma cells, i.e., RIN-5F cells, were cultured in the presence or absence of 50  $\mu$ M aspalathin or 350  $\mu$ g/mL of GRE (equaling ca 50  $\mu$ M aspalathin) for 24 h, and total RNA was prepared from each. More than 350 differentially expressed genes (DEGs) were detected in treated cells. In aspalathin-treated cells, 353 DEGs (117 upregulated and 236 downregulated) and in the GRE-treated cells, 381 DEGs (153 upregulated and 228 downregulated) were detected. Among those, 83 DEGs were common between aspalathin- and GRE-treated cells. Gene ontology (GO) term analyses of those DEGs revealed the involvement of metal ion binding proteins, transcription factors, and apoptosis-related proteins in aspalathin- or GRE-induced changes, suggesting that both aspalathin and GRE can protect  $\beta$ -cells by changing specific gene expression. Both aspalathin and GRE suppressed the expression of

synaptotagmin-like 4 gene (Syl4). Syl4 is reported to regulate the secretory pathway and to stimulate insulin secretion when its expression was suppressed (Izumi et al., 2007). We have already confirmed the stimulatory effects of aspalathin and GRE on insulin secretion in RIN-5F cells (Son et al., 2013). The transcriptomic analyses suggest that both aspalathin and GRE have protective effects at the transcription level.

Further, in the obese insulin resistant *ob/ob* mouse model, aspalathin reduced fasting blood glucose levels, increased adiponectin levels, and reduced hypertriglyceridemia and serum thiobarbituric acid reactive substances (TBARS) levels, a marker of ROS. In addition, enzymes related to gluconeogenesis, glycogenolysis, and lipogenesis were reduced by aspalathin, while the mRNA expression of glycogen synthase was increased in the liver of these obese IR mice (Son et al., 2013).

The inhibitory effects of O- and C-glycosyl flavonoids on SGLT2 and SGLT1 are crucial for understanding the value of these flavonoids as possible hypoglycemic agents. In silico docking scores of orientin, isoorientin, luteolin, and apigenin indicated these compounds to be better inhibitors of SGLT2 than dapagliflozin, a known inhibitor of SGLT2 (Annapurna et al., 2013). Phloridzin (phloretin-2'-O-glucoside), a nonselective SGLT1 and SGLT2 inhibitor, has been shown to protect against the deleterious effects of diabetic cardiomyopathy in *db/db* mice (Cai et al., 2013). However, O-glycosyl flavonoids are vulnerable to hydrolysis in the gut, while C-glycosyl derivatives such as aspalathin are generally more metabolically stable and should have greater bioactivity in vivo (Zhou et al., 2010). The activation of AMPK by aspalathin and other rooibos phenolic compounds, as well as PPAG, has profound metabolic implications. Similar to the effects of metformin, polyphenols





**Table 4.** Summary of in vitro and in vivo studies related to the anti-obesity, anti-diabetic, insulin resistance, anti-inflammatory, anti-hypertensive and enzyme inhibitory activity of rooibos polyphenols.

Compound	Bioactivity	Model	Effective concentration/dose	Outcome	Reference
Aspalathin	Antidiabetic	C2C12 muscle cells	1 – 100 $\mu\text{M}$	Increased glucose uptake	(Muller et al., 2012)
Aspalathin	Antidiabetic	L6 myotubes and RIN-5F pancreatic $\beta$ -cells	1 – 100 $\mu\text{M}$ for glucose uptake in L6 cells; 100 $\mu\text{M}$ for insulin secretion stimulation in RIN-5F cells	Increased glucose uptake in L6 myotubes; stimulated insulin secretion in RIN-5F cells	(Kawano et al., 2009)
Aspalathin	Antidiabetic	Diabetic <i>db/db</i> mice	Aspalathin dosed at 0.1% (initial two weeks) and 0.2% (subsequent three weeks) of food mixture; for IPGTT, aspalathin (20 mg/mL/kg BW) was administered by oral gavage	Decreased fasting plasma glucose; improved impaired glucose tolerance	(Kawano et al., 2009)
Aspalathin	Antidiabetic	L6 myocytes and RIN-5F pancreatic $\beta$ -cells	25 – 100 $\mu\text{M}$	Concentration dependently increased glucose uptake in L6 myocytes; protected RIN-5F cells against AGE-induced increase of ROS	(Son et al., 2013)
Aspalathin	Antidiabetic	Obese diabetic <i>ob/ob</i> mice	0.1% aspalathin mixed with food equalling ca. 106 mg/kg BW/day/mouse; for IPGTT aspalathin (10 mg/kg BW) in CMC was administered by oral gavage.	Suppressed increase in fasting blood glucose levels; improved glucose intolerance; decreased expression of hepatic gluconeogenic and lipogenic genes	(Son et al., 2013)
Aspalathin	Antidiabetic	Palmitate-induced insulin resistant C2C12 muscle, 3T3-L1 adipocytes and C3A liver cells	10 $\mu\text{M}$	Ameliorated palmitate-induced reduction in glucose uptake; mitochondrial activity and cellular ATP; inhibited NF- $\kappa$ B and PKC $\beta$ activation; enhanced activation of Akt and AMPK; increased Glut4 protein expression; decreased malonyl CoA; increased CPT-1 protein expression	(Mazibuko et al., 2015)
Isoorientin	Antidiabetic	STZ-induced diabetic rats	10 mg/kg BW (acute dose)	Reduced STZ-induced hyperglycemia	(Andrade-Cetto and Wriedenfeld, 2001)
Isoorientin	Antidiabetic	STZ-induced diabetic rats	15 and 30 mg/kg BW for 30 days	Improved hyperglycemia and hyperlipidemia; prevented weight loss	(Sezik et al., 2005)
Isoorientin	Antidiabetic	In silico computational analysis	50 $\mu\text{M}$	Ligands for PPAR- $\gamma$ and HMG CoA reductase	(Fidan et al., 2009)
Isoorientin	Antidiabetic	TNF- $\alpha$ -induced insulin resistant 3T3-F442A and human preadipocytes		Stimulated glucose uptake by enhancing insulin signaling and genes encoding for insulin signaling	(Alonso-Castro et al., 2012)
Isoorientin	Antiinflammatory	TNF- $\alpha$ treated HaCaT human keratinocytes and wound healing in 3T3 mouse fibroblasts	50 – 100 $\mu\text{M}$ for anti-inflammatory activity; 10 $\mu\text{M}$ for improved wound closure	Inhibited TNF- $\alpha$ -induced release of IL-8 and vascular endothelial growth factor; concentration-dependently reduced IL-6 levels; improved wound closure by up to 54% in 3T3 fibroblasts	(Wedler et al., 2014)
Orientin	Cardioprotective effect	Rat heart I/R model; isolated rat cardiomyocytes injured by hypoxia/reoxygenation	0.5 – 2.0 mg/kg BW pretreatment for ischemia/reperfusion; 3 – 30 $\mu\text{M}$ for isolated cardiomyocytes	Protected I/R myocardium by upregulation of BCL-2/BAX ratio; reduced cytochrome c and caspase-3 expression.	(Fu et al., 2006)
Orientin	Cardioprotective effect	H9c2 cardiomyocytes I/R injury by oxygen-glucose deprived solution	30 $\mu\text{M}$	Protected H9c2 cardiomyocytes against I/R-induced apoptosis by modulating the mPTP opening, possibly via PI3K/Akt signaling	(Lu et al., 2011)
Vitexin, isovitexin, isoorientin and luteolin	Antidiabetic	$\alpha$ -Glucosidase enzyme inhibition assay	IC <sub>50</sub> ( $\mu\text{M}$ ): vitexin 25.11, isovitexin 23.26, orientin 23.30, isoorientin 19.68 and luteolin 13.07	Strong mixed non-competitive and anti-competitive $\alpha$ -glucosidase inhibitory activity	(Li et al., 2009a)
Vitexin and isovitexin	Antidiabetic	Sucrose oral tolerance in normoglycemic mice	1 mg/kg BW (single dose)	Reduced sucrose induced glycemia in normoglycemic mice at 30 and 60 min	(Choo et al., 2012)
Vitexin and isovitexin	Antidiabetic	Sucrose oral tolerance in STZ-induced diabetic rats	200 mg/kg BW of vitexin or 100 mg/kg BW of isovitexin	Reduced sucrose induced hyperglycemia in STZ-induced diabetic rats at 30, 60 and 90 min	(Choi et al., 2014a)
Vitexin and isovitexin	Antidiabetic	Inhibition of RLAR, HRAR, AGE, and PTP1B	Isovitexin IC <sub>50</sub> ( $\mu\text{M}$ ) inhibitor values for RLAR 0.49, HRAR 0.13, AGE 175.66, and PTP1B 17.76. Vitexin IC <sub>50</sub> ( $\mu\text{M}$ ) inhibitor values for RLAR 1.47, HRAR 12.07, AGE 243.54, and PTP1B 7.62.	Isovitexin showed strong inhibitory activity against RLAR, HRAR and AGE formation; vitexin showed strong inhibitory activity against PTP1B	(Choi et al., 2014a)

Vitexin	Antiobesity	3T3-L1 adipocytes	50 – 100 $\mu$ M	Suppressed glycolysis via activation of ERK 1/2 MAPK signaling; inhibited adipose accumulation, glucose consumption and triglyceride synthesis; increased ERK 1/2 MAPK expression and suppressed adipogenic markers, Akt and PPAR- $\gamma$ in 3T3-L1 cells with vitexin and orientin the most effective; vitexin and orientin decreased C/EBP $\alpha$ and PPAR- $\gamma$ protein expression in 3T3-L1 cells and interacted with the glucose transporters SGLT2, GLUT4 and PPAR- $\gamma$ ; interaction compared well with that of the phase III drug, dapagliflozin; weak or partial PPAR- $\gamma$ agonists (luteolin and orientin)	(Lee et al., 2014)
Luteolin, Vitexin, and orientin	Antiobesity	3T3-L1 adipocytes	50 – 100 $\mu$ M	All compounds reduced lipid droplet accumulation in 3T3-L1 cells with vitexin and orientin the most effective; vitexin and orientin decreased C/EBP $\alpha$ and PPAR- $\gamma$ protein expression in 3T3-L1 cells and interacted with the glucose transporters SGLT2, GLUT4 and PPAR- $\gamma$ ; interaction compared well with that of the phase III drug, dapagliflozin; weak or partial PPAR- $\gamma$ agonists (luteolin and orientin)	(Kim et al., 2010)
Luteolin, orientin and isoorientin	Antidiabetic	In silico			(Annapurna et al., 2013)
Luteolin	Antidiabetic	RINm5F (RIN) rat insulinoma cells treated with IL-1 $\beta$ and IFN- $\gamma$	25 – 50 $\mu$ M	Protected against IL-1 $\beta$ and IFN- $\gamma$ -mediated cytotoxicity; suppressed NO production, iNOS messenger RNA and protein expression; inhibited NF- $\kappa$ B activation	(Kim et al., 2007)
Luteolin	Antiobesity	Polar screen PPAR- $\gamma$ ligand-binding competitive assay	IC <sub>50</sub> value of 3.9 $\mu$ M	Moderate PPAR- $\gamma$ antagonist activity	(Mueller and Jungbauer, 2009)
Luteolin	Cardioprotective	Rat cardiomyocytes (Langendorff heart perfusion apparatus)	Administered i.v. 10 mg/kg BW to STZ rats for 3 consecutive days	Protected against ischemic reperfusion injury; inhibited MPO expression and inflammatory cytokine production, including IL-6, IL-1 $\alpha$ and TNF- $\alpha$	(Sun et al., 2012)
Luteolin, orientin and isoorientin	Antidiabetic	PTP1B and RLAR inhibition	PTP1B inhibition IC <sub>50</sub> ( $\mu$ M) luteolin 6.70, isoorientin 24.54 orientin 57.1; RLAR IC <sub>50</sub> ( $\mu$ M) luteolin 0.78, isoorientin 1.85, orientin 1.79	Luteolin was the most potent PTP1B inhibitor followed by isoorientin and orientin; luteolin also exhibited the highest RLAR inhibition	(Choi et al., 2014b)
Luteolin, orientin and isoorientin	Antiinflammatory activities	NO production and iNOS protein expression in LPS-stimulated RAW 264.7 cells	5, 15, 30 $\mu$ M	Luteolin suppressed NO production and iNOS expression; orientin and isoorientin not effective at these doses	(Choi et al., 2014b)
Luteolin	Cardioprotective	Male Wistar rat heart perfusion model (Langendorff heart perfusion apparatus) and isolated cardiomyocytes	Pretreatment with 40 mM luteolin for 30 min before induction of ischemic reperfusion injury	Reduced infarct size, LDH activity, decreased apoptosis and increased BCL-2/BAX ratio; improved cardiomyocyte contractile function after ischemic reperfusion injury via an ERK1/2-PI3a-PLB-SERCA2a mediated mechanism independent of JNK signaling pathway	(Wu et al., 2013)
Luteolin	Hypolipidemic	Palmitate-induced steatosis in HepG2 liver cells	20 – 100 $\mu$ M	Enhanced phosphorylation of AMPK and acetyl-CoA carboxylase; decreased gene expression of SREBP-1c and FAS and increased CPT-1 gene expression leading to a decrease in the intracellular lipid levels in HepG2 cells; attenuated palmitate-induced ROS generation.	(Liu et al., 2011)
Luteolin	Antiinflammatory	Experimentally induced allergic encephalomyelitis (an experimental model of MS) induced acutely in Lewis rat and chronically in adult male Dark Agouti rats	50 mg/kg BW daily i.p. from day 6 to 18 during acute EAE induction and from day 6 to 24 or day 15 to 24 during chronic EAE induction; oral administration from day 3 after induction with 100 mg/kg BW luteolin	Suppressed clinical symptoms and prevented relapse when administered either before or after disease onset; reduced inflammation and axonal damage in the CNS by preventing monocyte migration across the brain endothelium modulated by Rho GTPases; oral administration of luteolin delayed the onset of clinical symptoms	(Hendriks et al., 2004)
Luteolin	Antiinflammatory	In vivo LPS-induced inflammation in mice	EC <sub>50</sub> 20 to 27 $\mu$ M	Reduced TNF- $\alpha$ production	(Ruiz and Haller, 2006)

(Continued on next page)



Table 4. (Continued).

Compound	Bioactivity	Model	Effective concentration/dose	Outcome	Reference
Luteolin	Antiinflammatory	LPS-activated murine microglial cells BV-2	50 $\mu$ M	Concentration-dependently suppressed pro-inflammatory and pro-apoptotic gene expression; induced phagocytic uptake, ramification, and chemotaxis; strongly reduced NO secretion	(Dirschel et al., 2010, 2012)
Luteolin	Antiinflammatory	NIH-3T3 and K562 cells; male JH-ICR mice carrageenan-induced paw inflammation model	20 – 30 $\mu$ M; 25 mg/kg BW	Inhibited TNF $\alpha$ -induced NF- $\kappa$ B activation and expression of CCL2 and CXCL1; inhibited acute carrageenan-induced paw edema in mice	(Funakoshi-Tago et al., 2011)
Luteolin	Antiinflammatory	LPS-activated RAW 264.7 cells	50 $\mu$ M	Inhibited production of NO and prostaglandin E2 and the enzymes: iNOS and COX-2; attenuated activation of NF- $\kappa$ B and AP-1; inhibited Akt phosphorylation	(Park and Song, 2013)
PPAG	Antidiabetic	In vitro glucose uptake in Chang cells; high-fat and sucrose/fructose fed obese insulin-resistant rats	Effective range 1.0 – 31.6 $\mu$ M (EC <sub>50</sub> = 3.6 $\mu$ M); oral dose of 0.3 mg/kg BW daily for two weeks; dose increased to 3 mg/kg BW on a third week	Enhanced glucose uptake in Chang cells; lowered fasting glucose concentrations and improved oral glucose tolerance values; increased mRNA expression of glucokinase, GLUT1, GLUT2, insulin receptor, PPAR- $\alpha$ , and SOCS-3 in the liver	(Muller et al., 2013)
PPAG	Antidiabetic	High-fat and sucrose/fructose fed obese RIP-CreERT/Rosa26-LSL-LacZ mice	10 mg/kg BW by oral gavage for six weeks	Protected obese mice from diet-induced hyperglycemia; increased pancreatic $\beta$ -cell mass; protected $\beta$ -cells protected against ER stress induced apoptosis by increasing BCL-2 expression	(Mathijs et al., 2014)

Abbreviations: AGE, advanced glycation end products; Akt also known as PKB, protein kinase B; AMPK, 5' AMP-activated protein kinase; AP-1, activator protein 1; ATP, adenosine triphosphate; BAX, BCL-2-like protein 4; BCL-2, antiapoptotic B-cell lymphoma 2; BW, body weight; CCL2, chemokine (C-C motif) ligand 2; C/EBP $\alpha$ , CCAAT-enhancer-binding proteins; CMC, carboxymethyl cellulose; CNS, central nervous system; COX-2, cyclo-oxygenase 2; CPT-1, carnitine palmitoyltransferase 1; CXCL1, chemokine (C-X-C motif) ligand 1; CYP, cytochrome P450; EAE, experimentally induced allergic encephalomyelitis; EC<sub>50</sub>, half maximal effective concentration; EAE, experimentally induced allergic encephalomyelitis; ER, endoplasmic reticulum; ERK 1/2, extracellular signal-regulated kinases 1 and 2; FAS, fatty acid synthase; GLUT2, glucose transporter 2; GLUT4, glucose transporter protein 4; GM-CSF, granulocyte macrophage-colony stimulating factor; HMG CoA, 3-hydroxy-3-methylglutaryl-coenzyme A; HRAR, human recombinant aldose reductase; IC<sub>50</sub>, half maximal inhibitory concentration; IL-1 $\alpha$ , interleukin 1 alpha; IL-1 $\beta$ , interleukin 1 beta; IL-6, interleukin 6; IL-8, interleukin 8; IFN- $\gamma$ , interferon  $\gamma$ ; iNOS, inducible nitric oxide synthase; i.p., intra-peritoneal; IPGT, intraperitoneal glucose tolerance test; I/R, ischemia/reperfusion; i.v., intravenous; JNK, c-Jun N-terminal kinases; LPS, lipopolysaccharide; MPO, myeloperoxidase; mPTP, mitochondrial permeability transition pore; MAPK, mitogen-activated protein kinases; NF- $\kappa$ B, nuclear factor kappa-light-chain-enhancer of activated B cells; NO, nitric oxide; PI3K/Akt, phosphatidylinositol 3'-kinase-Akt; PKC $\beta$ , protein kinase C-beta; PPAG, Z-2-( $\beta$ -D-glucopyranosyloxy)-3-phenylpropanoic acid; PP1 $\alpha$ , type 1 protein phosphatase; PLB, phospholamban; PPAR- $\alpha$ , peroxisome proliferator-activated receptor alpha; PPAR- $\gamma$ , peroxisome proliferator-activated receptor gamma; PIP1B, protein-tyrosine phosphatase 1B; RLAR, rat lens aldose reductase; ROS, reactive oxygen species; SERCA2a, cardiac sarcoplasmic reticulum Ca<sup>2+</sup>-ATPase; SGLT2, sodium-glucose linked transporter 2; SREBP-1c, sterol regulatory factor binding protein-1 c; STZ, streptozotocin; TNF- $\alpha$ , tumor necrosis factor alpha.

activate AMPK that inhibits proteolytic cleavage and activation of SREBP-1c and transcription of the lipogenic pathway in insulin-stimulated hepatocytes, cultured in the presence of high glucose concentrations (Li et al., 2011). AMPK suppression of SREBP-1c by polyphenols could account for some of the beneficial effects including enhanced glucose uptake and metabolism, and improved dyslipidemia, hepatic steatosis, and insulin resistance (Li et al., 2011).

### Antiobesity

Pancreatic lipase (triacylglycerol acyl hydrolase) is a key enzyme in the metabolism of dietary fat as it is responsible for the hydrolysis of 40–70% of triglycerides into monoacylglycerol and fatty acids, which are absorbed in the small intestine (Tucci et al., 2010). A recent approach for the treatment of obesity involves inhibition of dietary fat absorption via inhibition of pancreatic lipase to limit excess calorie intake (Birari and Bhutani, 2007). Luteolin, orientin, and isoorientin displayed pancreatic lipase inhibitory activity (Lee et al., 2010). Of these compounds luteolin was the least effective, indicating that the sugar moiety is an important structural feature for activity. Similarly to  $\alpha$ -glucosidase inhibitory activity, the position of the sugar moiety on the A-ring is important, but in this case C-glycosylation at the C-8 enhanced potency compared to C-glycosylation at the C-6 position with orientin more potent than isoorientin.

In vitro studies, performed in the 3T3-L1 preadipocyte cell line, have also demonstrated that adipocyte development can be inhibited by individual phenolic compounds found in rooibos (Choi et al., 2006), as well as by aqueous rooibos extracts (Sanderson et al., 2014). Mueller and Jungbauer (2009) found that rooibos was a PPAR- $\gamma$  antagonist and thus is able to modulate PPAR- $\gamma$ , a key regulator of adipocyte differentiation. Luteolin was demonstrated to be one of the constituents with moderate PPAR- $\gamma$  binding activity ( $IC_{50} = 3.9 \mu M$ ). Sanderson et al. (2014) demonstrated that a fermented rooibos extract suppressed the expression of PPAR- $\gamma$  and suppressed adipogenesis in undifferentiated 3T3-L1 cells, while Mazibuko et al. (2015) showed that this gene was activated both by rooibos and aspalathin in fully differentiated 3T3-L1 cells. This is an interesting finding in terms of adipogenesis as AMPK and PPAR- $\gamma$  play central, yet opposing roles regulating fatty acid synthesis and lipolysis, respectively (Daval et al., 2005). Activation of AMPK suppresses acetyl-CoA carboxylase (ACC) activity, the de novo rate limiting enzyme for fatty acid synthesis, regulated by PPAR- $\gamma$  (Georgiadi and Kersten, 2012). Suppression of ACC decreases malonyl-CoA allosteric inhibition of carnitine palmitoyl-transferase I (CPT1) activity, thereby enhancing fatty acid oxidation, while PPAR- $\gamma$  enhances fatty acid synthesis and adipogenesis (Rasmussen et al., 2002; Zammit, 2008). This would suggest that rooibos potentially suppresses differentiation of new adipocytes from adipose-derived stem cells within the adipose tissue, while also enhancing the lipid accumulation of existing adipocytes which could have important implications for the prevention of obesity driven by hyperplastic accumulation of adipocytes (Sun et al., 2011). In vivo treatment of obese *ob/ob* mice with aspalathin reduced fasting blood glucose levels, increased

adiponectin levels and reduced hypertriglyceridemia and serum TBARS levels, a marker of ROS (Son et al., 2013).

### Cardiovascular disease

Insulin resistance (IR) and resultant obesity are major risk factors for the development of T2D and CVD (Must et al., 1999). In South Africa in 2000, 87% of T2D, 68% of hypertensive disease, 45% of ischemic stroke, and 38% of ischaemic heart disease, were attributable to an elevated body mass index ( $\geq 21 \text{ kg/m}^2$ ) (Joubert et al., 2007). As discussed previously, inflammation, hyperinsulinemia, and an atherogenic lipoprotein profile, characteristics of IR and obesity, are causal factors in endothelial dysfunction and subendothelium deposition of LDL and the development atherosclerotic plaques (Yao et al., 2014). Localized cellular inflammatory processes involving macrophages, leukocytes, and other inflammatory cells produce high levels of proinflammatory cytokines and chemokines. Chemotactic recruitment and accumulation of these cells into arterial wall contribute directly to atherosclerosis progression (Yao et al., 2014). Aspalathin and nothofagin reduced LPS-induced upregulation of the endothelial adhesion molecules, vascular cell adhesion molecule 1 (VCAM-1), intercellular adhesion molecule 1 (ICAM-1) and E-selectin, thereby suppressing neutrophil adherence and migration across LPS-activated human umbilical vein endothelial cells (HUVECs) (Lee and Bae, 2015). Aspalathin and nothofagin also suppressed the inflammatory process induced by LPS in these human endothelial cells by reducing TNF- $\alpha$  and IL-6 secretion and inhibiting the activation of NF- $\kappa B$  and ERK1/2, initiators of proinflammatory response (Lee and Bae, 2015). These anti-inflammatory and vascular protective effects were confirmed in mice, manifested as reduced pulmonary injury after LPS injection (Lee and Bae, 2015). In a limited human study involving 40 healthy participants, six cups of rooibos tea per day for six weeks reduced serum levels of LDL-cholesterol and triacylglycerol and reduced oxidative stress by significantly decreasing lipid peroxidation (as measured by MDA) (Marnewick et al., 2011). In our own laboratory rooibos and aspalathin were also shown to protect cardiomyocytes isolated from diabetic rats against experimentally induced oxidative stress, suggesting that the consumption of rooibos, apart from reducing CVD risk, could also offer protection to the vulnerable cardiomyocyte in diabetics (Dludla et al., 2014). Luteolin protected isolated rat cardiomyocytes against ischemic reperfusion injury by inhibiting myeloperoxidase and the inflammatory cytokines, IL-6, IL-1 $\alpha$ , and TNF- $\alpha$  (Sun et al., 2012). Similar cardioprotective effects against ischemia were demonstrated in an isolated heart and cardiomyocyte model where luteolin improved cardiomyocyte contractile function, reduced infarct size and cell death as measured by LDH activity and decreased the rate of apoptosis by increasing the BCL-2/Bax ratio (Wu et al., 2013).

### Challenges to advance rooibos beyond a healthy beverage

Current evidence suggests that rooibos C-glucosyl flavonoids and PPAG play a major role in the health promoting properties of its extracts and infusions. The plant material exhibits large



inherent variation in phenolic content, with processing introducing further variation in phenolic content and profile. The use of nonstandardized extracts and infusions and lack of characterization of their phenolic composition using validated methods in many studies to date pose a major challenge in interpreting and validating results in follow-up studies. This lack of important scientific information, often overlooked in some papers, does not help to advance the knowledge base required for proper substantiation of the positive and/or negative outcomes of such studies. Research to date using different models confirmed rooibos as a healthy beverage, but it does not meet the regulatory criteria required to make health claims for a rooibos product. Future research should take this into account when designing studies with this purpose in mind. An alternative to the inherent compositional variation of rooibos is the use of pure compounds or combinations of compounds, isolated from the plant material, to ensure effective dose delivery and insight into their relative importance as rooibos bioactive compounds. Given the existing knowledge base and their predominance in rooibos, obvious choices are aspalathin, PPAG, orientin and isoorientin.

## Acknowledgments

Funding for collaboration was provided by the National Research Foundation (NRF) of South Africa (grants 75425, 85105 and 95520 to E. Joubert.) and the Japanese Society for the Promotion of Science (grants to K. Yagasaki and Y. Miura). The NRF grant holder (EJ) acknowledges that opinions, findings, and conclusions or recommendations expressed in any publication generated by the NRF supported research are those of the authors, and that the NRF accepts no liability whatsoever in this regard. The funding bodies had no involvement in the study design, collection, analysis and interpretation of data, writing of the manuscript, or decision to publish the work.

## ORCID

Christo J. F. Muller  <http://orcid.org/0000-0001-6821-2120>  
 Christiaan J. Malherbe  <http://orcid.org/0000-0001-8485-7877>  
 Nireshni Chellan  <http://orcid.org/0000-0001-9968-0998>  
 Kazumi Yagasaki  <http://orcid.org/0000-0002-9494-9887>  
 Yutaka Miura  <http://orcid.org/0000-0002-8113-8014>  
 Elizabeth Joubert  <http://orcid.org/0000-0002-9717-9769>

## References

Abdul-Ghani, M. A. and DeFronzo, R. A. (2010). Pathogenesis of insulin resistance in skeletal muscle. *J. Biomed. Biotechnol.* **2010**. DOI: 10.1155/2010/476279.

Alonso-Castro, A. J., Zapata-Bustos, R., Gómez-Espinoza, G. and Salazar-Olivo, L. A. (2012). Isoorientin reverts TNF- $\alpha$ -induced insulin resistance in adipocytes activating the insulin signaling pathway. *Endocrinology*. **153**:5222–5230.

Andrade-Cetto, A. and Wiedenfeld, H. (2001). Hypoglycemic effect of *Cecropia obtusifolia* on streptozotocin diabetic rats. *J. Ethnopharmacol.* **78**:145–149.

Annapurna, H. V., Apoorva, B., Ravichandran, N., Arun, K. P., Brindha, P., Swaminathan, S., Vijayalakshmi, M. and Nagarajan, A. (2013). Isolation and in silico evaluation of antidiabetic molecules of *Cynodon dactylon* (L.). *J. Mol. Graphics Modell.* **39**:87–97.

Anonymous. (2013). The labelling of rooibos and the rules of use for rooibos. Annexure, Notice 911 of 2013. The Government Gazette of South Africa. No 36820, 6 September 2013.

Anonymous. (2014). Geographical indications from the Republic of South Africa (2014/C 51/09). Official Journal of the European Union C051, 22 February 2014.

Beelders, T., Kalili, K. M., Joubert, E., de Beer, D. and de Villiers, A. (2012a). Comprehensive two-dimensional liquid chromatographic analysis of rooibos (*Aspalathus linearis*) phenolics. *J. Sep. Sci.* **35**:1808–1820.

Beelders, T., Sigge, G. O., Joubert, E., de Beer, D. and de Villiers, A. (2012b). Kinetic optimisation of the reversed phase liquid chromatographic separation of rooibos tea (*Aspalathus linearis*) phenolics on conventional high performance liquid chromatographic instrumentation. *J. Chromatogr. A.* **1219**:128–139.

Beltrán-Debón, R., Rull, A., Rodríguez-Sanabria, F., Iswaldi, I., Herranz-López, M., Aragonès, G., Camps, J., Alonso-Villaverde, C., Menéndez, J. A., Micol, V., Segura-Carretero, A. and Joven, J. (2011). Continuous administration of polyphenols from aqueous rooibos (*Aspalathus linearis*) extract ameliorates dietary-induced metabolic disturbances in hyperlipidemic mice. *Phytomedicine*. **18**:414–424.

Bensellam, M., Laybutt, D. R. and Jonas, J.-C. (2012). The molecular mechanisms of pancreatic  $\beta$ -cell glucotoxicity: Recent findings and future research directions. *Mol. Cell. Endocrinol.* **364**:1–27.

Birari, R. B. and Bhutani, K. K. (2007). Pancreatic lipase inhibitors from natural sources: Unexplored potential. *Drug Discov. Today*. **12**:879–889.

Blume, N., Skouv, J., Larsson, L. I., Holst, J. J. and Madsen, O. D. (1995). Potent inhibitory effects of transplantable rat glucagonomas and insulinomas on the respective endogenous islet cells are associated with pancreatic apoptosis. *J. Clin. Invest.* **96**:2227–2235.

Boath, A. S., Stewart, D. and McDougall, G. J. (2012). Berry components inhibit  $\alpha$ -glucosidase in vitro: Synergies between acarbose and polyphenols from black currant and rowanberry. *Food Chem.* **135**:929–936.

Bradley, R., Sherman, K., Catz, S., Calabrese, C., Jordan, L., Grothaus, L. and Cherkin, D. (2011). Survey of CAM interest, self-care, and satisfaction with health care for type 2 diabetes at group health cooperative. *BMC Complementary Altern. Med.* **11**:121.

Braune, A. and Blaut, M. (2011). Deglycosylation of puerarin and other aromatic C-glucosides by a newly isolated human intestinal bacterium. *Environ. Microbiol.* **13**:482–494.

Braune, A. and Blaut, M. (2012). Intestinal bacterium *Eubacterium cellulosolvens* deglycosylates flavonoid C- and O-glucosides. *Appl. Environ. Microbiol.* **78**:8151–8153.

Braune, A., Gütschow, M. I., Engst, W. and Blaut, M. (2001). Degradation of quercetin and luteolin by *Eubacterium ramulus*. *Appl. Environ. Microbiol.* **67**:5558–5567.

Breiter, T., Laue, C., Kressel, G., Gröll, S., Engelhardt, U. H. and Hahn, A. (2011). Bioavailability and antioxidant potential of rooibos flavonoids in humans following the consumption of different rooibos formulations. *Food Chem.* **128**:338–347.

Butler, A. E., Janson, J., Bonner-Weir, S., Ritzel, R., Rizza, R. A. and Butler, P. C. (2003).  $\beta$ -cell deficit and increased  $\beta$ -cell apoptosis in humans with type 2 diabetes. *Diabetes*. **52**:102–110.

Cai, Q., Li, B., Yu, F., Lu, W., Zhang, Z., Yin, M. and Gao, H. (2013). Investigation of the protective effects of phlorizin on diabetic cardiomyopathy in db/db mice by quantitative proteomics. *J. Diabetes Res.* **2013**. DOI: 10.1155/2013/263845.

Chen, Y.-G., Li, P., Li, P., Yan, R., Zhang, X.-Q., Wang, Y., Zhang, X.-T., Ye, W.-C. and Zhang, Q.-W. (2013).  $\alpha$ -Glucosidase inhibitory effect and simultaneous quantification of three major flavonoid glycosides in *Microctis folium*. *Molecules*. **18**:4221–4232.

Choi, I., Park, Y., Choi, H. and Lee, E. H. (2006). Anti-adipogenic activity of rutin in 3T3-L1 cells and mice fed with high-fat diet. *BioFactors*. **26**:273–281.

Choi, J. S., Islam, M. N., Ali, Y., Kim, E. J., Kim, Y. M. and Jung, H. A. (2014a). Effects of C-glycosylation on anti-diabetic, anti-Alzheimer's disease and anti-inflammatory potential of apigenin. *Food. Chem. Toxicol.* **64**:27–33.

Choi, J. S., Islam, N., Ali, Y., Kim, Y. M., Park, H. J., Sohn, H. S. and Hyun, A. J. (2014b). The effects of C-glycosylation of luteolin on its antioxidant, anti-Alzheimer's disease, anti-diabetic, and anti-inflammatory activities. *Arch. Pharm. Res.* **37**:1354–1363.

- Choo, C. Y., Sulong, N. Y., Man, F. and Wong, T. W. (2012). Vitexin and isovitexin from the leaves of *Ficus deltoidea* with in-vivo  $\alpha$ -glucosidase inhibition. *J. Ethnopharmacol.* **142**:776–781.
- Courts, F. L. and Williamson, G. (2009). The C-glycosyl flavonoid, aspalathin, is absorbed, methylated and glucuronidated intact in humans. *Mol. Nutr. Food Res.* **53**:1104–1111.
- Courts, F. L. and Williamson, G. (2013). The occurrence, fate and biological activities of C-glycosyl flavonoids in the human diet. *Crit. Rev. Food Sci. Nutr.* **55**:1352–1367.
- Danaei, G., Finucane, M. M., Lu, Y., Singh, G. M., Cowan, M. J., Paciorek, C. J., Lin, J. K., Farzadfar, F., Khang, Y.-H., Stevens, G. A., Rao, M., Ali, M. K., Riley, L. M., Robinson, C. A. and Ezzati, M. (2011). National, regional, and global trends in fasting plasma glucose and diabetes prevalence since 1980: Systematic analysis of health examination surveys and epidemiological studies with 370 country-years and 2.7 million participants. *Lancet.* **378**:31–40.
- Daval, M., Diot-Dupuy, F., Bazin, R., Hainault, I., Viollet, B., Vaulont, S., Hajduch, E., Ferré, P. and Foufelle, F. (2005). Anti-lipolytic action of AMP-activated protein kinase in rodent adipocytes. *J. Biol. Chem.* **280**:25250–25257.
- Dennis, T., Fanous, M. and Mousa, S. (2009). Natural products for chemopreventive and adjunctive therapy in oncologic disease. *Nutr. Cancer.* **61**:587–597.
- Dirscherl, K., Karlstetter, M., Ebert, S., Kraus, D., Hlawatsch, J., Walczak, Y., Moehle, C., Fuchshofer, R. and Langmann, T. (2010). Luteolin triggers global changes in the microglial transcriptome leading to a unique anti-inflammatory and neuroprotective phenotype. *J. Neuroinflammation.* **7**:3.
- Dirscherl, K., Karlstetter, M., Ebert, S., Kraus, D., Hlawatsch, J., Walczak, Y., Moehle, C., Fuchshofer, R. and Langmann, T. (2012). Correction: Luteolin triggers global changes in the microglial transcriptome leading to a unique anti-inflammatory and neuroprotective phenotype. *J. Neuroinflammation.* **9**:118.
- Dludla, P. V., Muller, C. J. F., Louw, J., Joubert, E., Salie, R., Opoku, A. R. and Johnson, R. (2014). The cardioprotective effect of an aqueous extract of fermented rooibos (*Aspalathus linearis*) on cultured cardiomyocytes derived from diabetic rats. *Phytomedicine.* **21**:595–601.
- Dong, H., Lin, W., Wu, J. and Chen, T. (2010). Flavonoids activate pregnane x receptor-mediated CYP3A4 gene expression by inhibiting cyclin-dependent kinases in HepG2 liver carcinoma cells. *BMC Biochem.* **11**:23.
- Dowman, J. K., Tomlinson, J. W. and Newsome, P. N. (2010). Pathogenesis of non-alcoholic fatty liver disease. *QJM Int. J. Med.* **103**:71–83.
- Ferré, P. and Foufelle, F. (2010). Hepatic steatosis: A role for de novo lipogenesis and the transcription factor SREBP-1c. *Diabetes, Obes. Metab.* **12**:83–92.
- Fidan, O., Aslan, M. and Mor, M. (2009). Computational evaluation of isorientin (C-glycosyl flavone) on PPAR-gamma receptors and HMG-CoA reductase using MOE 2008.10. *Planta Med.* **75**:PH27.
- Fraga, C. G., Galleano, M., Verstraeten, S. V. and Oteiza, P. I. (2010). Basic biochemical mechanisms behind the health benefits of polyphenols. *Mol. Aspects Med.* **31**:435–445.
- Francisco, N. M. (2010). Modulation of Postprandial Oxidative Stress by Rooibos (*Aspalathus linearis*) in Normolipidaemic Individuals. M. Tech thesis. Cape Peninsula University of Technology, Cape Town, South Africa.
- Fu, X. C., Wang, M. W., Li, S. P. and Wang, H. L. (2006). Anti-apoptotic effect and the mechanism of orientin on ischaemic/reperfused myocardium. *J. Asian Nat. Prod. Res.* **8**:265–272.
- Funakoshi-Tago, M., Nakamura, K., Tago, K. I., Mashino, T. and Kasahara, T. (2011). Anti-inflammatory activity of structurally related flavonoids, apigenin, luteolin and fisetin. *Int. Immunopharmacol.* **11**:1150–1159.
- Gehrmann, W., Elsner, M. and Lenzen, S. (2010). Role of metabolically generated reactive oxygen species for lipotoxicity in pancreatic  $\beta$ -cells. *Diabetes, Obes. Metab.* **12**:149–158.
- Georgiadi, A. and Kersten, S. (2012). Mechanisms of gene regulation by fatty acids. *Adv. Nutr.* **3**:127–134.
- Goldin, A., Beckman, J. A., Schmidt, A. M. and Creager, M. A. (2006). Advanced glycation end products. Sparking the development of diabetic vascular injury. *Circulation.* **114**:597–605.
- Grundy, S. M., Hansen, B., Smith, S. C., Cleeman, J. I., Kahn, R. A. for conference participants. (2004). Clinical management of metabolic syndrome: Report of the American Heart Association/National Heart, Lung, and Blood Institute/American Diabetes Association Conference on Scientific Issues Related to Management. *Arterioscler., Thromb., Vasc. Biol.* **24**:e19–e24.
- Gustafson, B., Gogg, S., Hedjazifar, S., Jenndahl, L., Hammarstedt, A. and Smith, U. (2009). Inflammation and impaired adipogenesis in hypertrophic obesity in man. *Am. J. Physiol. Endocrinol. Metab.* **297**:E999–E1003.
- Ha, T. J., Lee, J. H., Lee, M.-H., Lee, B. W., Kwon, H. S., Park, C.-H., Shim, K.-B., Kim, H.-T., Baek, I.-Y. and Jang, D. S. (2012). Isolation and identification of phenolic compounds from the seeds of *Perilla frutescens* (L.) and their inhibitory activities against  $\alpha$ -glucosidase and aldose reductase. *Food Chem.* **135**:1397–1403.
- Hasan, S. S., Loon, W. C. W., Ahmadi, K., Ahmed, S. I. and Bukhari, N. I. (2011). Reasons, perceived efficacy and factors associated with complementary and alternative medicine use among Malaysian patients with diabetes mellitus. *Br. J. Diabetes Vasc. Dis.* **11**:92–98.
- Hattori, M., Shu, Y.-Z., El-Sedawy, A. I. and Namba, T. (1988). Metabolism of homoorientin by human intestinal bacteria. *J. Nat. Prod.* **51**:874–878.
- Hays, N. P., Galassetti, P. R. and Coker, R. H. (2008). Prevention and treatment of type 2 diabetes: Current role of lifestyle, natural product, and pharmacological interventions. *Pharmacol. Ther.* **118**:181–191.
- Heine, R. J. and Dekker, J. M. (2002). Beyond postprandial hyperglycaemia: Metabolic factors associated with cardiovascular disease. *Diabetologia.* **45**:461–475.
- Heinrich, T., Willenberg, I. and Glomb, M. A. (2012). Chemistry of color formation during rooibos fermentation. *J. Agric. Food Chem.* **60**:5221–5228.
- Hendricks, R. and Pool, E. J. (2010). The in vitro effects of rooibos and black tea on immune pathways. *J. Immunoassay Immunochem.* **31**:169–180.
- Hendriks, J. J. A., Alblas, J., van der Pol, S. M. A., van Tol, E. A. F., Dijkstra, C. D. and de Vries, H. E. (2004). Flavonoids influence monocyte GTPase activity and are protective in experimental allergic encephalitis. *J. Exp. Med.* **200**:1667–1672.
- Huang, M., Du Plessis, J., Du Preez, J., Hamman, J. and Viloen, A. (2008). Transport of aspalathin, a rooibos tea flavonoid, across the skin and intestinal epithelium. *Phytother. Res.* **22**:669–704.
- Izumi, T., Kasai, K. and Gomi, H. (2007). Secretory vesicle docking to the plasma membrane: Molecular mechanism and functional significance. *Diabetes, Obes. Metab.* **9**:109–117.
- Jo, S.-H., Ka, E.-H., Lee, H.-S., Apostolidis, E., Jang, H.-D. and Kwon, Y.-I. (2009). Comparison of antioxidant potential and rat intestinal  $\alpha$ -glucosidase inhibitory activities of quercetin, rutin, and isoquercetin. *Int. J. Appl. Res. Nat. Prod.* **2**:52–60.
- Joubert, E. (1996). HPLC quantification of the dihydrochalcones, aspalathin and nothofagin in rooibos tea (*Aspalathus linearis*) as affected by processing. *Food Chem.* **55**:403–411.
- Joubert, E., Beelders, T., de Beer, D., Malherbe, C. J., de Villiers, A. J. and Sigge, G. O. (2012). Variation in phenolic content and antioxidant activity of fermented rooibos herbal tea infusions: Role of production season and quality grade. *J. Agric. Food Chem.* **60**:9171–9179.
- Joubert, E. and de Beer, D. (2011). Rooibos (*Aspalathus linearis*) beyond the farm gate: From herbal tea to potential phytopharmaceutical. *S. Afr. J. Bot.* **77**:869–886.
- Joubert, E. and de Beer, D. (2012). Phenolic content and antioxidant activity of rooibos food ingredient extracts. *J. Food Comp. Anal.* **27**:45–51.
- Joubert, E., de Beer, D., Malherbe, C. J., Muller, N., Bonnet, S. L., van der Westhuizen, J. H. and Ferreira, D. (2013). Occurrence and sensory perception of Z-2-( $\beta$ -D-glucopyranosyloxy)-3-phenylpropenoic acid in rooibos (*Aspalathus linearis*). *Food Chem.* **136**:1078–1085.
- Joubert, E., Gelderblom, W. C. A., Louw, A. and de Beer, D. (2008). South African herbal teas: *Aspalathus linearis*, *Cyclopia* spp. and *Athrixia phylloides*—A review. *J. Ethnopharmacol.* **119**:376–412.
- Joubert, E., Winterton, P., Britz, T. J. and Ferreira, D. (2004). Superoxide anion and  $\alpha$ ,  $\alpha$ -diphenyl- $\beta$ -picrylhydrazyl radical scavenging capacity of rooibos (*Aspalathus linearis*) aqueous extracts, crude phenolic fractions, tannin and flavonoids. *Food Res. Int.* **37**:133–138.

- Joubert, J., Norman, R., Bradshaw, D., Goedecke, J. H., Steyn, N. P., Puoane, T. and South African Comparative Risk Assessment Collaborating Group. (2007). Estimating the burden of disease attributable to excess body weight in South Africa in 2000. *S. Afr. Med. J.* **97**:683–690.
- Kahn, S. E., Cooper, M. E. and Del Prato, S. (2014). Pathophysiology and treatment of type 2 diabetes: Perspectives on the past, present and future. *Lancet*. **383**:1068–1083.
- Kahn, S. E., Hull, R. L. and Utzschneider, K. M. (2006). Mechanisms linking obesity to insulin resistance and type 2 diabetes. *Nature*. **444**:840–846.
- Kamakura, R., Son, M. J., de Beer, D., Joubert, E., Miura, Y. and Yagasaki, K. (2015). Antidiabetic effect of green rooibos (*Aspalathus linearis*) extract in cultured cells and type 2 diabetic model KK- $A_y$  mice. *Cyto-technology*. **67**:699–710.
- Kawano, A., Nakamura, H., Hata, S., Minakawa, M., Miura, Y. and Yagasaki, K. (2009). Hypoglycemic effect of aspalathin, a rooibos tea component from *Aspalathus linearis*, in type 2 diabetic model db/db mice. *Phytomedicine*. **16**:437–443.
- Khan, A.-U. and Gilani, A. H. (2006). Selective bronchodilatory effect of Rooibos tea (*Aspalathus linearis*) and its flavonoid, chrysoeriol. *Eur. J. Nutr.* **45**:463–469.
- Kim, E. K., Kwon, K. B., Song, M. Y., Han, M. J., Lee, J. H., Lee, Y. R., Lee, J. H., Ryu, D. G., Park, B. H. and Park, J. W. (2007). Flavonoids protect against cytokine-induced pancreatic beta-cell damage through suppression of nuclear factor  $\kappa$ B activation. *Pancreas*. **35**:e1–9.
- Kim, J.-S., Kwon, C.-S. and Son, K. H. (2000). Inhibition of alpha-glucosidase and amylase by luteolin, a flavonoid. *Biosci. Biotechnol. Biochem.* **64**:2458–2461.
- Kim, J., Lee, I., Seo, J., Jung, M., Kim, Y., Yim, N. and Bae, K. (2010). Vitexin, orientin and other flavonoids from *Spirodela polyrrhiza* inhibit adipogenesis in 3T3-L1 cells. *Phytother. Res.* **24**:1543–1548.
- Kim, M., Lee, J. and Han, J. (2014). Deglycosylation of isoflavone C-glycosides by newly isolated human intestinal bacteria. *J. Sci. Food Agric.* **95**:1925–1931.
- Koch, I. S., Muller, N., de Beer, D., Næs, T. and Joubert, E. (2013). Impact of steam pasteurization on the sensory profile and phenolic composition of rooibos (*Aspalathus linearis*) herbal tea infusions. *Food Res. Int.* **53**:704–712.
- Koeppen, B. H. and Roux, D. G. (1965). Aspalathin: A novel C-glycosylflavonoid from *Aspalathus linearis*. *Tetrahedron Lett.* **6**:3497–3503.
- Kraczyk, N. and Glomb, M. A. (2008). Characterization of phenolic compounds in rooibos tea. *J. Agric. Food Chem.* **56**:3368–3376.
- Kraczyk, N., Heinrich, T., Porzel, A. and Glomb, M. A. (2009a). Oxidation of the dihydrochalcone aspalathin leads to dimerization. *J. Agric. Food Chem.* **57**:6838–6843.
- Kraczyk, N., Woyand, F. and Glomb, M. A. (2009b). Structure–antioxidant relationship of flavonoids from fermented rooibos. *Mol. Nutr. Food Res.* **53**:635–642.
- Kreuz, S., Joubert, E., Waldmann, K.-H. and Ternes, W. (2008). Aspalathin, a flavonoid in *Aspalathus linearis* (rooibos), is absorbed by pig intestine as a C-glycoside. *Nutr. Res.* **28**:690–701.
- Kumar, S., Narwal, S., Kumar, V. and Prakash, O. (2011).  $\alpha$ -Glucosidase inhibitors from plants: A natural approach to treat diabetes. *Pharmacogn. Rev.* **5**:19–29.
- Kunishiro, K., Tai, A. and Yamamoto, I. (2001). Effects of rooibos tea extract on antigen-specific antibody production and cytokine generation in vitro and in vivo. *Biosci. Biotechnol. Biochem.* **65**:2137–2145.
- Kuo, S. M. (2002). Flavonoids and gene expression in mammalian cells. *Adv. Exp. Med. Biol.* **505**:191–200.
- Lee, E. M., Lee, S. S., Chung, B. Y., Cho, J.-Y., Lee, I. C., Ahn, S. R., Jang, S. J. and Kim, T. H. (2010). Pancreatic lipase inhibition by C-glycosidic flavones isolated from *Eremochloa ophiuroides*. *Molecules*. **15**:8251–8259.
- Lee, W. and Bae, J.-P. (2015). Anti-inflammatory effects of aspalathin and nothofagin from rooibos (*Aspalathus linearis*) in vitro and in vivo. *Inflammation*. **38**:1502–1516.
- Lee, Y.-H., Yang, S.-H., Chen, S.-L., Pan, Y.-F., Liu, C.-M., Li, M.-W., Chou, S. S., Chou, M.-Y. and Youn, S.-C. (2014). The anti-adipogenic effect of vitexin is via ERK 1/2 MAPK signaling in 3T3-L1 adipocytes. *Int. J. Pharmacol.* **6**:206–214.
- Li, D., Wang, Q., Yuan, Z., Zhang, L., Xu, L., Cui, Y. and Duan, K. (2008). Pharmacokinetics and tissue distribution study of orientin in rat by liquid chromatography. *J. Pharmaceut. Biomed. Anal.* **47**:429–434.
- Li, H., Song, F., Xing, J., Tsao, R., Liu, Z. and Liu, S. (2009a). Screening and structural characterization of  $\alpha$ -glucosidase inhibitors from hawthorn leaf flavonoids extract by ultrafiltration LC-DAD-MS<sup>n</sup> and SORI-CID FTICR MS. *J. Am. Soc. Mass Spectrom.* **20**:1496–1503.
- Li, Y., Xu, S., Mihaylova, M. M., Zheng, B., Hou, X., Jiang, B., Park, O., Luo, Z., Lefai, E., Shyy, John Y. J., Gao, B., Wierzbicki, M., Verbeuren, Tony J., Shaw, Reuben J., Cohen, Richard A. and Zang, M. (2011). AMPK phosphorylates and inhibits SREBP activity to attenuate hepatic steatosis and atherosclerosis in diet-induced insulin-resistant mice. *Cell Metab.* **13**:376–388.
- Li, Y. Q., Zhou, F. C., Gao, F., Bian, J. S. and Shan, F. (2009b). Comparative evaluation of quercetin, isoquercetin and rutin as inhibitors of  $\alpha$ -glucosidase. *J. Agric. Food Chem.* **57**:11463–11468.
- Lipinski, C. A., Lombardo, F., Dominy, B. W. and Feeney, P. J. (1997). Experimental and computational approaches to estimate solubility and permeability in drug discovery and development settings. *Adv. Drug Delivery Rev.* **23**:3–35.
- Liu, J.-F., Ma, Y., Wang, Y., Du, Z.-Y., Shen, J.-K. and Peng, H.-L. (2011). Reduction of lipid accumulation in HepG2 cells by luteolin is associated with activation of AMPK and mitigation of oxidative stress. *Phytother. Res.* **25**:588–596.
- Lu, N., Sun, Y. and Zheng, X. (2011). Orientin-induced cardioprotection against reperfusion is associated with attenuation of mitochondrial permeability transition. *Planta Med.* **77**:984–991.
- Malviya, N., Jain, S. and Malviya, S. (2010). Antidiabetic potential of medicinal plants. *Acta Pol. Pharm.* **67**:113–118.
- Manach, C., Williamson, G., Morand, C., Scalbert, A. and Rémésy, C. (2005). Bioavailability and bioefficacy of polyphenols in humans. I. Review of 97 bioavailability studies. *Am. J. Clin. Nutr.* **81**:230S–242S.
- Manley, M., Joubert, E. and Botha, M. (2006). Quantification of the major phenolic compounds, soluble solid content and total antioxidant activity of green rooibos (*Aspalathus linearis*) by means of near infrared spectroscopy. *J. Near Infrared Spectrosc.* **14**:213–222.
- Mansukhani, R., Volino, L. and Varghese, R. (2014). Natural products for the treatment of type 2 diabetes mellitus. *Pharmacol. Pharm.* **5**:487–503.
- Marais, C., Janse van Rensburg, W., Ferreira, D. and Steenkamp, J. A. (2000). (S)- and (R)-Eriodictyol-6-C- $\beta$ -D-glucopyranoside, novel keys to the fermentation of rooibos (*Aspalathus linearis*). *Phytochemistry*. **55**:43–49.
- Marais, C., Steenkamp, J. A. and Ferreira, D. (1996). Occurrence of phenylpyruvic acid in woody plants: Biosynthetic significance and synthesis of an enolic glucoside derivative. *J. Chem. Soc., Perkin Trans.* **1**:2915–2918.
- Marais, S. S., Marais, C., Steenkamp, J. A., Malan, E. and Ferreira, D. (1998). Progress in the investigation of rooibos tea extractives. In: *Abstracts of the 3rd Tannin Conference*, Bend, Oregon, USA, pp. 129–130.
- Marnewick, J. L., Rautenbach, F., Venter, I., Neethling, H., Blackhurst, D. M., Wolmarans, P. and Macharia, M. (2011). Effects of rooibos (*Aspalathus linearis*) on oxidative stress and biochemical parameters in adults at risk for cardiovascular disease. *J. Ethnopharmacol.* **133**:46–52.
- Mathijs, I., Da Cunha, D. A., Himpe, E., Ladriere, L., Chellan, N., Roux, C. R., Joubert, E., Muller, C., Cnop, M., Louw, J. and Bouwens, L. (2014). Phenylpropanoic acid glucoside augments pancreatic beta cell mass in high-fat diet-fed mice and protects beta cells from ER stress-induced apoptosis. *Mol. Nutr. Food Res.* **58**:1980–1990.
- Matsui, T., Kobayashi, M., Hayashida, S. and Matsumoto, K. (2002). Luteolin, a flavone, does not suppress postprandial glucose absorption through an inhibition of  $\alpha$ -glucosidase action. *Biosci. Biotechnol. Biochem.* **66**:689–692.
- Mazibuko, S. E., Joubert, E., Johnson, R., Louw, J., Opoku, A. R. and Muller, C. J. F. (2015). Aspalathin improves glucose and lipid metabolism in 3T3-L1 adipocytes exposed to palmitate. *Mol. Nutr. Food Res.* **59**:2199–2208.



- Mazibuko, S. E., Muller, C. J. F., Joubert, E., de Beer, D., Johnson, R., Opoku, A. R. and Louw, J. (2013). Amelioration of palmitate-induced insulin resistance in C2C12 muscle cells by rooibos (*Aspalathus linearis*). *Phytomedicine*. **20**:813–819.
- Memon, R. A., Feingold, K. R., Moser, A. H., Fuller, J. and Grunfeld, C. (1998). Regulation of fatty acid transport protein and fatty acid translocase mRNA levels by endotoxin and cytokines. *Am. J. Physiol. Endocrinol. Metab.* **274**:E210–E217.
- Miranda, P. J., DeFronzo, R. A., Califf, R. M. and Guyton, J. R. (2005). Metabolic syndrome: Evaluation of pathological and therapeutic outcomes. *Am. Heart J.* **149**:20–32.
- Montane, J., Cadavez, L. and Novials, A. (2014). Stress and the inflammatory process: A major cause of pancreatic cell death in type 2 diabetes. *Diabetes, Metab. Syndr. Obes.: Targets Ther.* **7**:25–34.
- Mueller, M., Hobiger, S. and Jungbauer, A. (2010). Anti-inflammatory activity of extracts from fruits, herbs and spices. *Food Chem.* **122**:987–996.
- Mueller, M. and Jungbauer, A. (2009). Culinary plants, herbs and spices – A rich source of PPAR $\gamma$  ligands. *Food Chem.* **117**:660–667.
- Muller, C. J. F., Joubert, E., de Beer, D., Sanderson, M., Malherbe, C. J., Fey, S. J. and Louw, J. (2012). Acute assessment of an aspalathin-enriched green rooibos (*Aspalathus linearis*) extract with hypoglycemic potential. *Phytomedicine*. **20**:32–39.
- Muller, C. J. F., Joubert, E., Pfeiffer, C., Ghoor, S., Sanderson, M., Chellan, N., Fey, S. J. and Louw, J. (2013). Z-2-( $\beta$ -D-glucopyranosyloxy)-3-phenylpropenoic acid, an  $\alpha$ -hydroxy acid from rooibos (*Aspalathus linearis*) with hypoglycemic activity. *Mol. Nutr. Food Res.* **57**:2216–2222.
- Must, A., Spadano, J., Coakley, E. H., Field, A. E., Colditz, G. and Dietz, W. H. (1999). The disease burden associated with overweight and obesity. *JAMA*. **282**:1523–1529.
- Nakamura, K. T. N., Jin, J.-S., Ma, C.-M., Komatsu, K., Iwashima, M. and Hattori, M. (2011). The C-glucosyl bond of puerarin was cleaved hydrolytically by a human intestinal bacterium strain PUE to yield its aglycone daidzein and an intact glucose. *Chem. Pharm. Bull.* **59**:23–27.
- Ng, M., Fleming, T., Robinson, M., Thomson, B., Graetz, N., Margono, C., Mullany, E. C., Biryukov, S., Abbafati, C., Abera, S. F., Abraham, J. P., Abu-Rmeileh, N. M. E., Achoki, T., AlBuhairan, F. S., Alemu, Z. A., Alfonso, R., Ali, M. K., Ali, R., Guzman, N. A., Ammar, W., Anwar, P., Banerjee, A., Barquera, S., Basu, S., Bennett, D. A., Bhutta, Z., Blore, J., Cabral, N., Nonato, I. C., Chang, J.-C., Chowdhury, R., Courville, K. J., Criqui, M. H., Cundiff, D. K., Dabhadkar, K. C., Dandona, L., Davis, A., Dayama, A., Dharmaratne, S. D., Ding, E. L., Durrani, A. M., Esteghamati, A., Farzadfar, F., Fay, D. F. J., Feigin, V. L., Flaxman, A., Forouzanfar, M. H., Goto, A., Green, M. A., Gupta, R., Hafezi-Nejad, N., Hankey, G. J., Harewood, H. C., Havmoeller, R., Hay, S., Hernandez, L., Husseini, A., Idrisov, B. T., Ikeda, N., Islami, F., Jahangir, E., Jassal, S. K., Jee, S. H., Jeffreys, M., Jonas, J. B., Kabagambe, E. K., Khalifa, S. E. A. H., Kengne, A. P., Khader, Y. S., Khang, Y.-H., Kim, D., Kimokoti, R. W., Kinge, J. M., Kokubo, Y., Kosen, S., Kwan, G., Lai, T., Leinsalu, M., Li, Y., Liang, X., Liu, S., Logroscino, G., Lotufo, P. A., Lu, Y., Ma, J., Mainoo, N. K., Mensah, G. A., Merriman, T. R., Mokdad, A. H., Moschandreas, J., Naghavi, M., Naheed, A., Nand, D., Narayan, K. M. V., Nelson, E. L., Neuhouser, M. L., Nisar, M. I., Ohkubo, T., Oti, S. O., Pedroza, A., Prabhakaran, D., Roy, N., Sampson, U., Seo, H., Sepanlou, S. G., Shibuya, K., Shiri, R., Shiue, I., Singh, G. M., Singh, J. A., Skirbekk, V., Stapelberg, N. J. C., Sturua, L., Sykes, B. L., Tobias, M., Tran, B. X., Trasande, L., Toyoshima, H., van de Vijver, S., Vasankari, T. J., Veerman, J. L., Velasquez-Melendez, G., Vlassov, V. V., Vollset, S. E., Vos, T., Wang, C., Wang, X., Weiderpass, E., Werdecker, A., Wright, J. L., Yang, Y. C., Yatsuya, H., Yoon, J., Yoon, S.-J., Zhao, Y., Zhou, M., Zhu, S., Lopez, A. D., Murray, C. J. L. and Gakidou, E. (2014). Global, regional, and national prevalence of overweight and obesity in children and adults during 1980–2013: A systematic analysis for the Global Burden of Disease Study 2013. *Lancet*. **384**:766–781.
- Oki, T., Matsui, T. and Matsumoto, K. (2000). Evaluation of  $\alpha$ -glucosidase inhibition by using an immobilized assay system. *Biol. Pharm. Bull.* **23**:1084–1087.
- Oki, T., Matsui, T. and Osajima, Y. (1999). Inhibitory effect of  $\alpha$ -glucosidase inhibitors varies according to its origin. *J. Agric. Food Chem.* **47**:550–553.
- Park, C. M. and Song, Y.-S. (2013). Luteolin and luteolin-7-O-glucoside inhibit lipopolysaccharide-induced inflammatory responses through modulation of NF- $\kappa$ B/AP-1/PI3K-Akt signaling cascades in RAW 264.7 cells. *Nutr. Res. Pract.* **7**:423–429.
- Patwardhan, B. and Mashelkar, R. A. (2009). Traditional medicine-inspired approaches to drug discovery: Can Ayurveda show the way forward? *Drug Discov. Today*. **14**:804–811.
- Peer, N., Steyn, K., Lombard, C., Lambert, E. V., Vythilingum, B. and Levitt, N. S. (2012). Rising diabetes prevalence among urban-dwelling black South Africans. *PLoS ONE*. **7**:e43336.
- Persson, I. A. L. (2012). The pharmacological mechanism of angiotensin-converting enzyme inhibition by green tea, rooibos and enalaprilat—A study on enzyme kinetics. *Phytother. Res.* **26**:517–521.
- Persson, I. A. L., Josefsson, M., Persson, K. and Andersson, R. G. G. (2006). Tea flavanols inhibit angiotensin-converting enzyme activity and increase nitric oxide production in human endothelial cells. *J. Pharm. Pharmacol.* **58**:1139–1144.
- Persson, I. A. L., Persson, K., Hägg, S. and Anderson, R. G. G. (2010). Effects of green tea, black tea and Rooibos tea on angiotensin converting enzyme and nitric oxide in healthy volunteers. *Publ. Health Nutr.* **13**:730–737.
- Peters, S. E., Huxley, R. and Woodward, M. (2014). Diabetes as risk factor for incident coronary heart disease in women compared with men: A systematic review and meta-analysis of 64 cohorts including 858,507 individuals and 28,203 coronary events. *Diabetologia*. **57**:1542–1551.
- Petersen, K. F., Dufour, S., Befroy, D., Garcia, R. and Shulman, G. I. (2004). Impaired mitochondrial activity in the insulin-resistant offspring of patients with type 2 diabetes. *N. Engl. J. Med.* **350**:664–671.
- Pinent, M., Castell, A., Baiges, I., Montagut, G., Arola, L. and Ardévol, A. (2008). Bioactivity of flavonoids on insulin-secreting cells. *Comp. Rev. Food Sci. Food Saf.* **7**:299–308.
- Potterat, O. and Hamburger, M. (2013). Concepts and technologies for tracking bioactive compounds in natural product extracts: Generation of libraries, and hyphenation of analytical processes with bioassays. *Nat. Prod. Rep.* **30**:546–564.
- Rasmussen, B. B., Holmbäck, U. C., Volpi, E., Morio-Liondore, B., Padon-Jones, D. and Wolfe, R. R. (2002). Malonyl coenzyme A and the regulation of functional carnitine palmitoyltransferase-1 activity and fat oxidation in human skeletal muscle. *J. Clin. Invest.* **110**:1687–1693.
- Reagan-Shaw, S., Nihal, M. and Ahmad, N. (2008). Dose translation from animal to human studies revisited. *FASEB J.* **22**:659–661.
- Reaven, G. (2005). Compensatory hyperinsulinemia and the development of an atherogenic lipoprotein profile: The price paid to maintain glucose homeostasis in insulin-resistant individuals. *Endocrinol. Metab. Clin. North Am.* **34**:49–62.
- Rein, M. J., Renouf, M., Cruz-Hernandez, C., Actis-Goretta, L., Thakkar, S. K. and Da Silva Pinto, M. (2012). Bioavailability of food components: A challenging journey to bioefficacy. *Br. J. Clin. Pharmacol.* **75**:588–602.
- Rosen, E. D. and MacDougald, O. A. (2006). Adipocyte differentiation from the inside out. *Nat. Rev. Mol. Cell Biol.* **7**:885–896.
- Ruiz, P. A. and Haller, D. (2006). Functional diversity of flavonoids in the inhibition of the proinflammatory NF- $\kappa$ B, IRF, and Akt signaling pathways in murine intestinal epithelial cells. *J. Nutr.* **136**:664–671.
- Sanderson, M., Mazibuko, S. E., Joubert, E., de Beer, D., Johnson, R., Pfeiffer, C., Louw, J. and Muller, C. J. F. (2014). Effects of fermented rooibos (*Aspalathus linearis*) on adipocyte differentiation. *Phytomedicine*. **21**:109–117.
- Sangul, K., Akao, T., Li, Y., Kakiuchi, N., Nakamura, N. and Hattori, M. (2005). Isolation of a human intestinal bacterium that transforms mangiferin to norathyriol and inducibility of the enzyme that cleaves a C-glucosyl bond. *Biol. Pharm. Bull.* **28**:1672–1678.
- Scheen, A. J. (2003). Is there a role for  $\alpha$ -glucosidase inhibitors in the prevention of type 2 diabetes mellitus? *Drugs*. **63**:933–951.
- Schloms, L., Storbeck, K.-H., Swart, P., Gelderblom, W. C. A. and Swart, A. C. (2012). The influence of *Aspalathus linearis* (Rooibos) and dihydrochalcones on adrenal steroidogenesis: Quantification of steroid intermediates and end products in H295R cells. *J. Steroid Biochem. Mol. Biol.* **128**:128–138.



- Schoefer, L., Mohan, R., Schwartz, A., Braune, A. and Blaut, M. (2003). Anaerobic degradation of flavonoids by *Clostridium orbiscindens*. *Appl. Environ. Microbiol.* **69**:5849–5854.
- Semplicini, A., Ceolotto, G., Massimino, M., Valle, R., Serena, L., De Toni, R., Pessina, A. C. and Dal Palù, C. (1994). Interactions between insulin and sodium homeostasis in essential hypertension. *Am. J. Med. Sci.* **307**:S43–S46.
- Sezik, E., Aslan, M., Yesilada, E. and Ito, S. (2005). Hypoglycaemic activity of *Gentiana olivieri* and isolation of the active constituent through bioassay-directed fractionation techniques. *Life Sci.* **76**:1223–1238.
- Shaw, J. E., Sicree, R. A. and Zimmet, P. Z. (2009). Global estimates of the prevalence of diabetes for 2010 and 2030. *Diabetes Res. Clin. Pract.* **87**:4–14.
- Silveira, L. R., Fiamoncini, J., Hirabara, S. M., Procópio, J., Cambiaghi, T. D., Pinheiro, C. H., Lopes, L. R. and Curi, R. (2008). Updating the effects of fatty acids on skeletal muscle. *J. Cell. Physiol.* **217**:1–12.
- Sirikul, B., Gower, B. A., Hunter, G. R., Larson-Meyer, D. E. and Newcomer, B. R. (2006). Relationship between insulin sensitivity and in vivo mitochondrial function in skeletal muscle. *Am. J. Physiol. Endocrinol. Metab.* **294**:E724–E728.
- Snel, M., Jonker, J. T., Hammer, S., Kerpershoek, G., Lamb, H. J., Meinders, A. E., Pijl, H., de Roos, A., Romijn, J. A., Smit, J. W. A. and Jazet, I. M. (2012). Long-term beneficial effect of a 16-week very low calorie diet on pericardial fat in obese type 2 diabetes mellitus patients. *Obesity.* **20**:1572–1576.
- Snijman, P. W., Joubert, E., Ferreira, D., Li, X.-C., Ding, Y., Green, I. R. and Gelderblom, W. C. A. (2009). Antioxidant activity of the dihydrochalcones aspalathin and nothofagin and their corresponding flavones in relation to other rooibos (*Aspalathus linearis*) flavonoids, epigallocatechin gallate, and trolox. *J. Agric. Food Chem.* **57**:6678–6684.
- Son, M. J., Minakawa, M., Miura, Y. and Yagasaki, K. (2013). Aspalathin improves hyperglycemia and glucose intolerance in obese diabetic ob/ob mice. *Eur. J. Nutr.* **52**:1607–1619.
- South African Rooibos Council (2013). Available at <https://sarooibos.co.za>.
- Stalmach, A., Mullen, W., Pecorari, M., Serafini, M. and Crozier, A. (2009). Bioavailability of C-linked dihydrochalcone and flavanone glucosides in humans following ingestion of unfermented and fermented rooibos teas. *J. Agric. Food Chem.* **57**:7104–7111.
- Sun, D., Huang, J., Zhang, Z., Gao, H., Li, J., Shen, M., Cao, F. and Wang, H. (2012). Luteolin limits infarct size and improves cardiac function after myocardium ischemia/reperfusion injury in diabetic rats. *PLoS ONE.* **7**:e33491.
- Sun, K., Kusminski, C. M. and Scherer, P. E. (2011). Adipose tissue remodeling and obesity. *J. Clin. Invest.* **121**:2094–2101.
- Thielecke, F. and Boschmann, M. (2009). The potential role of green tea catechins in the prevention of the metabolic syndrome - a review. *Phytochemistry.* **70**:11–24.
- Tiwari, A. K. and Rao, J. M. (2002). Diabetes mellitus and multiple therapeutic approaches of phytochemicals: Present status and future prospects. *Curr. Sci.* **83**:30–38.
- Tucci, S. A., Boyland, E. J. and Halford, J. C. G. (2010). The role of lipid and carbohydrate digestive enzyme inhibitors in the management of obesity: A review of current and emerging therapeutic agents. *Diabetes, Metab. Syndr. Obes.: Targets Ther.* **3**:125–143.
- Ulicná, O., Vancová, O., Bozek, P., Cársky, J., Sebeková, K., Boor, P., Nakano, M. and Greksák, M. (2006). Rooibos tea (*Aspalathus linearis*) partially prevents oxidative stress in streptozotocin-induced diabetic rats. *Physiol. Res.* **55**:157–164.
- Van de Laar, F. A., Lucassen, P. L., Akkermans, R. P., van de Lisdonk, E. H., Rutten, G. E. and van Weel, C. (2005).  $\alpha$ -Glucosidase inhibitors for patients with type 2 diabetes. Results from a Cochrane systematic review and meta-analysis. *Diabetes Care.* **28**:154–163.
- Van de Laar, F. A., Lucassen, P. L. B. J., Akkermans, R. P., Van de Lisdonk, E. H. and De Grauw, W. J. C. (2006). Alpha-glucosidase inhibitors for people with impaired glucose tolerance or impaired fasting blood glucose. *Cochrane Database Syst. Rev.* doi: 10.1002/14651858.CD005061.pub2.
- Van der Merwe, J. D., Joubert, E., Manley, M., De Beer, D., Malherbe, C. J. and Gelderblom, W. C. A. (2010). In vitro hepatic biotransformation of aspalathin and nothofagin, dihydrochalcones of rooibos (*Aspalathus linearis*), and assessment of metabolite antioxidant activity. *J. Agric. Food Chem.* **58**:2214–2220.
- Veber, D. F., Johnson, S. R., Cheng, H. Y., Smith, B. R., Ward, K. W. and Kopple, K. D. (2002). Molecular properties that influences the oral bioavailability of drug candidates. *J. Med. Chem.* **45**:2615–2623.
- Von Gadow, A., Joubert, E. and Hansmann, C. F. (1997). Comparison of the antioxidant activity of aspalathin with that of other plant phenols of rooibos tea (*Aspalathus linearis*),  $\alpha$ -tocopherol, BHT, and BHA. *J. Agric. Food Chem.* **45**:632–638.
- Wedler, J., Daubitz, T., Schlotterbeck, G. and Butterweck, V. (2014). In vitro anti-inflammatory and wound-healing potential of a *Phyllostachys edulis* leaf extract – Identification of isoorientin as an active compound. *Planta Med.* **80**:1678–1684.
- WHO. (2012). Global status report on noncommunicable diseases 2010.
- WHO. (2015). Obesity and overweight. Fact sheet N° 311.
- Williams, R. J., Spencer, J. P. E. and Rice-Evans, C. (2004). Flavonoids: Antioxidants or signalling molecules? *Free Radical Biol. Med.* **36**:838–849.
- Wolfram, S., Wang, Y. and Thielecke, F. (2006). Anti-obesity effects of green tea: From bedside to bench. *Mol. Nutr. Food Res.* **50**:176–187.
- Wu, X., Xu, T., Li, D., Zhu, S., Chen, Q., Hu, W., Pan, D., Zhu, H. and Sun, H. (2013). ERK/PP1a/PLB/SERCA2a and JNK pathways are involved in luteolin-mediated protection of rat hearts and cardiomyocytes following ischemia/reperfusion. *PLoS ONE.* **8**:e82957.
- Xiao, J., Kai, G., Yamamoto, K. and Chen, X. (2012). Advance in dietary polyphenols as  $\alpha$ -glucosidases inhibitors: A review on structure-activity relationship aspect. *Crit. Rev. Food Sci. Nutr.* **53**:818–836.
- Yao, L., Herlea-Pana, O., Heuser-Baker, J., Chen, Y. and Barlic-Dicen, J. (2014). Roles of the chemokine system in development of obesity, insulin resistance, and cardiovascular disease. *J. Immunol. Res.* doi: 10.1155/2014/181450.
- Yao, Y., Cheng, X., Wang, L., Wang, S. and Ren, G. (2011). A determination of potential  $\alpha$ -glucosidase inhibitors from azuki beans (*Vigna angularis*). *Int. J. Mol. Sci.* **12**:6445–6451.
- Yun, J. W. (2010). Possible anti-obesity therapeutics from nature—A review. *Phytochemistry.* **71**:1625–1641.
- Zammit, V. A. (2008). Carnitine palmitoyltransferase 1: Central to cell function. *IUBMB Life.* **60**:347–354.
- Zhang, Y., Xiaowei, T., Bili, B., Xiaoqin, W. and Ying, Z. (2007). Metabolism of flavone C-glucosides and p-coumaric acid from antioxidant of bamboo leaves (AOB) in rats. *Br. J. Nutr.* **97**:484–494.
- Zhou, H., Danger, D. P., Dock, S. T., Hawley, L., Roller, S. G., Smith, C. D. and Handlon, A. L. (2010). Synthesis and SAR of benzisothiazole- and indolizine- $\beta$ -D-glucopyranoside inhibitors of SGLT2. *ACS Med. Chem. Lett.* **1**:19–23.
- Zimmet, P., Magliano, D., Matsuzawa, Y., Alberti, G. and Shaw, J. (2005). The metabolic syndrome: A global public health problem and a new definition. *J. Atheroscler. Thromb.* **12**:295–300.