An emerging trend in functional foods for the prevention of cardiovascular disease and diabetes: Marine algal polyphenols

Margaret Murray[1], Aimee L Dordevic[1], Lisa Ryan[2], Maxine P Bonham[1]

margaret.murray@monash.edu

aimee.dordevic@monash.edu

lisa.ryan@gmit.ie

maxine.bonham@monash.edu

[1]Department of Nutrition & Dietetics, Monash University, VIC, Australia

[2]Department of Natural Sciences, Galway-Mayo Institute of Technology, Galway, Ireland

Corresponding Author: Dr Maxine Bonham Department of Nutrition & Dietetics Monash University Level 1, 264 Ferntree Gully Rd Notting Hill, VIC 3168 Australia Email: maxine.bonham@monash.edu

Abstract

Marine macroalgae are gaining recognition among the scientific community as a significant source of functional food ingredients. Due to the harsh environments in which macroalgae survive, they produce unique bioactive compounds that are not found in terrestrial plants. Polyphenols are the predominant bioactive compound in brown algae and are accountable for the majority of its biological activity. Phlorotannins are a type of polyphenol that are unique to

marine sources and have exhibited protective effects against hyperglycaemia, hyperlipidaemia, inflammation and oxidative stress, known risk factors for cardiovascular disease and diabetic complications, in cell culture, animal studies and some human studies. This review updates the information on marine polyphenols, with a particular focus on phlorotannins and their potential health benefits in relation to the prevention and treatment of risk factors for type 2 diabetes and cardiovascular diseases.

Key words

anti-inflammatory, antioxidant, hyperglycaemia, hyperlipidaemia, macroalgae, phlorotannin, polyphenol

Introduction

Polyphenols are a highly heterogeneous group of compounds (Naczk and Shahidi 2004) that are synthesized in terrestrial plants (Manach et al. 2004, Naczk and Shahidi 2004) and marine algae (Murugan et al. 2015). Their natural function is predominantly to act as the defence system of the organism, protecting against ultra-violet radiation (Manach et al. 2004, Naczk and Shahidi 2004, Bocanegra et al. 2009, Heffernan et al. 2015), infection (Manach et al. 2004, Naczk and Shahidi 2004, Heffernan et al. 2015) and consumption by herbivores (Bocanegra et al. 2009, Heffernan et al. 2015). Over 8000 structurally different polyphenols have been identified, from simple monomer units to complex polymerised structures (Kris-Etherton et al. 2002, Crozier et al. 2009). However, only several hundred of those varieties exist in edible plants (Manach et al. 2004), and those from terrestrial sources have been extensively reviewed (Scalbert and Williamson 2000, Yang et al. 2001, Kris-Etherton et al. 2002, Higdon and Frei 2003, Manach et al. 2004, Naczk and Shahidi 2004, Manach et al. 2005, Williamson and Manach 2005, D'Archivio et al. 2007, Crozier et al. 2009). This review investigates polyphenols from marine macroalgae, their dietary intake levels and key dietary sources, their potential as functional food ingredients and potential role as mediators of cardiovascular disease and diabetes.

A variety of polyphenols, including catechins, flavonols and phlorotannins, can all be found in marine macroalgae (Murugan et al. 2015). However phlorotannins, the predominant polyphenol in macroalgae, are unique to marine sources (Heffernan et al. 2015). Phlorotannins are synthesised in marine macroalgae through the acetate-malonate pathway by the polymerisation of phloroglucinol monomer units (1,3,5-tri hydroxybenzene) (Shibata et al. 2004, Chowdhury et

al. 2014, Heffernan et al. 2015, Murugan et al. 2015) (Figure 1). Phlorotannins are highly hydrophilic molecules that contain both phenyl (C_6H_5-) and phenoxy (C_6H_5O-) groups (Figure 1) and range in size from 126 Da to 650 kDa (Murugan et al. 2015), a much broader range than terrestrial polyphenols (up to 30 kDa) (Bravo 1998). Phlorotannins vary in structure and degree of polymerisation (Bocanegra et al. 2009), and are classified into four subclasses based on the chemical bonds they contain (Murugan et al. 2015). The subclasses are 1) fucols which have a phenyl linkage; 2) fuhalols and phlorethols which contain an ether linkage; 3) fucophloroethols which have a mixture of a phenyl and ether linkage; and 4) eckols which contain a dibenzodioxin linkage (Figure 1) (Murugan et al. 2015). However the literature often defines phlorotannins based on their source or specific type (e.g. Phlorofucofuroeckol A, dieckol) rather than which subclass they belong to.

Sources

There exist around 10,000 species of marine macroalgae, which are classified into three categories based on their pigmentation; Chlorophyta (green algae), Rhodophyta (red algae) and Phaeophyta (brown algae). Of the three varieties, brown algae contain the highest levels of polyphenols (Bocanegra et al. 2009, Heffernan et al. 2015) (5-30% of the dry weight (Heffernan et al. 2015)) the majority of which are phlorotannins (Chowdhury et al. 2014, Hamed et al. 2015). Due to the harsh environments in which marine macroalgae exist, including exposure to varying light intensity, salinity, pressure and temperatures, they produce a variety of unique and potent bioactive substances, which are not found in terrestrial plants (Hamed et al. 2015).

Different algal species contain varying combinations and concentrations of phlorotannins, and within a single species of marine alga a range of low and high molecular weight phlorotannins can be found (Heffernan et al. 2015) (Table 1). Phlorotannin content can vary between individuals of the same algal population, even within an individual algal body (Bocanegra et al. 2009) and phlorotannins are generally more concentrated in the outer layers of the organism, where it is exposed to the environment (Shibata et al. 2004). Environmental factors such as ultraviolet radiation, salinity, light and nutrient availability and herbivore grazing are likely causes for differences in phlorotannin content (Bocanegra et al. 2009). The location on the shore at which brown algae is grown may also affect phlorotannin content. Species grown in the intertidal zones have the highest phlorotannin content, whereas those grown at lower and upper levels of the shore have lower phlorotannin content (Connan et al. 2004), likely due to differences in exposure to environmental factors. The phlorotannin content of brown algae also varies according to season, and the degree of seasonal variation differs among species (Connan et al. 2004). The *Fucales* genus, *Pelvetia canaliculata* and *Ascophyllum nodosum* species exhibit maximal phenolic content in summer, whereas the *Laminariales* genus has a higher content in winter, and *Fucus vesiculosus* and *Ecklonia radiata* have highest levels in spring (Steinberg 1995, Connan et al. 2004).

Due to the structural complexity and polymeric nature of phlorotannins -- variations in the number of monomer units, their positions, and chemical bonds with which they are joined -- there is currently limited understanding of the array of phlorotannins in marine algae, and the distribution of phlorotannins within specific algal species (Heffernan et al. 2015). Historically, only low molecular weight phlorotannins (2-8 phloroglucinol units) could be characterised and

the isomeric complexity of high molecular weight phlorotannins was unable to be elucidated (Heffernan et al. 2015). However, recent technological advancements in chromatographic and mass spectrometric techniques allow for more thorough study of the complex structures and distribution of phlorotannins in marine algae, with phlorotannin isomers of up to 16 monomer units successfully detected (Heffernan et al. 2015).

Phlorotannin Intake

There is currently no known literature that outlines average population intake of marine phlorotannins. However, macroalgae consumption is documented in Asian countries, such as Japan, where it is a traditional part of the diet (Besada et al. 2009, Mouritsen et al. 2013). In 2006 Japanese households consumed 450 g per year of the seaweed Kombu (*Laminaria japonica* -- a brown macroalgae), although generally consumption was four times higher in elders than in young adults (< 29 years) (Zava and Zava 2011). However, while Kombu consumption has decreased over the last 50 years in Japan, daily seaweed intake has remained relatively stable; 4.3 g/day in 1955 and 5.3 g/day in 1995 with an increase in Wakame (*Undaria pinnatifida* - a brown macroalgae) and Nori (*Porphyra* genus -- a red algae) varieties making up for the decline in Kombu (Zava and Zava 2011). An average intake of 5.3g of seaweed per day equates to approximately 160 mg of phlorotannins per day from seaweed (Connan et al. 2004, Shibata et al. 2004), however this value would vary depending on individual intake, seaweed variety and bioavailability. From the red alga family, *Porphyra* is the genus that is most frequently consumed (Nori). From the brown algae, the *Laminaria japonica* (Kombu), *Undaria pinnatifida*

(Wakame) and *Hizikia fusiforme* (Hiziki) species are the most commonly consumed (Besada et al. 2009, Zava and Zava 2011).

In most western cultures seaweed is relatively new to the diet, but consumption has been steadily increasing since the early 1980s (Besada et al. 2009, Mouritsen et al. 2013) due to consumer demand for interesting, natural and sustainable food products (Mouritsen et al. 2013). However there is limited literature regarding actual daily intakes of seaweed among western cultures. The red seaweed *Palmaria palmata* is common in Atlantic waters and is one of the few algal species that is documented to have been used for human consumption in Europe (Mouritsen et al. 2013). However, there are now polyphenol-rich seaweed extracts that are commercially available as health food products in the United States of America, Canada and Korea. These supplements may dramatically increase the average population intake of marine polyphenols in these countries. Especially as these products carry claims of antioxidant and anti-inflammatory activity, improvement of lipid balance, weight loss and protection against cardiovascular disease and diabetes. These claims are as yet unsubstantiated in human populations, but there is some support for their role in certain health outcomes based on evidence from *in vitro* and animal studies.

Accurate estimation of polyphenol intake based on dietary intake data, like any other dietary component, is difficult. Collection of dietary data is predominantly through self-report and therefore is likely to be inexact and carry bias. Perceived ‘unhealthy’ foods are often under-reported, while perceived ‘healthy’ foods are typically over-reported (Spencer et al. 2008), resulting in an overestimation of polyphenol intake. The difficulty of estimating polyphenol

intake is further exacerbated as the polyphenol content of foods is not included in most food composition databases. There is an online European database that provides information on the polyphenol content of 459 common foods, but as yet this is limited to polyphenols from terrestrial food sources (includes 500 different polyphenols) and does not include phlorotannins (Phenol-Explorer, Version 3.6, http://phenol-explorer.eu/). Furthermore the variation in the phlorotannin content of algal species increases the difficulty of accurate intake estimation, as the recorded phlorotannin content of seaweeds in food databases may not be an accurate representation of all individuals in that species. The use of biomarkers, such as urinary excretion or plasma levels of polyphenols, are becoming more widely used may be more useful measures to determine polyphenol intake and make conclusions about the potential health effects of polyphenols (Wang et al. 2015).

Potential of marine algal polyphenols as a functional food

A functional food or functional ingredient is defined as a "natural or processed food that contains known or unknown biologically-active compounds; which, in defined, effective non-toxic amounts, provide a clinically proven and documented health benefit for the prevention, management, or treatment of chronic disease" (Martirosyan and Singh 2015). The value of seaweeds, and their constituents, as functional food products is rapidly increasing as science uncovers their many biological activities and potential health benefits. There are also a number of benefits to utilising marine sources, as opposed to land-based sources, to attain biologically active compounds. Recent trends have shown an increase in consumer preferences for natural and sustainable health products and functional foods (Blandon et al. 2007, Mouritsen et al.

2013), thus there is interest in marine-based food products (Murugan et al. 2015). With the ocean making up more than 70% of the Earth's surface (Hamed et al. 2015), it provides an abundant source of marine products, and algal species are easy to harvest from the wild as well as to culture in the sea and in pools on land (Mouritsen et al. 2013). The cultivation of marine algae has a number of advantages over terrestrial plant cultivation; it requires less fresh water, produces a higher biomass, can be grown in lower quality agricultural environments, and can be grown in seawater avoiding the need for herbicides and pesticides (Buono et al. 2014).

Additionally, recent advances in biotechnological tools for the extraction and identification of bioactive compounds from marine algae, has led to an upward trend in the use of these products as functional food ingredients (Murugan et al. 2015). Therefore there is likely to be a large market for marine polyphenols as a functional food ingredient if efficacy can be demonstrated.

While drugs are the current accepted treatment for blood sugar and cholesterol control, long term use of oral antidiabetic and anti-hyperlipidaemic drugs can cause unpleasant side effects, including muscle cramping, fatigue, muscle breakdown, vomiting and diarrhoea (Golomb and Evans 2008, Di Stasi et al. 2010, Bahadoran et al. 2013, Murugan et al. 2015). Whereas marine polyphenols are thought to be relatively safe for consumption (Zaragoza et al. 2008, Heo et al. 2009, Yeo et al. 2012, Yang et al. 2014, Kang et al. 2015, Kellogg et al. 2015) and lack unpleasant side effects (Paradis et al. 2011, Bahadoran et al. 2013, Murugan et al. 2015). The safety of a polyphenol-rich supplement from *Fucus vesiculosus* has been demonstrated at up to 750 mg/kg/day, in rats, over four weeks (Zaragoza et al. 2008). The phlorotannin diphlorethohydroxycarmalol (DPHC) has also shown no cytotoxicity in human umbilical vein

epithelial cells (HUVECs) at concentrations up to 3.91 mM after 20 hours incubation (Heo et al. 2009). It should be noted, however, that green tea polyphenols have been shown to cause hepatotoxicity and other adverse effects at high doses; 500 mg/kg/day of pure epigallocatechin gallate (EGCG) for 13 weeks increased bilirubin and decreased fibrinogen in rats. The risk of toxicity is increased when ingested in the fasting state or over long periods of time, or when the polyphenols are administered intraperitoneally to animals (Mazzanti et al. 2009). If the safety of marine polyphenols and efficacy for blood glucose or cholesterol control, or inflammation reduction can be shown in a human population, then marine polyphenols have great potential for commercialisation as a functional food ingredient.

Marine algal polyphenols and chronic disease

Polyphenols from seaweeds are thought to help reduce hyperglycaemia, hyperlipidaemia, oxidative damage and chronic inflammation; metabolic abnormalities that increase the risk of cardiovascular diseases (CVDs) and diabetic complications (Bahadoran et al. 2013). Polyphenols from terrestrial sources have been linked to positive health effects regarding a number of risk factors for chronic conditions including obesity, diabetes and cardiovascular diseases (Kris-Etherton et al. 2002, Higdon and Frei 2003, Crozier et al. 2009, Hursel et al. 2009, Hanhineva et al. 2010, Hursel et al. 2011, Rains et al. 2011, Bahadoran et al. 2013). Recent research has extended to marine macroalgae, possibly as a result of epidemiological data from Asian countries which indicate a diet rich in seaweed is associated with longevity and a decreased risk for cardiovascular disease, some cancers, and other chronic diseases (Miyagi et al. 2003, Willcox et al. 2009, Gavrilova and Gavrilov 2012).

Anti-hyperglycaemic effects

Impaired carbohydrate metabolism, insulin resistance, increased gluconeogenesis, β-cell dysfunction and defects in insulin signalling pathways are all potential causes of hyperglycaemia and risk factors for type 2 diabetes (Bahadoran et al. 2013). Both acute and chronic high blood glucose cause overloading of the metabolic pathways with glucose, resulting in oxidative stress and free radical formation, cardiovascular disorders, nephropathy, retinopathy, neuropathy, foot and leg ulcers, and limb amputation (Barde et al. 2015, Murugan et al. 2015). Alpha-amylase, located in the pancreas, and α-glucosidase, at the brush border of intestinal cells, are two key enzymes involved in carbohydrate metabolism (Kim et al. 2000, Benalla et al. 2010, Murugan et al. 2015). These enzymes break down carbohydrates into monosaccharides that are absorbed into the bloodstream, resulting in a rise in blood glucose following a meal (Kim et al. 2000, Benalla et al. 2010, Murugan et al. 2015). Enzyme inhibition reduces the rate at which glucose is released from carbohydrate foods following a meal, and can be an effective strategy for managing postprandial blood glucose (Kim et al. 2000, Benalla et al. 2010, Murugan et al. 2015). Oral glucosidase inhibitor drugs are the common clinical treatment for type 2 diabetes, however long term use can result in side effects such as renal tumours, acute hepatitis and serious hepatic injury (Murugan et al. 2015). Marine polyphenols may be a safer alternative (Heo et al. 2009, Paradis et al. 2011, Bahadoran et al. 2013, Murugan et al. 2015).

In vitro studies

One of the key mechanisms by which marine polyphenols exert protective effects against type 2 diabetes is through the inhibition of α-amylase and α-glucosidase. Polyphenolic-rich extracts from the marine macroalgae *Alaria*, *Pulmaria* and *Ascophyllum* exhibited some α-amylase

inhibitory activity. The extract from *Ascophyllum* demonstrated the strongest α-amylase inhibition (IC_{50} approximately 0.1 µg/mL gallic acid equivalents (GAE)) and was the only extract to also inhibit α-glucosidase activity (IC_{50} approximately 20 µg/mL GAE) (Nwosu et al. 2011). An *Ascophyllum nodosum* extract has also been shown to induce dose-dependent α-amylase and α-glucosidase inhibition (Apostolidis and Lee 2010). Furthermore, the α-glucosidase inhibition observed from the phlorotannin-rich *Ascophyllum nodosum* extract (0.24 µg phenolics) was greater than that of acarbose (0.37 µg), a current antidiabetic drug (Apostolidis and Lee 2010). DPHC, a phlorotannin extracted from *Ishige okamurae,* also dose-dependently inhibited α-amylase and α-glucosidase activity (IC_{50} values of 0.53 and 0.16 mM, respectively) in a chemical assay to a greater extent than acarbose (Heo et al. 2009).

Marine polyphenols also act on other enzymes involved in carbohydrate metabolism to reduce hyperglycaemia. The phlorotannins phlorofucofuroeckol A, dieckol and 8,8'-bieckol extracted from *Eisenia bicyclis* inhibited α-fucosidase, β-galactosidase and β-mannosidase, enzymes involved in carbohydrate break down, *in vitro*. Whereas the phlorotannins phloroglucinol, eckol and an unidentified tetramer were only weakly active against the enzymes (Shibata et al. 2002). An additional mechanism by which phlorotannins from *Ascophyllum nodosum* (400 µg/mL extract) have demonstrated to potentially reduce hyperglycaemia is to increase basal glucose uptake in 3T3-L1 adipocytes. During a 20-minute incubation period glucose uptake increased by approximately 3-fold (Zhang et al. 2007). Additionally, marine polyphenols from *Ecklonia cava* have been shown to activate the AMP-activated protein kinase/ acetyl-CoA carboxylase (AMPK/ACC) signal transduction pathways in C_2C_{12} myoblasts (Kang et al. 2010), which results

in increased glucose uptake into the cells and is another potential mechanism for a reduction in blood glucose levels (Park et al. 2002) (Figure 2).

Animal studies

Six weeks of supplementation with a diet containing 0.5% w/w of a polyphenol-rich extract from *Ishige okamurae* reduced hepatic glucose-6-phosphatase (G6Pase) and phosphoenolpyruvate carboxykinase (PEPCK) activity and increased hepatic glycogen production in mice, which resulted in reduced fasting blood glucose level (Min et al. 2011). The treated mice also presented with reduced hyperinsulinemia and HbA1c, compared with control (Min et al. 2011). Similarly, diabetic KK-A^{γ} mice that were administered 16.42 or 81.20 mg/day (0.2% or 1% of diet, respectively) of a phlorotannin extract from *Ecklonia stolonifera* for 4 weeks maintained blood glucose and insulin levels at a close-to-normal level in a dose-dependent manner, compared with control mice whose blood glucose and insulin levels increased over time (Iwai 2008). Kang et al (2013), also identified that supplementation with dieckol (20 mg/kg body weight/day for 14 days), from *Ecklonia cava*, reduced blood glucose and insulin levels in C57BL/KsJ-*db/db* diabetic mice. Interestingly, a dose-dependent treatment effect on insulin levels but not blood glucose levels was observed (Kang et al. 2013). Park et al (2012) reported that supplementation with 200 mg/kg body weight/day of an *Ecklonia cava* polyphenol extract for 7 weeks reduced fasting blood glucose in obese C57BL/6 mice compared with placebo.

Diabetic mice administered with 200 mg/kg body weight of a crude extract or enriched extract (purified polyphenolic fraction) from *Ascophyllum nodosum* for up to 4 weeks, exhibited reduced fasting blood glucose following both doses compared with placebo. However, only the enriched extract dampened the postprandial rise in blood glucose following an oral sucrose tolerance test

(Zhang et al. 2007). Similarly, a dieckol-rich extract from *Ecklonia cava* (0.5 g/100 g diet) reduced fasting blood glucose levels, HbA1c levels and plasma insulin levels in C57Bl/6/KsJ-*db/db(db/db)* mice after 6 weeks, compared with a control diet (Lee et al. 2012). The effect observed from the dieckol-rich extract was comparable to that of rosiglitazone (0.005 g/100 g diet), a current antidiabetic drug. Glucose tolerance also improved in the mice as a result of phlorotannin supplementation; the blood glucose area under the curve (AUC) was significantly reduced following phlorotannin treatment compared with control (Lee et al. 2012). Obese mice administered with an *Ecklonia cava* phlorotannin-rich extract (28.2 ± 0.58% polyphenols) five times a week for 12 weeks (Eo et al. 2015), also presented with reduced postprandial blood glucose AUC following both the 100 mg/kg body weight/day and 500 mg/kg body weight/day doses, compared with placebo. The high dose group exhibited significantly lower plasma insulin and HOMA-IR after 12 weeks compared with placebo. However, polyphenol supplementation had no effect on fasting blood glucose (Eo et al. 2015). Furthermore, when fed to streptozotocin-induced diabetic mice, a 100 mg/kg body weight single dose of DPHC diminished postprandial blood glucose AUC to 2022 (113.0) mmol/min, compared with 2210 (125.2) mmol/min in the control mice (Heo et al. 2009).

Human studies

In a randomised controlled trial, Shin et al (2012) gave 97 overweight adults a daily dose of either 72 mg or 144 mg of a polyphenol-rich extract (polyphenol content 98.5%) from *Ecklonia cava*, or a placebo, for 12 weeks. A reduction in fasting blood glucose was observed, but only in the high dose group (Shin et al. 2012). Conversely, another randomised controlled trial showed that three months of an oral supplement (500 mg/day) containing 5% marine polyphenols

increased plasma insulin levels, HOMA β-cell and HOMA-IR, compared with placebo, in overweight and obese adults. However, no change was observed in fasting blood glucose levels or blood glucose levels following an oral glucose tolerance test (OGTT) (Hernandez-Corona et al. 2014). Lee and Jeon (2015) administered 690 mg polyphenols or a placebo to 73 adults with high fasting blood glucose (100 to 180 mg/dL) for 12 weeks. While an improvement in postprandial blood glucose control and significant reduction in fasting blood insulin levels was observed following supplementation, there was, again, no change in fasting blood glucose level. Furthermore, Paradis et al (2011) demonstrated a reduction in three hour postprandial insulin incremental area under the curve (iAUC) and increased insulin sensitivity in 23 non-diabetic adults following consumption of a phlorotannin-rich blend of the brown seaweeds *Ascophyllum nodosum* and *Fucus vesiculosus*. Participants consumed either 500 mg seaweed capsules (containing at least 10% polyphenols) or placebo capsules 30 minutes prior to 50g of available carbohydrates from bread. There was no significant effect on postprandial blood glucose iAUC (Paradis et al. 2011).

Summary

Marine polyphenols inhibit the action of α-amylase and α-glucosidase *in vitro*, and reduce the postprandial rise in blood glucose and insulin levels in animals. However, reductions in postprandial blood glucose and insulin have not been consistently demonstrated in humans (Table 2). Marine polyphenols also reduce fasting blood glucose in animals, and one study has shown this same effect in humans (Shin et al. 2012). Some evidence suggests that a dose-dependent relationship exists between polyphenol intake and the anti-hyperglycaemic effects, yet

the variation in dosages, timeframes and species examined between studies make interpretation difficult.

Anti-hyperlipidaemic effects

Dyslipidaemia occurs in diabetes and contributes to CVD risk (Grundy et al. 1999, Rader 2007, Musunuru 2010, Bahadoran et al. 2013). One of the key protective activities of terrestrial polyphenols on the cardiovascular system is the improvement of dyslipidaemia. Polyphenols reduce digestion and absorption of dietary lipids, decrease synthesis and secretion of apolipoprotein B, inhibit cholesterol esterification and intestinal lipoprotein production, and inhibit key enzymes in lipid biosynthesis pathways (Bahadoran et al. 2013) resulting in an improved lipid profile and lowered cardiovascular risk. Emerging research indicates that marine polyphenols may have similar lipid lowering actions.

In vitro studies

In vitro research suggests a number of mechanisms by which marine polyphenols may exert anti-hyperlipidaemic activity (Figure 2). Both Seapolynol^{TM} (a polyphenol extract containing 98.5% unspecified polyphenols) and the isolated phlorotannin dieckol from *Ecklonia cava*, dose-dependently (0 - 200 µg/mL) inhibited adipocyte differentiation and lipid accumulation in 3T3-L1 preadipocytes, (which contributes to reduced intracellular triglyceride (TG) levels) and inhibited activity of 3-hydroxy-3-methylglutaryl-coenzyme A (HMGCoA) reductase (an enzyme involved in cholesterol production) in a HMGCoA reductase assay kit (Yeo et al. 2012). The phlorotannins phloroglucinol, eckol and phlorofucofuroeckol A from *Ecklonia stolonifera* also dose-dependently (12.5 -- 100 µM) inhibited lipid accumulation in 3T3-L1 adipocytes and did

not affect cell viability at 0 -- 200 μM for 24 h (Jung et al. 2014). Phloroglucinol, eckol, dieckol, dioxinodehydroeckol and phlorofucofuroeckol A also reduced expression levels of adipocyte marker genes peroxisome proliferator activated receptor γ (PPARγ) and CCAAT/enhancer-binding protein α (C/EBPα), which suggests that phlorotannins regulate adipogenesis and inhibit adipocyte differentiation via modulation of PPARγ and C/EBPα expression (Jung et al. 2014). This mechanism has been suggested as a way of managing obesity via reduction of the formation of mature adipocytes and adipose tissue (Furuyashiki et al. 2004, Huang et al. 2006, Ejaz et al. 2009, Jung et al. 2014), and has been associated with reduced body weight gain in obese mice (Ejaz et al. 2009).

Animal studies

High fat diet-fed mice supplemented with Seapolynol^{TM} (1.25, 2.5 or 5 mg/ day) or isolated dieckol, from *Ecklonia cava* (0.5, 1 or 2 mg/day) for 5 weeks exhibited reduced serum total cholesterol (TC), TG and low density lipoprotein cholesterol (LDL-C) levels compared with mice fed a high fat diet only (Yeo et al. 2012). Similarly, in diabetic mice, a dieckol rich extract from *Ecklonia cava* (0.5 g dieckol/100 g diet) reduced TC levels and free fatty acids (FFAs) after 6 weeks, compared with placebo, reduced TG levels to an extent similar to treatment with rosiglitazone (0.005 g/100 g diet), and increased high density lipoprotein cholesterol (HDL-C) compared with rosiglitazone treatment (Lee et al. 2012). Doses of 100 mg/kg body weight/day and 500 mg/kg body weight/day of a phlorotannin-rich extract from *Ecklonia cava* (28.2 ± 0.58% polyphenols) also reduced TG and TC levels in obese mice after 12 weeks, compared with mice fed a high fat diet alone, however there was no change in HDL-C (Eo et al. 2015). A phlorotannin-rich extract (100 -- 250 mg/kg body weight/day) or isolations of the phlorotannins

eckol and dieckol (10 or 20 mg/kg body weight/day) from *Ecklonia stolonifera,* were administered to hyperlipidaemic rats (Yoon et al. 2008). The phlorotannin-rich extract reduced TG, TC and LDL-C levels and increased HDL-C levels in a dose-dependent manner after 3 days of treatment. Both eckol and dieckol isolations reduced TG, TC and LDL-C levels after 3 days. Dieckol treatment alone produced a greater hypolipidaemic effect than lovastatin (50 mg/kg) and increased HDL-C levels in the hyperlipidaemic rats after 3 days (Yoon et al. 2008). Conversely, when Park et al (2012) administered polyphenol-rich extracts from *Ecklonia cava,* grown in two different geographical areas in Korea; Jeju and Gijang, to obese mice at 200 mg/kg body weight/day for 8 weeks, treatment with the extract from Jeju had no effect on TC, TG, LDL-C or HDL-C levels. However, the extract from Gijang reduced TC level compared with placebo (Park et al. 2012), highlighting the potential for differences in polyphenol content based on location, even within the same species of algae (Connan et al. 2004, Bocanegra et al. 2009).

Human studies

In a randomised controlled trial in 97 overweight adults, consumption of a phlorotannin-rich extract from *Ecklonia cava*, at doses of 72 or 144 mg polyphenols per day, reduced TC levels, LDL-C levels and TC to HDL-C ratio, in a dose-dependent manner following 12 weeks of treatment, compared with placebo. An increase in HDL-C levels was only observed following the highest dose (Shin et al. 2012). A comparable trial in 80 adults with raised cholesterol (>200 mg/dL TC, or >110 mg/dL LDL-C) demonstrated that consumption of a dieckol-rich *Ecklonia cava* extract (400 mg/day, 8.2% dieckol) for 12 weeks resulted in reduced TC and LDL-C, compared with placebo, without change in TG or HDL-C levels (Choi et al. 2015). Conversely, in a randomised controlled trial of 25 overweight or obese volunteers, no changes were reported

in TC, TG or HDL-C levels following 500 mg of a polyphenol-containing oral supplement (5% polyphenols) daily for 3 months. However, LDL-C levels were reduced following the supplement treatment compared with no change from placebo (Hernandez-Corona et al. 2014).

Summary

Similar to the anti-hyperglycaemic evidence, marine polyphenols improved dyslipidaemia in animal models and *in vitro* via a number of mechanisms, although results in humans are few and inconsistent (Table 3). Marine polyphenols have potential as an anti-hyperlipidaemic agent in humans, but due to factors such as bioavailability and dosing, which differ considerably between humans and animals, further research is required to determine a consistent effect and appropriate dosage and treatment schedule in humans.

Anti-inflammatory effects

Increased inflammatory mediators and chronic sub-clinical inflammation are key risk factors for CVD (Osiecki 2004, Willerson and Ridker 2004, Libby 2006, Bahadoran et al. 2013) and promote the progression of long-term complications of diabetes (Elmarakby et al. 2010, Bahadoran et al. 2013, Roy et al. 2013, Jialal and Devaraj 2014, Roy et al. 2015). There are a number of important mediators (tumour necrosis factor α (TNF-α), interleukin 6 (IL-6), interleukin-1β (IL-1β) and monocyte chemoattractant protein-1 (MCP-1)) that play a role in the regulation of inflammation and may be affected by polyphenols. The anti-inflammatory effects of marine polyphenols are presented in Table 4.

In vitro studies

Phlorotannin-rich extracts from the seaweeds *Dictyopteris divaricate, Dictyopteris prolifera, Prionitis cornea, Grateloupia lanceolate* and *Grateloupia filicina* exhibited anti-inflammatory effects on RAW 264.7 macrophages under lipopolysaccharide (LPS) stimulation. All five species strongly inhibited nitric oxide (NO) production after 18 hours, with IC_{50} of 18.0 µg/mL, 38.36 µg/mL, 38.43 µg/mL, 32.81 µg/mL and 37.14 µg/mL, respectively. All extracts also dose-dependently reduced inducible NO synthase (iNOS) and cyclooxygenase-2 (COX2) protein levels, and decreased secretion of TNF-α and IL-6 cytokines (Yang et al. 2014). Furthermore, all extracts except *Grateloupia lanceolate* reduced secretion of prostaglandin E_2 in a dose-dependent manner (Yang et al. 2014). DPHC, isolated from *Ishige okamurae,* also demonstrated anti-inflammatory effects in LPS stimulated RAW 264.7 macrophages. Compared with control conditions, DPHC potently reduced the secretion of the pro-inflammatory cytokine IL-6 through suppression of the phosphorylation of nuclear factor kappa B (NF-κB), downregulation of the Janus kinase/signal transducers and activators of transcription (Jak2-STAT5) pathway and upregulation of suppressor of cytokine signalling 1 (SOCS1) regulator. However DPHC had no effect on levels of secreted TNF-α (Kang et al. 2015). Phlorotannins from the brown algae *Fucus distichus, Alaria marginate, Saccharina groenlandica* and *Saccharina latissimi,* have demonstrated anti-inflammatory activity in RAW 263.7 macrophages. All extracts inhibited expression of the pro-inflammatory genes COX2, iNOS, TNF-α, interleukin 10 (IL-10) and MCP-1 (Kellogg et al. 2015). Further refined phlorotannin extracts also reduced expression of toll-like receptors TLR4 and TLR9 (Kellogg et al. 2015).

Animal studies

In a rat model, phlorotannins from three different algal species; *Cystoseira crinita* (56.5 mg GAE/g dried sample), *Cystoseira sedoides* (50.3 mg GAE/g dried sample) and *Cystoseira compressa* (61.0 mg GAE/g dried sample), administered at doses of 25 or 50 mg/kg body weight exhibited anti-inflammatory activity against carrageenan-induced rat paw oedema in a dose-dependent manner at 1 hour, 3 hours and 5 hours post administration. This level of inhibition was similar to that of known anti-inflammatory mediators (300 mg/kg acetylsalicylic of lysine (aspirin) and 1 mg/kg dexamethasone (an anti-inflammatory steroid medication)) (Mhadhebi et al. 2014). Obese mice fed a phlorotannin-rich extract (28.2 ± 0.58% polyphenols) from *Ecklonia cava* five times a week for 12 weeks, at a dose of 500 mg/kg body weight, showed reductions in protein levels of inflammatory markers MCP1, TNF-α, IL-1β, NF-κB and COX2. Whereas mice that received 100 mg/kg body weight only showed reductions in protein levels of NFκB and COX2, compared with a high fat diet alone (Eo et al. 2015). Another study in high fat diet-induced obese mice demonstrated anti-inflammatory effects of polyphenol-rich extracts from *Ecklonia cava* (79.70 mg/g of polyphenols), from the geographical area of Gijang, Korea (Park et al. 2012). Following doses of 200 mg/kg body weight daily for 8 weeks, the mice showed reductions in mRNA expression levels of TNF-α, IL-1β and F4/80 in the epididymal adipose tissue, compared with mice who received a placebo (Park et al. 2012).

Summary

There is evidence that marine polyphenols reduce inflammation *in vitro* and in animal models in a dose-dependent manner (Table 4). To date, there is currently no research that has examined the anti-inflammatory effects of marine polyphenols in humans. Further research to investigate role

of marine polyphenols as anti-inflammatory agents, to reduce the chronic low grade inflammation that contributes to diabetes progression and cardiovascular diseases in humans is warranted (Osiecki 2004, Willerson and Ridker 2004, Libby 2006, Elmarakby et al. 2010, Bahadoran et al. 2013, Roy et al. 2013, Jialal and Devaraj 2014, Roy et al. 2015).

Antioxidant effects

Tissue damage caused by oxidation contributes to the progression and pathogenesis of inflammation, hypertension, and diabetes, and also increases the risk of CVD (Chowdhury et al. 2014, Murugan et al. 2015). Marine polyphenols may have antioxidant effects that protect cellular constituents against oxidative stress and reduce tissue injuries, either by direct free radical scavenging or through enhancing the actions of endogenous reducing agents (Scalbert et al. 2005, Murugan et al. 2015). Much research has been conducted *in vitro* to examine the antioxidant properties of polyphenol-rich extracts from marine macroalgae (Table 5).

In vitro studies

Phlorotannin extracts from the brown seaweeds *Padina antillarum, Caulerpa racemose* and *Kappaphycus alvarezzi* showed high antioxidant activity as measured by β-carotene bleaching. *Padina antillarum* was determined to have the highest total phenolic content as well as ascorbic acid equivalent antioxidant activity (1140 ± 85 mg AA/100g), highest reducing power (15.7 ± 2.6 mg GAE/g) according to the ferric reducing antioxidant power (FRAP) assay and highest chelating ability in the ferrous ion chelating (FIC) assay (Chew et al. 2008). Kumar Chandini et al (2008) examined the reducing power and radical scavenging ability of phlorotannin extracts from three brown seaweeds; *Sargassum marginatum, Padina tetrastomatica* and *Turbinaria*

conoides. The antioxidant activity of the extracts varied depending on the type of extraction solvent used, likely due to variation in the phlorotannins extracted by each solvent. The ethyl acetate extract from *Sargassum marginatum* had the highest total antioxidant activity according to the phosphomolybdenum method (39.62 mg as ascorbic acid equivalents/g extract) and highest 1,1-diphenyl-2-picrylhydrazyl (DPPH) scavenging activity (23.16%). All three phlorotannin extracts extracted using methanol exhibited dose-dependent antioxidant activity, with the reducing power of *Turbinaria conoides* and *Padina tetrastomatica* greater than that of α-tocopherol (Kumar Chandini et al. 2008). Likewise, a phlorotannin extract from *Ishige okamurae* exhibited dose-dependent radical scavenging activity as measured by ESR spectrophotometer (Heo and Jeon 2008). Phlorotannin extracts from *Turbinaria conoides* and *Turbinaria ornate* also exhibited antioxidant activity *in vitro*. However, while a higher total phenolic content was identified in *Turbinaria conoides*, the *Turbinaria ornata* extract had significantly higher antioxidant potential as determined by radical scavenging ability, Fe^{2+} ion chelating ability and reducing potential (Chakraborty et al. 2013).

Eckol, dieckol, 8,8'-bieckol and Phlorofucofuroeckol A extracted from *Ecklonia cava, Ecklonia kurome* and *Eisenia bicyclis* showed potent inhibition of phospholipid peroxidation in a liposome system at a concentration of 1 μM (Shibata et al. 2008). They also exhibited significant radical scavenging ability against the superoxide anion (50% effective concentration value: 6.5-8.4 μM) and DPPH (50% effective value: 12-26 μM) in chemical assay; where the antioxidant activities of the four phlorotannins were more effective than antioxidants ascorbic acid and α-tocopherol at similar concentrations (Shibata et al. 2008). Similarly, an extract from *Fucus vesiculosus* of high molecular weight phlorotannins had strong DPPH quenching activity, comparable to that of

ascorbic acid and butylated hydroxytoluene, and greater than α-tocopherol in chemical assay (Wang et al. 2012). Furthermore, bifuhalol, a phlorotannin isolated from *Sargassum ringgoldianum,* exhibited superoxide anion radical scavenging activity in chemical assay approximately five times stronger than catechin, a polyphenol in terrestrial plants that is marketed as an antioxidant supplement (Nakai et al. 2006).

In L5178 mouse T-cell lymphoma cell lines, phloroglucinol, eckol and dieckol phlorotannin isolations exerted a dose-dependent protective effect against H_2O_2-mediated DNA damage, in comet assay, and exhibited free radical scavenging activity (Ahn et al. 2007). The eckol isolation had the highest radical scavenging ability, scavenging 93% of DPPH at 0.25, 0.5 and 1.0 mg/mL concentrations after 2 minutes (Ahn et al. 2007). Likewise, phlorotannins from *Fucus vesiculosus* exhibited reducing power and radical scavenging ability in non-cellular systems, and reduced production of reactive oxygen species (ROS) and NO by RAW 264.7 macrophages in a dose-dependent manner (Zaragoza et al. 2008). Li et al (2009) also showed antioxidant capacity and free radical scavenging activity, in a linoleic acid model system, of 7-phloro eckol, 6,6'-bieckol, phloroglucinol, eckol, fucodiphloroethol G, phlorofucofuroeckol A and dieckol phlorotannin isolations from *Ecklonia cava* in the RAW 264.7 cell line. Where 6,6'-bieckol, dieckol and fucodiphloroethol exhibited significantly stronger radical scavenging activities compared with the other phlorotannins (Li et al. 2009). Furthermore, when high glucose-induced oxidative stress in HUVECs was treated with 10 μg/mL and 50 μg/mL dieckol, isolated from *Ecklonia cava,* glucose-induced cytotoxicity and intracellular ROS generation was inhibited, and thiobarbituric acid reactive substances (TBARS) and NO level were reduced after 20 hours of incubation (Lee et al. 2010).

Animal studies

In C57BL/KsJ-*db/db* type 2 diabetic mice, treatment with dieckol from *Ecklonia cava* at doses of 10 and 20 mg/kg body weight for 14 days, increased, though not significantly, the activity of endogenous antioxidant enzyme superoxide dismutase (SOD), with no effect on the enzymes catalase (CAT) and glutathione peroxidase (GSH-px) (Kang et al. 2013).

Summary

Antioxidant activity of marine polyphenols has been demonstrated *in vitro* (Table 5). Some studies found that marine polyphenol antioxidant activities were comparable to, or stronger than, that of widely used antioxidants ascorbic acid (Shibata et al. 2008, Wang et al. 2012), α-tocopherol (Kumar Chandini et al. 2008, Shibata et al. 2008, Wang et al. 2012), butylated hydroxytoluene (Wang et al. 2012) or catechin (Nakai et al. 2006), *in vitro*. There is evidence that the antioxidant activity of marine polyphenols may be dose-dependent, but this was not consistently shown. It is worth noting that the concentrations of marine polyphenols tested *in vitro* are far greater than concentrations that would be present in human blood and tissues. Therefore, despite strong evidence from *in vitro* studies, antioxidant activity of marine polyphenols is less likely in humans due to comparatively low absorption rates and serum concentrations (Williamson and Manach 2005, Crozier et al. 2009, Lee 2013), however this has not been investigated.

Health effects according to algal species

Phlorotannin-rich extracts from the *Ascophyllum nodosum, Ecklonia stolonifera, Fucus vesiculosus, Ishige okamurae* and *Ecklonia cava* macroalgae varieties are the most predominantly tested with relation to their potential health effects. Table 6 outlines the health

effects of these algal species. While there are numerous different seaweed species, the research to date has tended to focus on the aforementioned species with a particular emphasis on *Ecklonia cava*, despite it not being a commonly consumed seaweed. Phlorotannins from *Ecklonia cava* have demonstrated all of the health effects examined in this review and are beginning to be tested in human populations (Shin et al. 2012, Choi et al. 2015, Lee and Jeon 2015). Future research is warranted to continue to investigate the health effects of *Ecklonia cava* phlorotannins particularly in human populations, as they show great potential to be used as a functional food ingredient. However, the potential of other species of macroalgae that are not yet as well investigated should not be neglected.

Limitations

It is important to measure the bioavailability of marine polyphenols in humans to properly assess their biological functioning (D'Archivio et al. 2007). Most evidence for the biological activity of marine polyphenols to date has been in cultured cells or animal models, which do not account for the effects of other dietary components, or digestion and absorption in humans, and therefore may not represent the biological actions of polyphenols in humans. Thus polyphenols that have exhibited strong biological activity *in vitro* may not have the same effect in the human body (Crozier et al. 2009, Lee 2013).

The bioavailability of any compound is affected by its ability to cross membranes, withstand pH changes in the gastrointestinal tract and maintain its structural integrity (Barditch-Crovo et al. 1998, Lee 2013). Factors that affect the absorption and metabolism of polyphenols from food

include their chemical structure (degree of glycosylation/acylation, molecular size, degree of polymerization) (Bravo 1998, Scalbert and Williamson 2000, Manach et al. 2004, D'Archivio et al. 2007); dietary factors (interactions with proteins and polysaccharides, transit time, intestinal fermentations, biliary excretion) (Manach et al. 2004); behavioural factors (such as smoking) (Higdon and Frei 2003); individual variation in enzyme activity (Higdon and Frei 2003); and whether absorption takes place in the small intestine or colon (Manach et al. 2004, Bahadoran et al. 2013). The multitude of factors that impact on polyphenol bioavailability result in large variations in bioavailability, and thus biological activity, from one polyphenol to another (Scalbert and Williamson 2000, D'Archivio et al. 2007).

During digestion, polyphenols are metabolized in the small intestine, some in the large intestine by colonic microflora, in the liver and other organs whereby they go through numerous structural modifications (Manach et al. 2004, Williamson and Manach 2005, D'Archivio et al. 2007, Bahadoran et al. 2013). Therefore human body tissues are not exposed to polyphenols in their original form (Crozier et al. 2009, Lee 2013), so *in vitro* studies that examine polyphenol extracts which have not undergone digestion are not a true representation of the activity or concentration of the metabolites present in the human body (Williamson and Manach 2005, Crozier et al. 2009, Lee 2013). Polyphenol studies need to take into account the changes in structure and concentration that occur when the compounds enter the human body (Crozier et al. 2009, Lee 2013). To further complicate the issue, personal variations in intestinal microflora may also impact an individuals' metabolism and absorption of polyphenols, but this area is not yet well understood (Lee 2013). These issues highlight the need for studies to be performed in humans.

Conclusion

Under experimental conditions polyphenols from marine macroalgae have many positive health-related effects. There is strong evidence in cell and animal models for the anti-hyperglycaemic, anti-hyperlipidaemic, anti-inflammatory and antioxidant effects of marine polyphenols. However, there are currently only five studies known to have investigated the anti-hyperglycaemic and anti-hyperlipidaemic effects in humans, and none the anti-inflammatory or antioxidant effects. When translating the research to humans it is important to consider that doses given in animal model studies are likely to be much higher than those in human studies and so the same positive effects may not be observed. It is also important to consider the effects of digestion and metabolism throughout the human digestive tract as this may result in the compounds being altered differently to how they may be within an animal or cell model. More research is required to understand the bioavailability of marine polyphenols and mechanisms of action within the human body and how this differs from cell and animal models. Randomised controlled trials should be performed to examine different doses of marine polyphenols and the effects of different types of marine polyphenols on health outcomes in human populations. *Ecklonia cava* has shown great potential as a source of bioactive marine polyphenols, with evidence for anti-hyperglycaemic, anti-hyperlipidaemic, anti-inflammatory and antioxidant effects, and trials already completed in human populations. However, other seaweed species should not be ignored in the search for functional food ingredients with health benefits.

Conflicts of interest

none to declare

Acknowledgments

none to declare

References

<BIBL>

Ahn, G.-N., K.-N. Kim, S.-H. Cha, C.-B. Song, J. Lee, M.-S. Heo, I.-K. Yeo, N.-H. Lee, Y.-H. Jee, J.-S. Kim, M.-S. Heu and Y.-J. Jeon (2007). Antioxidant activities of phlorotannins purified from Ecklonia cava on free radical scavenging using ESR and H2O2-mediated DNA damage. *Eur Food Res Technol* **226**: 71-79.

Apostolidis, E. and C. Lee (2010). In Vitro Potential of Ascophyllum nodosum Phenolic Antioxidant-Mediated α-Glucosidase and α-Amylase Inhibition. *Journal of Food and Science* **75**(3): H97-H102.

Bahadoran, Z., P. Mirmiran and F. Azizi (2013). Dietary polyphenols as potential nutraceuticals in management of diabetes: a review. *Journal of Diabetes & Metabolic Disorders* **12**(43): 1-9.

Barde, S. R., R. S. Sakhare, S. B. Kanthale, P. G. Chandak and P. G. Jamkhande (2015). Marine bioactive agents: a short review on new marine antidiabetic compounds. *Asian Pacific Journal of Tropical Biomedicine* **5**(Suppl 1): S209-S213.

Barditch-Crovo, P. A., B. G. Petty, J. Gambertoglio, L. J. Nerhood, S. Kuwahara, R. Hafner, P. S. Lietman and D. M. Kornhauser (1998). The effect of increasing gastric pH upon the bioavailability of orally-administered foscarnet. *Antiviral Research* **38**(3): 209-212.

Benalla, W., S. Bellahcen and M. Bnouham (2010). Antidiabetic Medicinal Plants as a Source of Alpha Glucosidase Inhibitors. *Current Diabetes Reviews* **6**(4): 247-254.

Besada, V., J. M. Andrade, F. Schultze and J. J. Gonzalez (2009). Heavy metals in edible seaweeds commercialised for human consumption. *Journal of Marine Systems* **75**: 305-313.

Blandon, J., J. Cranfield and S. Henson (2007). Functional Food and Natural Health Product Issues: The Canadian and International Context Department of Food Agricultural and Resource Economics, International Food Economy Research Group

Bocanegra, A., S. Bastida, J. Benedi, S. Rodenas and F. J. Sanchez-Muniz (2009). Characteristics and Nutritional and Cardiovascular-Health Properties of Seaweeds. *Journal of Medicinal Food* **12**(2): 236-258.

Bravo, L. (1998). Polyphenols: Chemistry, dietary sources, metabolism, and nutritional significance. *Nutrition Reviews* **56**(11): 317-333.

Buono, S., A. L. Langellotti, A. Martello, F. Rinna and V. Fogliano (2014). Functional ingredients from microalgae†. *Food & Function* **5**: 1669-1685.

Chakraborty, K., N. K. Praveen, K. K. Vijayan and G. S. Rao (2013). Evaluation of phenolic contents and antioxidant activities of brown seaweeds belonging to Turbinaria spp. (Phaeophyta, Sargassaceae) collected from Gulf of Mannar. *Asian Pacific Journal of Tropical Biomedicine* **3**(1): 8-16.

Chew, Y., Y. O. Lim, M and K. Khoo (2008). Antioxidant activity of three edible seaweeds from two areas in South East Asia. *LWT* **41**: 1067-1072.

Choi, E.-K., S.-H. Park, K.-C. Ha, S.-O. Noh, S.-J. Jung, H.-J. Chae, S.-W. Chae and T.-S. Park (2015). Clinical Trial of the Hypolipidemic Effects of a Brown Alga Ecklonia cava Extract in Patients with Hypercholesterolemia. *Int J Pharm* **11**(7): 798-805.

Chowdhury, M. T. H., I. Bangoura, J.-Y. Kang, J. Y. Cho, J. Joo, Y. S. Choi, D. S. Hwang and Y.-K. Hong (2014). Comparion of Ecklonia cava, Ecklonia stolonifera and Eisenia bicyclis for phlorotannin extraction. *Journal of Environmental Biology* **35**: 713-719.

Connan, S., F. Goulard, V. Stiger, E. Deslandes and E. A. Gall (2004). lnterspecific and temporal variation in phlorotannin levels in an assemblage of brown algae. *Botanica Marina* **47**: 410-416.

Creis, E., L. Delage, S. Charton, S. Goulitquer, C. Leblanc, P. Potin and E. A. Gall (2015). Constitutive or Inducible Protective Mechanisms against UV-B Radiation in the Brown Alga Fucus vesiculosus? A Study of Gene Expression and Phlorotannin Content Responses. *PLOS ONE*.

Crozier, A., I. B. Jaganath and M. N. Clifford (2009). Dietary phenolics: chemistry, bioavailability and effects on health. *Nat. Prod. Rep* **26**: 1001-1043.

Cuong, D. X., V. N. Boi, T. T. T. Van and L. N. Hau (2015). Effect of storage time on phlorotannin content and antioxidant activity of six Sargassum species from Nhatrang Bay, Vietnam. *Journal of Applied Phycology* **published online**.

D'Archivio, M., C. Filesi, R. Di Benedetto, R. Gargiulo, C. Giovannini and R. Masella (2007). Polyphenols, dietary sources and bioavailability. *Ann Ist Super Sanita* **43**(4): 348-361.

Di Stasi, S. L., T. D. MacLeod, J. D. Winters and S. A. Binder-Macleod (2010). Effects of Statins on Skeletal Muscle: A Perspective for Physical Therapists. *Physical Therapy* **90**(10): 1530-1542.

Ejaz, A., D. Wu, P. Kwan and M. Meydani (2009). Curcumin Inhibits Adipogenesis in 3T3-L1 Adipocytes and Angiogenesis and Obesity in C57/BL Mice. *The Journal of Nutrition* **139**(5): 919-925.

Elmarakby, A. A., R. Abdelsayed, J. Y. Liu and M. S. Mozaffari (2010). Inflammatory cytokines as predictive markers for early detection and progression of diabetic nephropathy. *EPMA Journal* **1**: 117-129.

Eo, H., Y.-J. Jeon, M. Lee and Y. Lim (2015). Brown Alga Ecklonia cava Polyphenol Extract Ameliorates Hepatic Lipogenesis, Oxidative Stress, and Inflammation by Activation of AMPK and SIRT1 in High-Fat Diet-Induced Obese Mice. *Journal of Agricultural and Food Chemistry* **63**: 349-359.

Furuyashiki, T., H. Nagayasu, Y. Aoki, H. Bessho, T. Hashimoto, K. Kanazawa and H. Ashida (2004). Tea Catechin Suppresses Adipocyte Differentiation Accompanied by Down-regulation of PPARγ2 and C/EBPα in 3T3-L1 Cells. *Bioscience, Biotechnology, and Biochemistry* **68**(11): 2353-2359.

Gavrilova, N. S. and L. A. Gavrilov (2012). Comments on Dietary Restriction, Okinawa Diet and Longevity. *Gerontology* **58**(3): 221-223.

Golomb, B. A. and M. A. Evans (2008). Statin Adverse Effects: A Review of the Literature and Evidence for a Mitochondrial Mechanism. *American journal of cardiovascular drugs : drugs, devices, and other interventions* **8**(6): 373-418.

Grundy, S. M., I. J. Benjamin, G. L. Burke, A. Chait, R. H. Eckel, B. V. Howard, W. Mitch, S. C. Smith and J. R. Sowers (1999). Diabetes and Cardiovascular Disease: A Statement

for Healthcare Professionals From the American Heart Association. *Circulation* **100**(10): 1134-1146.

Hamed, I., F. Ozogul, Y. Ozogul and J. M. Regenstein (2015). Marine Bioactive Compounds and Their Health Benefits: A Review. *Comprehensive Reviews in Food Science and Food Safety* **14**: 446-465.

Hanhineva, K., R. Torronen, I. Bondia-Pons, J. Pekkinen, M. Kolehmainen, H. Mykkanen and K. Poutanen (2010). Impact of dietary polyphenols on carbohydrate metabolism. *International Journal of Molecular Sciences* **11**: 1365-1402.

Heffernan, N., N. P. Brunton, R. J. FitzGerald and T. J. Smyth (2015). Profiling of the Molecular Weight and Structural Isomer Abundance of Macroalgae-Derived Phlorotannins. *Marine Drugs* **13**: 509-528.

Heo, S.-J., J.-Y. Hwang, J.-I. Choi, J.-S. Han, H.-J. Kim and Y.-J. Jeon (2009). Diphlorethohydroxycarmalol isolated from Ishige okamurae, a brown algae, a potent α-glucosidase and α-amylase inhibitor, alleviates postprandial hyperglycemia in diabetic mice. *European Journal of Pharmacology* **615**: 252-256.

Heo, S.-J. and Y.-J. Jeon (2008). Radical scavenging capacity and cytoprotective effect of enzymatic digests of Ishige okamurae. *Journal of Applied Phycology* **20**(1087-1095).

Hernandez-Corona, D. M., E. Martinez-Abundis and M. Gonzalez-Ortiz (2014). Effect of Fucoidan Administration on Insulin Secretion and Insulin Resistance in Overweight or Obese Adults. *Journal of Medicinal Food* **17**(7): 830-832.

Higdon, J. V. and B. Frei (2003). Tea catechins and polyphenols; health effects, metabolism, and antioxidant functions. *Critical Reviews in Food Science and Nutrition* **43**(1): 89-143.

Huang, C., Y. Zhang, Z. Gong, X. Sheng, Z. Li, W. Zhang and Y. Qin (2006). Berberine inhibits 3T3-L1 adipocyte differentiation through the PPARγ pathway. *Biochemical and Biophysical Research Communications* **348**(2): 571-578.

Hursel, R., W. Viechtbauer, A. Dulloo, A. T. Tremblay, L, W. Rumpler and M. Westerterp-Plantenga (2011). The effects of catechin rich teas and caffeine on energy expenditure and fat oxidation: a meta-analysis. *Obesity Reviews* **12**: e573-e581.

Hursel, R., W. Viechtbauer and M. Westerterp-Plantenga (2009). The effects of green tea on weight loss and weight maintenance: a meta-analysis. *International Journal of Obesity* **33**: 956-961.

Iwai, K. (2008). Antidiabetic and Antioxidant Effects of Polyphenols in Brown Alga Ecklonia stolonifera in Genetically Diabetic KK-Ay Mice. *Plant Foods Hum Nutr* **63**: 163-169.

Jialal, I. and S. Devaraj (2014). Baseline Markers of Inflammation Are Associated With Progression to Macroalbuminuria in Type 1 Diabetic Subjects. *Diabetes Care* **37**: e106-e107.

Jung, H. A., H. J. Jung, H. Y. Jeong, H. J. Kwon, M. Y. Ali and J. S. Choi (2014). Phlorotannins isolated from the edible brown alga Ecklonia stolonifera exert anti-adipogenic activity on 3T3-L1 adipocytes by downregulating C/EBPα and PPARγ. *Fitoterapia* **92**: 260-269.

Kang, C., Y. B. Jin, H. Lee, M. Cha, E.-t. Sohn, J. Moon, C. Park, S. Chun, E.-S. Jung, J.-S. Hong, S. B. Kim, J.-S. Kim and E. Kim (2010). Brown alga Ecklonia cava attenuates type 1

diabetes by activating AMPK and Akt signaling pathways. *Food and Chemical Toxicology* **48**: 509-516.

Kang, M.-C., W. Wijesinghe, S.-H. Lee, S.-M. Kang, S.-C. Ko, X. Yang, N. Kang, B.-T. Jeon, J. Kim, D.-H. Lee and Y.-J. Jeon (2013). Dieckol isolated from brown seaweed Ecklonia cava attenuates type II diabetes in db/db mouse model. *Food and Chemical Toxicology* **53**: 294-298.

Kang, N.-J., S.-C. Hun, G.-J. Kang, D.-H. Koo, Y.-S. Koh, J.-W. Hyun, N.-H. Lee, M.-H. Ko, H.-K. Kang and E.-S. Yoo (2015). Diphlorethohydroxycarmalol Inhibits Interleukin-6 Production by Regulating NF-κB, STAT5 and SOCS1 in Lipopolysaccharide-Stimulated RAW264.7 Cells. *Marine Drugs* **13**: 2141-2157.

Kellogg, J., D. Esposito, M. H. Grace, S. Komarnytsky and M. A. Lila (2015). Alaskan seaweeds lower inflammation in RAW 264.7 macrophages and decrease lipid accumulation in 3T3-L1 adipocytes. *Journal of Functional Foods* **15**: 396-407.

Kim, J.-S., C.-S. Kwon and K. H. Son (2000). Inhibition of Alpha-glucosidase and Amylase by Luteolin, a Flavonoid. *Bioscience, Biotechnology, and Biochemistry* **64**(11): 2458-2461.

Kim, J., M. Yoon, H. Yang, J. Jo, D. Han, Y.-J. Jeon and S. Cho (2014). Enrichment and purification of marine polyphenol phlorotannins using macroporous adsorption resins. *Food Chemistry* **162**: 135-142.

Kim, S. M., K. Kang, J.-S. Jeon, E. H. Jho, C. Y. Kim, C. W. Nho and B.-H. Um (2011). Isolation of Phlorotannins from Eisenia bicyclis and Their Hepatoprotective Effect against Oxidative Stress Induced by tert-Butyl Hyperoxide. *Appl Biochem Biotechnol* **165**: 1296-1307.

Kris-Etherton, P. M., K. D. Hecker, A. Bonanome, S. M. Coval, A. E. Binkoski, K. F. Hilpert, A. E. Griel and T. D. Etherton (2002). Bioactive Compounds in Foods: Their Role in the Prevention of Cardiovascular Disease and Cancer. *Am J Med* **113**(9B): 71S-88S.

Kumar Chandini, S., P. Ganesan and N. Bhaskar (2008). In vitro antioxidant activities of three selected brown seaweeds of India. *Food Chemistry* **107**: 707-713.

Lee, C. Y. (2013). Challenges in providing credible scientific evidence of health benefits of dietary polyphenols. *Journal of Functional Foods* **5**: 524-526.

Lee, S.-H., J.-S. Han, S.-J. Heo, J.-Y. Hwang and Y.-J. Jeon (2010). Protective effects of dieckol isolated from Ecklonia cava against high glucose-induced oxidative stress in human umbilical vein endothelial cells. *Toxicology in Vitro* **24**: 375-381.

Lee, S.-H. and Y.-J. Jeon (2015). Efficacy and safety of a dieckol-rich extract (AG-dieckol) of brown algae, Ecklonia cava, in pre-diabetic individuals: a double-blind, randomized, placebo-controlled clinical trial. *Food Funct* **6**(853-858).

Lee, S.-H., K.-H. Min, J.-S. Han, D.-H. Lee, D.-B. Park, W.-K. Jung, P.-J. Park, B.-T. Jeon, S.-K. Kim and Y.-J. Jeon (2012). Effects of brown alga, Ecklonia cava on glucose and lipid metabolism in C57BL/KsJ-db/db mice, a model of type 2 diabetes mellitus. *Food and Chemical Toxicology* **50**: 575-582.

Li, Y., Z.-J. Qian, B. Ryu, S.-H. Lee, M.-M. Kim and S.-K. Kim (2009). Chemical components and its antioxidant properties in vitro: An edible marine brown alga, Ecklonia cava. *Bioorganic & Medicinal Chemistry* **17**: 1963-1973.

Libby, P. (2006). Inflammation and cardiovascular disease mechanisms. *American Journal of Clinical Nutrition* **83**(suppl): 456S-460S.

Manach, C., A. Scalbert, C. Morand, C. Rémésy and L. Jime´nez (2004). Polyphenols: food sources and bioavailability. *Am J Clin Nutr* **79**: 727-747.

Manach, C., G. Williamson, C. Morand, A. Scalbert and C. Rémésy (2005). Bioavailability and bioefficacy of polyphenols in humans. I. Review of 97 bioavailability studies. *Am J Clin Nutr* **81**(suppl): 230S-242S.

Martirosyan, D. M. and J. Singh (2015). A new definition of functional food by FFC: what makes a new definition unique? *Functional Foods in Health and Disease* **5**(6): 209-223.

Mazzanti, G., F. Menniti-Ippolito, P. A. Moro, F. Cassetti, R. Raschetti, C. Santuccio and S. Mastrangelo (2009). Hepatotoxicity from green tea: a review of the literature and two unpublished cases. *European Journal of Clinical Pharmacology* **65**(4): 331-341.

Mhadhebi, L., A. Mhadhebi, J. Robert and A. Bouraoui (2014). Antioxidant, Anti-inflammatory and Antiproliferative Effects of Aqueous Extracts of Three Mediterranean Brown Seaweeds of the Genus Cystoseira. *Iranian Journal of Pharmaceutical Research* **13**(1): 207-220.

Min, K.-H., H.-J. Kim, Y.-J. Jeon and J.-S. Han (2011). Ishige okamurae ameliorates hyperglycemia and insulin resistance in C57BL/KsJ-db/db mice. *Diabetes Research and Clinical Practice* **93**: 70-76.

Miyagi, S., N. Iwama, T. Kawabata and K. Hasegawa (2003). Longevity and Diet in Okinawa, Japan: The Past, Present and Future. *Asia-Pacific Journal of Public Health* **15**(1 suppl): S3-S9.

Mouritsen, O. G., C. Dawezynski, L. Duelund, G. Jahreis, W. Vetter and M. Schroder (2013). On the human consumption of the red seaweed dulse (Palmaria palmata (L.) Weber & Mohr). *J Appl Phycol* **25**.

Murugan, A. C., M. R. Karim, M. B. M. Yusoff, S. H. Tan, M. F. B. F. Asras and S. S. Rashid (2015). New insights into seaweed polyphenols on glucose homeostasis. *Pharmaceutical Biology* **53**(8): 1087-1097.

Musunuru, K. (2010). Atherogenic Dyslipidemia: Cardiovascular Risk and Dietary Intervention. *Lipids* **45**(10): 907-914.

Naczk, M. and F. Shahidi (2004). Extraction and analysis of phenolics in food. *Journal of Chromatography A* **1054**: 95-111.

Nakai, M., N. Kageyama, K. Nakahara and W. Miki (2006). Phlorotannins as Radical Scavengers from the Extract of Sargassum ringgoldianum. *Marine Biotechnology* **8**: 409-414.

National Institute of Diabetes and Digestive and Kidney Diseases National Institutes of Health. NIDDK Image Library. Retrieved February, 2016, from https://catalog.niddk.nih.gov/imagelibrary/searchresults.cfm?type=recordtype&recordtype=1.

Nwosu, F., J. Morris, V. A. Lund, D. Stewart, H. A. Ross and G. J. McDougall (2011). Anti-proliferative and potential anti-diabetic effects of phenolic-rich extracts from edible marine algae. *Food Chemistry* **126**: 1006-1012.

Osiecki, H. (2004). The Role of Chronic Inflammation in Cardiovascular Disease and its Regulation by Nutrients. *Altern Med Rev* **9**(1): 32-53.

Paradis, M.-E., P. Couture and B. Lamarche (2011). A randomised crossover placebo-controlled trial investigating the effect of brown seaweed (Ascophyllum nodosum and Fucus vesiculosus) on postchallenge plasma glucose and insulin levels in men and women. *Appl Physiol Nutr Metab* **36**: 913-919.

Park, E. Y., E. H. LKim, M. H. Kim, Y. W. Seo, J. I. Lee and H. S. Jun (2012). Polyphenol-Rich Fraction of Brown Alga Ecklonia cava Collected from Gijang, Korea, Reduces Obesity and Glucose Levels in High-Fat Diet-Induced Obese Mice. *Evidence-Based Complementary and Alternative Medicine* **2012**: 11.

Park, S. H., S. R. Gammon, J. D. Knippers, S. R. Paulsen, D. S. Rubink and W. W. Winder (2002). Phosphorylation-activity relationships of AMPK and acetyl-CoA carboxylase in muscle. *Journal of Applied Physiology* **92**(6): 2475-2482.

Rader, D. J. (2007). Effect of Insulin Resistance, Dyslipidemia, and Intra-abdominal Adiposity on the Development of Cardiovascular Disease and Diabetes Mellitus. *The American Journal of Medicine* **120**(3, Supplement 1): S12-S18.

Rains, T. M., S. Agarwal and K. C. Maki (2011). Antiobesity effects of green tea catechins: a mechanistic review. *Journal of Nutritional Biochemistry* **22**: 1-7.

Roy, M. S., M. N. Janal, J. Crosby and R. Donnelly (2013). Inflammatory Biomarkers and Progression of Diabetic Retinopathy in African Americans With Type 1 Diabetes. *Investigative Ophthalmology & Visual Science* **54**: 5471-5480.

Roy, M. S., M. N. Janal, J. Crosby and R. Donnelly (2015). Markers of endothelial dysfunction and inflammation predict progression of diabetic nephropathy in African Americans with type 1 diabetes. *Kidney International* **87**: 427-433.

Scalbert, A., C. Manach, C. Morand, C. Rémésy and L. Jiménez (2005). Dietary Polyphenols and the Prevention of Diseases. *Critical Reviews in Food Science and Nutrition* **45**(4): 287-306.

Scalbert, A. and G. Williamson (2000). Dietary intake and bioavailability of polyphenols. *The Journal of Nutrition* **130**(8S): 2073S-2085S.

Shibata, T., K. Ishimaru, S. Kawaguchi, H. Yoshikawa and Y. Hama (2008). Antioxidant activities of phlorotannins isolated from Japanese Laminariaceae. *Journal of Applied Phycology* **20**(705-711).

Shibata, T., S. Kawaguchi, Y. Hama, M. Inagaki, K. Yamaguchi and T. Nakamura (2004). Local and chemical distribution of phlorotannins in brown algae. *Journal of Applied Phycology* **16**: 291-296.

Shibata, T., K. Yamaguchi, K. Nagayama, S. Kawaguchi and T. Nakamura (2002). Inhibitory activity of brown algal phlorotannins against glycosidases from the viscera of the turban shell Turbo cornutus. *European Journal of Phycology* **37**(4): 493-500.

Shin, H.-C., S. H. Kim, Y. Park, B. H. Lee and H. J. Hwang (2012). Effects of 12‐week Oral Supplementation of Ecklonia cava Polyphenols on Anthropometric and Blood Lipid Parameters in Overweight Korean Individuals: A Double‐blind Randomized Clinical Trial. *Phytotherapy Research*: 363-368.

Spencer, J. P., M. M. Abd El Mohsen, A.-M. Minihane and J. C. Mathers (2008). Biomarkers of the intake of dietary polyphenols: strengths, limitations and application in nutrition research. *British Journal of Nutrition* **99**: 12-22.

Steinberg, P. D. (1995). Seasonal variation in the relationship between growth rate and phlorotannin production in the kelp Ecklonia radiata. *Oecologia* **102**: 169-173.

Wang, J., L. Tang and J.-S. Wang (2015). Biomarkers of Dietary Polyphenols in Cancer Studies: Current Evidence and Beyond. *Oxidative Medicine and Cellular Longevity* **2015**: 14.

Wang, T., R. Jonsdottir, H. Liu, L. Gu, H. G. Kristinsson, S. Raghavan and G. Olafsdottir (2012). Antioxidant Capacities of Phlorotannins Extracted from the Brown Algae Fucus vesiculosus. *Journal of Agricultural and Food Chemistry* **60**: 5874-5883.

Willcox, D. C., B. J. Willcox, H. Todoriki and M. Suzuki (2009). The Okinawan Diet: Health Implications of a Low- Calorie, Nutrient-Dense, Antioxidant-Rich Dietary Pattern Low in Glycemic Load. *Journal of the American College of Nutrition* **28**(sup4): 500S-516S.

Willerson, J. T. and P. M. Ridker (2004). Inflammation as a Cardiovascular Risk Factor. *Circulation* **109**(suppl II): II-2-II-10.

Williamson, G. and C. Manach (2005). Bioavailability and bioefficacy of polyphenols in humans. II. Review of 93 intervention studies. *Am J Clin Nutr* **81**(suppl): 243S-255S.

Yang, C. S., J. M. Landau, M.-T. Huang and H. L. Newmark (2001). Inhibition of carcinogenesis by dietary polyphenolic compounds. *Annu Rev Nutr* **21**: 381-406.

Yang, E.-J., J.-Y. Moon, S. S. Kim, K.-W. Yang, W. J. Lee, N. H. Lee and C.-G. Hyun (2014). Jeju seaweeds suppress lipopolysaccharide-stimulated proinflammatory response in RAW 264.7 murine macrophages. *Asian Pacific Journal of Tropical Biomedicine* **4**(7): 529-537.

Yeo, A.-R., J. Lee, I. H. Tae, S.-R. Park, Y. H. Cho, B. H. Lee, H.-C. Shin, S. H. Kim and Y. C. Yoo (2012). Anti-hyperlipidemic Effect of Polyphenol Extract (SeapolynolTM) and Dieckol Isolated from Ecklonia cava in in vivo and in vitro Models. *Prev Nutr Food Sci* **17**: 1-7.

Yoon, N. Y., H. R. Kim, H. Y. Chung and J. S. Choi (2008). Anti-hyperlipidemic Effect of an Edible Brown Algae, Ecklonia stolonifera, and its Constituents on Poloxamer 407-Induced Hyperlipidemic and Cholesterol-fed Rats. *Arch Pharm Res* **31**(12): 1564-1571.

Zaragoza, M., D. Lopez, M. Saiz, M. Poquet, J. Perez, P. Puig-Parellada, F. Marmol, P. Simonetti, C. Gardana, Y. Lerat, P. I. Burtin, C, I. Rousseau, M. Besnard and M. Mitjavila (2008). Toxicity and Antioxidant Activity in Vitro and in Vivo of Two Fucus vesiculosus Extracts. *Journal of Agricultural and Food Chemistry* **56**: 7773-7780.

Zava, T. T. and D. T. Zava (2011). Assessment of Japanese iodine intake based on seaweed consumption in Japan: A literaturebased analysis. *Thyroid Research* **4**(14): 1-7.

Zhang, J., C. Tiller, J. Shen, C. Wang, G. S. Girouard, D. Dennis, C. J. Barrow, M. Miao and H. S. Ewart (2007). In Vitro Potential of Ascophyllum nodosum Phenolic Antioxidant-Mediated α-Glucosidase and α-Amylase Inhibition. *Can J Physiol Pharmacol* **85**: 1116-1123.

</BIBL>

Table 1 Summary of phlorotannins isolated from marine algal species.

Seaweed species	Reported phlorotannin content	Reference
Ascophyllum nodosum	Approx. 5.80% of dry weight	(Connan et al. 2004)
Bifurcaria bifurcata	3.73 (0.57)% of dry weight	(Connan et al. 2004)
Cystoseira nodicaulis	89.14 (2.57) g phloroglucinol equivalents (PGE)/mg sample	(Heffernan et al. 2015)
Ecklonia cava	3.3% crude phlorotannins: • 4.7% phloroglucinol • 0.7% phloroglucinol tetramer • 6.4% eckol • 16.6% phlorofucofuroeckol A • 22.2% dieckol • 12.4% 8,8'-Bieckol	(Shibata et al. 2004)
Ecklonia cava	Dieckol - 1.52 mg/g dry weight	(Chowdhury et al. 2014)
Ecklonia cava	Phlorofucofuroeckol A - 0.93 mg/g dry weight	(Chowdhury et al. 2014)
Ecklonia cava	Total phlorotannin content - 3.39 mg PGE/mL in crude phlorotannin extract solution	(Kim et al. 2014)
Ecklonia kurome	3.0% crude phlorotannins: • 2.6% phloroglucinol • 0.3% phloroglucinol tetramer • 9.2% eckol • 28.6% phlorofucofuroeckol A • 24.6% dieckol	(Shibata et al. 2004)
Ecklonia kurome	7.8% 8,8'-Bieckol	(Shibata et al. 2004)
Ecklonia stolonifera	Dieckol - 1.52 mg/g dry weight	(Chowdhury et al. 2014)
Ecklonia stolonifera	Phlorofucofuroeckol A - approx. 1.20 mg/g dry weight	(Chowdhury et al. 2014)
Eisenia bicyclis	3.1% crude phlorotannins: • 0.9% phloroglucinol • 4.4% phloroglucinol tetramer • 7.5% eckol	(Shibata et al. 2004)

	• 21.9% phlorofucofuroeckol A • 23.4% dieckol • 24.6% 8,8'-Bieckol	
Eisenia bicyclis	Dieckol - 1.33 mg/g dry weight	(Chowdhury et al. 2014)
Eisenia bicyclis	Phlorofucofuroeckol A - 1.30 mg/g dry weight	(Chowdhury et al. 2014)
Eisenia bicyclis	Contains Eckol	(Kim et al. 2011)
Eisenia bicyclis	Contains 6,6'-Bieckol	(Kim et al. 2011)
Eisenia bicyclis	Contains 8,8'-Bieckol	(Kim et al. 2011)
Eisenia bicyclis	Contains Dieckol	(Kim et al. 2011)
Eisenia bicyclis	Contains Phlorofucofuroeckol A	(Kim et al. 2011)
Fucus serratus	4.27 (1.12)% of dry weight	(Connan et al. 2004)
Fucus serratus	180.55 (16.98) µg PGE/mg sample	(Heffernan et al. 2015)
Fucus spiralis	3.88 (0.65)% of dry weight	(Connan et al. 2004)
Fucus vesiculosus	Phlorotannins approx. 5.80% of dry weight	(Connan et al. 2004)
Fucus vesiculosus	231.95 (8.97) µg PGE/mg sample	(Heffernan et al. 2015)
Fucus vesiculosus	Total phlorotannins ranged from 12 to 23 mg/g dry weight	(Creis et al. 2015)
Himanthalia elongata	2.17 (1.40)% of dry weight	(Connan et al. 2004)
Himanthalia elongata	198.28 (9.17) µg PGE/mg sample	(Heffernan et al. 2015)
Laminaria digitata	0.13 (0.03)% of dry weight	(Connan et al. 2004)
Pelvetia canaliculata	3.39 (0.64)% of dry weight	(Connan et al. 2004)
Sargassum aquifolium	6.770 (0.001) mg phlorotannins/g dry weight	(Cuong et al. 2015)
Sargassum denticarpum	0.978 (0.004) mg phlorotannins/g dry weight	(Cuong et al. 2015)
Sargassum mcclurei	2.057 (0.003) mg phlorotannins/g dry weight	(Cuong et al. 2015)
Sargassum oligocystum	2.369 (0.004) mg phlorotannins/g dry weight	(Cuong et al. 2015)
Sargassum serratum	1.305 (0.008) mg phlorotannins/g dry weight	(Cuong et al. 2015)
Sargassum polycystum	0.735 (0.002) mg phlorotannins/g dry weight	(Cuong et al. 2015)

PGE -- phloroglucinol equivalents

Table 2 Anti-hyperglycaemic effects of marine polyphenols.

Seaweed species	Polyphenol	Dosage and duration	Subject/medium	Anti-diabetic effect	Reference
Alaria, Palmaria, Ascophyllum	Polyphenolic rich extracts	NA	Chemical assay	α-amylase inhibition α-glucosidase inhibition	(Nwosu et al. 2011)
Ascophyllum nodosum	Does not name specific polyphenols	NA	Chemical assay	Dose dependent Strong α-glucosidase inhibitionDose dependent α-amylase inhibition	(Apostolidis and Lee 2010)
Eisenia bicyclis	Phloroglucinol Eckol Phlorofucofuroeckol A Dieckol 8,8'-bieckol An unidentified tetramer	NA	Chemical assay	Glycosidase enzyme inhibition	(Shibata et al. 2002)
Ascophyllum nodosum	Does not name specific polyphenols	NA 400 µg/mL extract, 20 minute incubation 200 mg/kg body weight for 4 weeks	Chemical assay 3T3-L1 adipocytes streptozotocin-diabetic mice	Dose dependent α-glucosidase inhibition Glucose uptake stimulated Fasting blood glucose reduced Postprandial blood glucose rise blunted	(Zhang et al. 2007)
Ecklonia stolonifera	Does not name specific polyphenols	NA 16.42 or 81.20 mg for 4 weeks	Chemical assay Genetically non-insulin dependent diabetic KK-	Strong α-glucosidase inhibition in vitro Suppressed postprandial	(Iwai 2008)

			A^y male mice	blood glucose and insulin	
Ishige okamurae	Diphlorethohydroxycarmalol (DPHC)	NA 100 mg/kg body weight (single dose)	Chemical assay Streptozotocin-induced diabetic mice	Strong α-glucosidase and α-amylase inhibition Supressed postprandial blood glucose	(Heo et al. 2009)
Ecklonia cava	Does not name specific polyphenols	50--300 µg/ml, 1 hour incubation 300 mg/kg body weight for 3 weeks	C_2C_{12} myoblasts Streptozotocin-induced type 1 diabetes mellitus rats	Activated AMPK/ACC and P13/Akt signal transduction pathways Fasting blood glucose reduced Insulin concentration increased	(Kang et al. 2010)
Ecklonia cava	Polyphenol extract (28.2 ± 0.58% polyphenols): Dieckol 2,7''-phloroglucinol-6,6'-bieckol, Pyrygallol-phloroglucinol-6,6'-bieckol, Phlorofucofuroeckol A	100 or 500 mg/kg body weight for 12 weeks	C57BL/6 male mice	Reduced blood glucose one hr after injection Reduced postprandial glucose AUC High dose reduced plasma insulin and HOMA-IR	(Eo et al. 2015)
Ecklonia cava	CA extract -- 68.78mg/g polyphenols G-CA extract -- 79.70mg/g polyphenols	200 mg/kg body weight for 7 weeks	C57BL/6 mice	Reduced fasting blood glucose	(Park et al. 2012)

				Improved glucose tolerance	
Ecklonia cava	Dieckol rich extract	0.5 g/100 g diet for 6 weeks	C57BL/KsJ-*db/db (db/db)* male mice	Reduced fasting blood glucose Reduced plasma insulin Reduced HbA1c	(Lee et al. 2012)
Ecklonia cava	Dieckol	20 mg/kg body weight for 14 days	C57BL/KsJ-*db/db*, a type II diabetes mice	Reduced blood glucose Dose dependently reduced plasma insulin	(Kang et al. 2013)
Ishige okamurae	Does not mention specific polyphenols	0.5% w/w for 6 weeks	C57BL/KsJ-*db/db* mice	Reduced G6Pase and PEPCK activity Increased hepatic glycogen production Reduced fasting blood glucose Reduced HbA1c	(Min et al. 2011)
Ascophyllum nodosum, Fucus vesiculosus	Commercially available blend of brown seaweeds containing a minimum of 10% polyphenols	50 mg polyphenols, single dose prior to postprandial testing	23 non-diabetic adults	Reduced postprandial insulin iAUC Increased insulin sensitivity	(Paradis et al. 2011)
Ecklonia cava	Polyphenol extract	72 or 144 mg/day for 12 weeks	97 non-diabetic overweight	Reduced fasting blood	(Shin et al. 2012)

			adults	glucose (high dose only)	
Ecklonia cava	Dieckol-rich extract	690 mg/day for 12 weeks	73 adults with high fasting blood glucose	Improved postprandial blood glucose control Reduced fasting blood insulin level No change in fasting blood glucose level	(Lee and Jeon 2015)
Not specified	Polyphenol containing (5%) oral supplement	25 mg polyphenols for 3 months	25 non-diabetic overweight or obese volunteers	Increased plasma insulin, HOMA β-cell and HOMA -IR	(Hernandez-Corona et al. 2014)

AMPK/ACC -- AMP-activated protein kinase/acetyl-CoA carboxylase

AUC -- area under the curve

G6Pase -- glucose-6-phosphatase

HbA1c -- glycated haemoglobin

HOMA-IR -- homeostatic model assessment -- insulin resistance

HOMA β-cell -- homeostatic model assessment -- beta cell function

iAUC -- incremental area under the curve

NA -- not available/not applicable

P13/Akt -- phosphatidylinositol 3-kinase/Akt (a serine/threonine protein kinase)

PEPCK -- phosphoenolpyruvate carboxykinase

Table 3 Anti-hyperlipidaemic effects of marine polyphenols.

Seaweed species	Polyphenol	Dosage and duration	Subjects/medium	Anti-hyperlipidaemic effect	Reference
Ecklonia cava	Generic polyphenol extract (Seapolynol™ -- 98.5% polyphenols) and Dieckol	NA ~200 µg/ml, 4 days incubation 1.25, 2.5 or 5.0 mg Seapolynol™, or 0.5, 1.0 or 2.0 mg dieckol for 4 weeks	Chemical assay 3T3-L1 preadipocytes ICR mice	Inhibited HMGCoA reductase Inhibited adipocyte differentiation and lipid accumulation Reduced TC, TG and LDL-C	(Yeo et al. 2012)
Ecklonia stolonifera	Phloroglucinol Eckol Dieckol Dioxinodehydroeckol Phlorofucofuroeckol A	Concentrations from 12.5 to 100 µM, 8 days incubation	3T3-L1 preadipocytes	Dose dependently inhibited lipid accumulation. Reduced expression of adipocyte marker genes	(Jung et al. 2014)
Ecklonia cava	Dieckol-rich extract	0.5 g/100 g diet for 6 weeks	C57BL/KsJ-*db/db (db/db)* male mice	Reduced TC, TG and FFA Increased HDL-C	(Lee et al. 2012)
Ecklonia cava	Polyphenol extract (28.2 ± 0.58% polyphenols): Dieckol 2,7''-phloroglucinol-6,6'-bieckol, Pyrygallol-phloroglucinol-6,6'-bieckol, Phlorofucofuroeckol-A	100 or 500 mg/kg body weight for 12 weeks	Obese C57BL/6 male mice	Reduced TG and TC levels No change in HDL-C	(Eo et al. 2015)
Ecklonia cava	CA extract -- 68.78mg/g polyphenols G-CA extract --	200 mg/kg body weight for 8 weeks	Obese C57BL/6 mice	G-CA extract reduced TC	(Park et al. 2012)

	79.70mg/g polyphenols				
Ecklonia stolonifera	Polyphenol extract Eckol Dieckol	100 to 250 mg/kg body weight (polyphenol extract), or 10 or 20 mg/kg body weight (eckol & dieckol) for 3 days	Hyperlipidaemic rats	Both treatments dose dependently reduced TC, TG, and LDL-C and increased HDL-C. Different extraction techniques yielded different actions.	(Yoon et al. 2008)
Ecklonia cava	Polyphenol extract	72 or 144 mg for 12 weeks	97 overweight adults	Dose dependently reduced TC, LDL-C, and TC/HDL-C ratio. High dose increased HDL-C	(Shin et al. 2012)
Ecklonia cava	Dieckol-rich extract (8.2% dieckol)	400 mg/day (32.8 mg dieckol) for 12 weeks	80 adults with raised cholesterol	Reduced TC and LDL-C levels Intervention had no effect on HDL-C or TG levels	(Choi et al. 2015)
Not specified	Polyphenol containing (5%) oral supplement	25 mg polyphenols for 3 months	25 overweight or obese volunteers	Reduced LDL-C only	(Hernandez-Corona et al. 2014)

TC -- total cholesterol

LDL-C -- low density lipoprotein cholesterol

HDL-C -- high density lipoprotein cholesterol

TG -- triglyceride

HMGCoA -- 3-hydroxy-3-methylglutaryl-coenzyme A

CA -- Jeju geographical area, Korea

G-CA -- Gijang geographical area, Korea

Table 4 Anti-inflammatory effects of marine polyphenols.

Seaweed species	Polyphenol	Dosage and duration	Subject/medium	Anti-inflammatory effect	Reference
Ishige okamurae	Diphlorethohydroxycarmalol	312.4 pg/mL at 6 hours incubation 918.3 pg/mL at 24 hours incubation	RAW264.7 Cells	Dose dependently reduced production of IL-6, downregulated Jak2-STAT5, upregulated SOCS1	(Kang et al. 2015)
Fucus distichus, Alaria marginata, Saccharina groenlandica, Saccharina latissimi	Phlorotannin sub fraction	12.5 to 50 µg/mL	RAW264.7 macrophages	Reduced expression of COX2 iNOS, TNF-α, IL-10 and MCP-1 pro-inflammatory genes	(Kellogg et al. 2015)
Dictyopteris divaricata, Dictyopteris prolifera, Prionitis cornea, Grateloupia lanceolata, and *Grateloupia filicina*	Polyphenol rich extracts	12.5, 25, 50 or 100 µg/mL, 18 hours incubation	RAW 264.7 murine macrophages	Dose dependently reduced NO production, COX2 protein levels, TNF-α and IL-6 cytokines	(Yang et al. 2014)
Cystoseira crinita, Cystoseira sedoides, Cystoseira compressa	Aqueos extracts Total phenolic content *C. sedoides* - 50.3 mg GAE/g dried sample *C. crinita* -- 56.5 mg GAE/g dried sample *C. compressa* - 61.0 mg GAE/g dried sample	25 or 50 mg/kg body weight (single dose)	Rat paw oedema assay	Dose dependently reduced inflammation	(Mhadhebi et al. 2014)

Ecklonia cava	Polyphenol extract (28.2 ± 0.58% polyphenols) Dieckol 2,7''-phloroglucinol-6,6'-bieckol, Pyrygallol-phloroglucinol-6,6'-bieckol, Phlorofucofuroeckol-A	100 or 500 mg/kg body weight for 12 weeks	Obese C57BL/6 male mice	Dose dependently reduced MCP-1, TNF-α, IL-1β, NF-κB and COX2 protein levels	(Eo et al. 2015)
Ecklonia cava	CA extract -- 68.78 mg/g polyphenols G-CA extract -- 79.70 mg/g polyphenols	200 mg/kg body weight for 8 weeks	Obese C57BL/6 mice	Reduced expression of TNF-α, IL-1β and F4/80	(Park et al. 2012)

CA -- Jeju geographical area, Korea

COX2 -- cyclooxygenase-2

G-CA -- Gijang geographical area, Korea

GAE -- gallic acid equivalents

IL-1β -- interleukin 1β

IL-10 -- interleukin 10

IL-6 -- interleukin 6

iNOS -- inducible nitric oxide synthase

Jak2-STAT5 -- Janus kinase signal transducer and activator of transcription

MCP-1 -- monocyte chemoattractant protein-1

NF-κB -- nuclear factor κB

NO -- nitric oxide

SOCS1 - suppressor of cytokine signalling 1

TNF-α -- tumour necrosis factor α

Table 5 Antioxidant effects of marine polyphenols.

Seaweed species	Polyphenol	Dosage and duration	Subject/medium	Antioxidant effect	Reference
Fucus vesiculosus	High molecular weight phlorotannins	Concentrations from 0.1 to 5.0 mg/mL	Chemical assay	Strong antioxidant activity	(Wang et al. 2012)
Ishige okamurae	Does not name specific polyphenols	60 µL	Chemical assay	Dose dependent antioxidant activity	(Heo and Jeon 2008)
Padina antillarum, Caulerpa racemosa and *Kappaphycus alvarezzi*	Does not name specific polyphenols	Concentrations of 1.0 to 7.0 mg/mL, incubation periods of 10 to 60 minutes	Chemical assay	Strong antioxidant activity	(Chew et al. 2008)
Sargassum marginatum, Padina tetrastomatica and *Turbinaria conoides*	Does not name specific polyphenols	Incubation periods of 20 minutes to 4 hours	Chemical assay	Dose dependent strong antioxidant activity	(Kumar Chandini et al. 2008)
Sargassum ringgoldianum	Bifuhalol	NA	Chemical assay	Strong antioxidant activity	(Nakai et al. 2006)
Turbinaria conoides and *Turbinaria ornata*	Does not name specific polyphenols	Concentrations of 0.1 to 0.6 mg/mL, 45 minute incubation	Chemical assay	Antioxidant activity	(Chakraborty et al. 2013)
Eisenia bicylis, Ecklonia cava and *Ecklonia kurome*	Eckol Phlorofucofuroeckol A Dieckol 8,8'-bieckol	Concentrations from 1 µM to 26 µM, 30 minute incubation	Liposome system	Strong antioxidant activity	(Shibata et al. 2008)
Ecklonia cava	7-phloro eckol, 6,6'-bieckol, Phloroglucinol, Eckol,	Concentrations from 1 to 50 µM, incubation up	RAW264.7 cell line	Antioxidant activity	(Li et al. 2009)

	Fucodiphloroethol G, Phlorofucofuroeckol A, Dieckol	to 7 days			
Ecklonia cava	Phloroglucinol, eckol and dieckol	Concentrations of 0.25, 0.5 and 1.0 mg/mL	L5178 mouse T-cell lymphoma cell lines	Dose dependent Strong (eckol) Antioxidant activity	(Ahn et al. 2007)
Ecklonia cava	Dieckol	Concentrations of 10 and 50 µg/mL, 20 hours incubation	HUVECs	Antioxidant activity	(Lee et al. 2010)
Fucus vesiculosus	28.8% polyphenol content extract 18% polyphenol content extract	Variety of extract concentrations, 1 hour incubation	Activated RAW264.7 macrophages	Antioxidant activity	(Zaragoza et al. 2008)
Ecklonia cava	Dieckol	10 and 20 mg/body weight for 14 days	C57BL/KsJ-*db/db* type II diabetic mice	Antioxidant activity	(Kang et al. 2013)

HUVECs -- human umbilical vein endothelial cells

Table 6 Health effects of marine polyphenols according to algal species.

Species	Effect	Subject/medium	Reference
Ascophyllum nodosum	Anti-hyperglycaemic	Chemical assay	(Nwosu et al. 2011)
			(Apostolidis and Lee 2010)
			(Zhang et al. 2007)
		3T3-L1 adipocytes	(Zhang et al. 2007)
		Diabetic mice	(Zhang et al. 2007)
		Non-diabetic adults	(Paradis et al. 2011)
Ecklonia cava	Anti-hyperglycaemic	C_2C_{12} myoblasts	(Kang et al. 2010)
		Diabetic rats	(Kang et al. 2010)
		Mice	(Eo et al. 2015)
			(Park et al. 2012)
		Diabetic mice	(Lee et al. 2012)
			(Kang et al. 2013)
		Non-diabetic overweight adults	(Shin et al. 2012)
		Pre-diabetic adults	(Lee and Jeon 2015)
	Anti-hyperlipidaemic	Chemical assay	(Yeo et al. 2012)
		3T3-L1 preadipocytes	(Yeo et al. 2012)
		Mice	(Yeo et al. 2012)
		Diabetic mice	(Lee et al. 2012)
		Obese mice	(Eo et al. 2015)
			(Park et al. 2012)
		Overweight adults	(Shin et al. 2012)
		Adults with raised cholesterol	(Choi et al. 2015)
	Anti-inflammatory	Obese mice	(Eo et al. 2015)
			(Park et al. 2012)
	Antioxidant	Liposome system	(Shibata et al. 2008)
		RAW264.7 cell line	(Li et al. 2009)
		L5178 mouse T-cell lymphoma cell lines	(Ahn et al. 2007)
		HUVECs	(Lee et al. 2010)
		Diabetic mice	(Kang et al. 2013)
Ecklonia stolonifera	Anti-hyperglycaemic	Chemical assay	(Iwai 2008)
		Diabetic mice	(Iwai 2008)
	Anti-hyperlipidaemic	3T3-L1 preadipocytes	(Jung et al. 2014)
		Hyperlipidaemic rats	(Yoon et al. 2008)
Fucus vesiculosus	Anti-hyperglycaemic	Non-diabetic adults	(Paradis et al. 2011)
	Antioxidant	Chemical assay	(Wang et al. 2012)

		RAW264.7 macrophages	(Zaragoza et al. 2008)
Ishige okamurae	Anti-hyperglycaemic	Chemical assay	(Heo et al. 2009)
		Diabetic mice	(Heo et al. 2009)
			(Min et al. 2011)
	Anti-inflammatory	RAW264.7 Cells	(Kang et al. 2015)
	Antioxidant	Chemical assay	(Heo and Jeon 2008)

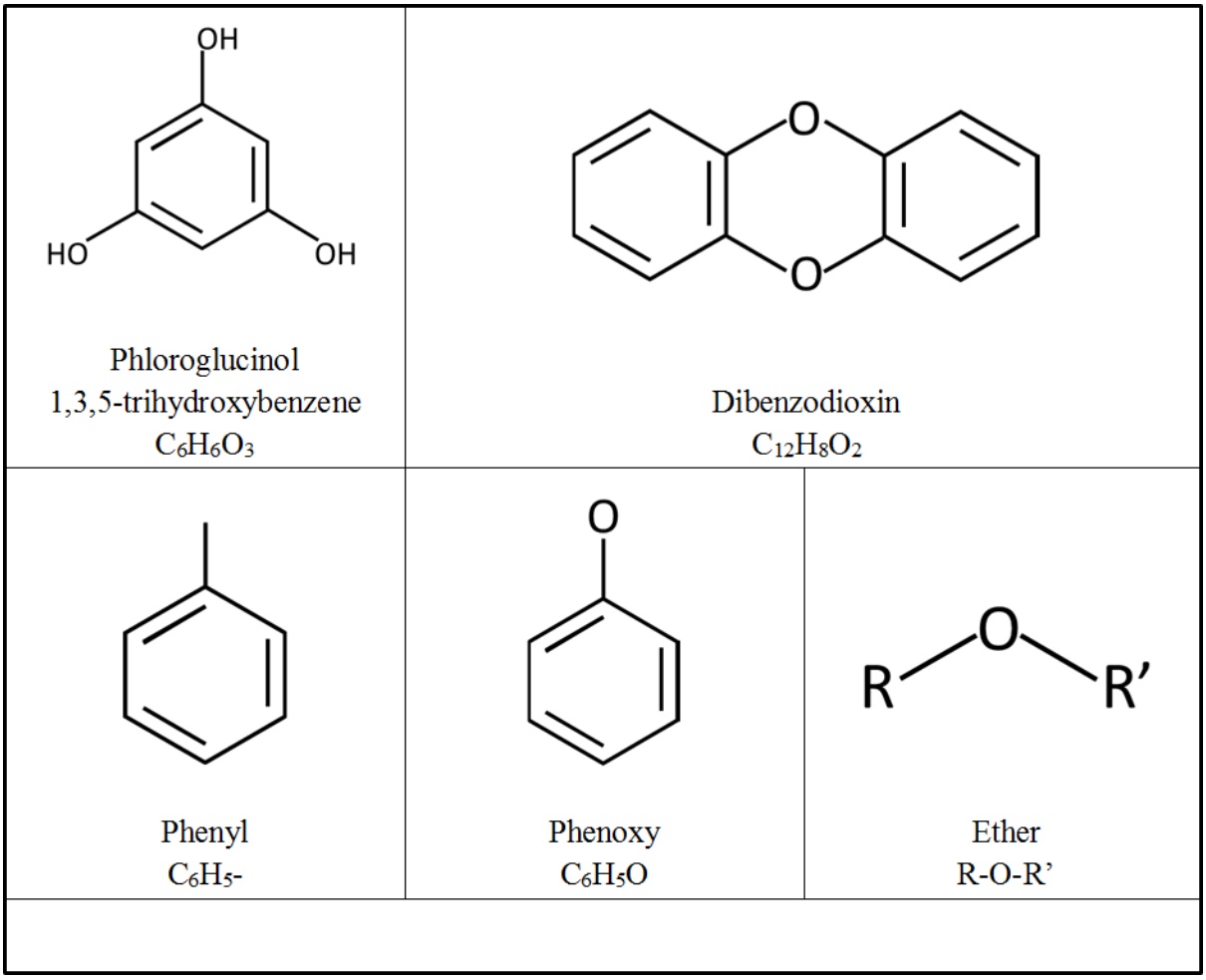

Figure 1 Chemical structures of phloroglucinol, dibenzodioxin, phenyl, phenoxy and ether groups

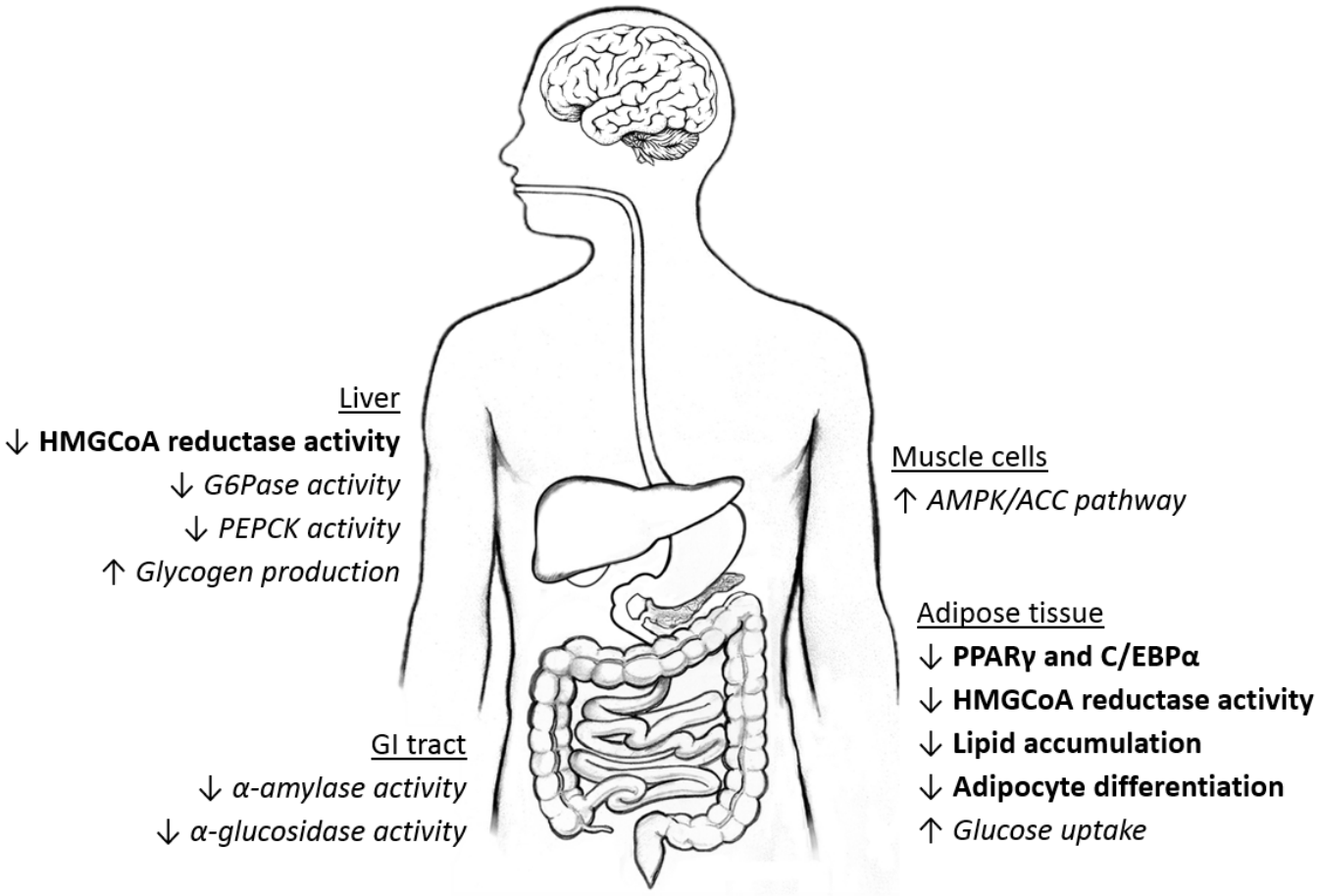

Figure 2 The proposed anti-hyperglycaemic and anti-hyperlipidaemic effects of marine polyphenols