**Introduction**

Diabetes mellitus is a group of human metabolic disorders causing elevated blood glucose levels due to a lack of insulin secretion and/or the development of insulin resistance in peripheral tissues. It is one of the most common chronic diseases in the world, with an estimated 422 million adults globally living with diabetes in 2014, compared with 108 million in 1980 (World Health Organization, 2016). The estimated global direct health expenditure on diabetes in 2019 was USD 760 billion (Williams et al., 2020). Targets of the UN’s Sustainable Development Goal 3 include reducing the mortality rate attributed to cardiovascular disease, cancer, diabetes and chronic respiratory disease by one-third by 2020 (https://sustainabledevelopment.un.org/sdg3).

Currently, type 2 diabetes mellitus (T2DM) accounts for more than 90% of all cases of diabetes, so there is a focus on treatment or management of this disease (World Health Organization, 2016). T2DM has many features but a key characteristic is hyperglycaemia, in which an excessive amount of postprandial glucose is absorbed into the bloodstream as a result of impaired insulin production/signalling (Muskiet & Tonneijck, 2017). A major source of this glucose is the intestinal hydrolysis of dietary carbohydrates carried out by starch-converting enzymes, in particular alpha-glucosidase. Thus, inhibition of alpha-glucosidase delays carbohydrate hydrolysis and in turn reduces post-prandial hyperglycaemia (Muskiet & Tonneijck, 2017). Glucose absorption is further affected by the action of sodium glucose transporter-2 (SGLT-2). These transport proteins are found in the renal tubular epithelium and are quantitatively the most important for glucose reabsorption in the kidney (Wright, Hirayama, & Loo, 2007). Inhibition of SGLT-2 has proven to lower blood glucose in a dose-dependent manner (Rosenstock et al., 2012). Furthermore, stimulating the secretion of glucagon-like peptide-1 (GLP-1), a hormone released from L-cells which elicits postprandial insulin secretion and thus has a significant role in glucose homeostasis, could have a considerable impact on T2DM patients. The administration of GLP-1 to type 2...
diabetic patients effectively lowered blood glucose levels (Gutniak, Orskov, Holst, Ahren, & Efendic, 1992). Finally, inhibiting dipeptidyl peptidase-4 (DPP-4), a ubiquitous type II transmembrane glycoprotein responsible for GLP-1 and glucose-dependent insulino tropic polypeptide (GIP) degradation, has also proven fundamental as a management tool for T2DM (Green et al., 2003; Green, Irwin, Gault, FPM, & Flatt, 2005). Improved glucose tolerance was observed in DPP-4 knockout mice, in correlation with increased GLP-1 levels and enhanced insulin secretion, after oral administration of glucose (Marguet et al., 2000). A combination of all of these strategies could allow effective management of T2DM in affected patients.

With the increasing global incidence and cost of managing T2DM, further action must be taken to source alternatives to the costly, synthetic drugs already available and which can be easily made accessible to patients on a global scale. As such, naturally derived marine products with anti-diabetic activity may be exploited as an efficient and cost-effective approach to managing this disease (Nguyen et al., 2019). Previous studies have demonstrated that extracts from brown, red and green seaweed species, collected primarily in tropical areas, have the ability to inhibit alpha-glucosidase (e.g. Li, Niu, Fan, & Han, 2005; Lee, Karadeniz, Kim, Kim, & Kim, 2009; Apostolidis & Lee, 2010; Nwosu et al., 2011; Lee et al., 2012a, 2012b; Kawamura-Konishi et al., 2012; Kang et al., 2013; Schultz Moreira et al., 2014; Lauritano & Ianora, 2016; Shannon & Abu-Ghannam, 2019). The hypoglycemic effects of seaweed extracts and their secondary metabolites have been demonstrated in normal and diabetic animals (e.g. Iwai, 2008; Lopes, Andrade, & Valentao, 2017; Roy et al., 2011; Xu et al., 2012). To date, comparatively few naturally occurring compounds have been discovered which inhibit DPP-4. Berberine, an isoquinoline alkaloid from the Chinese herb Coptis chinensis, inhibited human recombinant DPP-4 (Al-Masri, Mohammad, & Tahaa, 2009). Procyanidins from grape seed inhibited intestinal DPP-4 in vivo (Gonzalez-Abuin et al., 2012, 2014) and protein hydrolysates from the red seaweed Palmaria palmata inhibited DPP-4 in vitro with IC₅₀ values of 1.65–4.60 mg ml⁻¹ (Harnedy, FitzGerald, & FitzGerald, 2015). Growing interest over the last 15 years in the effects of natural products (with phlorizin obtained from higher plants as a model) on SGLT-2 inhibition and GLP-1 secretion (Ehrenkranz, Lewis, Kahn, & Roth, 2005) has led to the development and testing of new drugs as next-generation antihyperglycemic agents (reviewed by Blaschek, 2017; Choi, 2016).

Marine organisms are currently the subject of intense research effort into bioactives including those with anti-diabetic properties (reviewed by Cotas, Leandro, Pacheco, Gonçalves, & Pereira, 2020; Lauritano & Ianora, 2016; Shannon & Abu-Ghannam, 2019). Brown algae are of particular interest due to their production of phlorotannins, a class of polyphenols exclusively produced by brown seaweeds (Gunathilaka, Samarakoon, Ranasinghe, & Peiris, 2020; Lopes et al., 2017). The majority of studies to date have focussed on tropical species (Gunathilaka et al., 2020), although recently the effects of dried seaweeds from Norway were investigated in a diabetic mouse model (Sørensen, Jeppesen, Christiansen, Hermansen, & Gregersen, 2019). Here we screened extracts from eleven common edible species of seaweeds (three red, two green and six brown algae) collected in Ireland for an array of anti-diabetic effects. The aim was to ascertain whether edible seaweeds could act through known and established therapeutic mechanisms, which included inhibition of alpha-glucosidase, inhibition of DPP-4, inhibition of SGLT-2 transporters and promotion of GLP-1 synthesis and secretion. We examined the effects of seaweed extracts on an important type of intestinal cell (the enteroendocrine L-cell). We used the widely established STC-1 murine cell model which produces significant amounts of the insulino tropic hormone GLP-1 (Gillespie, Pan, Marcocamell, Meharg, & Green, 2017; McCarthy et al., 2015). In a final phase, oral glucose tolerance tests were performed in vivo on promising seaweed extracts. We limited ourselves to using only food grade solvents to permit the future prospect of extracts being used as food additives/ingredients, with the aim of exploiting these natural products to be used as dietary supplements, medicinal foods or bio-therapeutics for cost-effective management of T2DM and hence potentially contribute to reducing premature mortality. In order to ensure reproducibility, one of our goals was to use samples that had been carefully identified, with publicly available voucher specimens, to determine the potential therapeutic value of these seaweeds.

Materials and methods

Collection and identification of seaweeds

Edible seaweed species were collected at Doaghbeg, Fanad Head, County Donegal, Ireland (55°14.90’N, 7°37.01’W). Species under investigation were two green seaweeds, Ulva rigida C.Agardh and Codium fragile (Suringar) Hariot subsp. fragile, three red seaweeds, Palmaria palmata (Linnaeus) F.Weber & D.Mohr, Porphyra linearis Greville and Chondrus crispus Stackhouse, and six brown seaweeds, Himanthalia elongata (Linnaeus) S.F.Gray, Saccharina latissima (Linnaeus) C.E.Lane, C.Mayes, Druehl & G.W.Saunders (synonym Laminaria saccharina), Laminaria digitata (Hudson) J.V.Lamouroux, Fucus vesiculosus Linnaeus, Ascophyllum nodosum (Linnaeus) Le
Jolis and *Alaria esculenta* (Linnaeus) Greville (Supplementary figs S1–2).

Several of these species have been subject to extensive taxonomic and genetic investigation at this site: *Codium fragile* subsp. *fragile* (Provan, Wattier, & Maggs, 2005a); *Palmaria palmata* (Provan, Murphy, & Maggs, 2005b); *Chondrus crispus* (Provan, Glendinning, Kelly, & Maggs, 2013) and *Ulva* spp. (Brodie, Maggs, & John, 2007; Hughey et al., 2019). Voucher specimens of the readily identifiable species *F. vesiculosus, L. digitata, H. elongata* and *A. esculenta* have been deposited in the algal herbarium (BM) at the Natural History Museum (Supplementary table S1). *Ulva* species are extremely difficult (arguably impossible) to identify morphologically (Brodie et al., 2007; Hughey et al., 2019); therefore, samples of the Doaghbeg population were sequenced for the *rbcL* marker. Samples for molecular identification were collected on 22 September 2002 and processed as described in Krupnik et al. (2018), using PCR to obtain *rbcL* sequences from the samples. Identifications were made by BLAST searches using only quality-assured sequences in GenBank. The majority of the Fanad material was *U. rigida*, whereas *U. fenestrata* Postels & Ruprecht (formerly known as *U. lactuca*) was relatively uncommon.

**Preparation and extraction of seaweed extracts**

Seaweed was rinsed under cold fresh water to remove excess sand and grit. Samples were dried with paper towels, frozen to −80°C then lyophilized for two days in a Moduloyd freeze dryer (Milford, USA). Freeze-dried samples were blended to a fine powder in a blender (IKA®-Werke GmbH & Co. KG, Germany), milled and stored in airtight containers at room temperature for less than 6 months prior to use.

Only water and ethanol extractions were performed as these solvents are classified as food grade solvents. 1.25 g of dried seaweed powder was added to 50 ml of boiling absolute ethanol (> 99.5%), placed on a rotary mixer for 30 min then centrifuged at 4000 g for 10 min after which the supernatant was removed. Ethanolic extracts were dried in a MiVac sample concentrator (Genevac, Ipswich, UK) and water extracts freeze-dried. The extracted seaweed powder was stored at −20°C until further use. On experimental days, dried seaweed extracts were reconstituted in appropriate buffer for experimentation.

**Alpha-glucosidase assay**

Alpha-glucosidase enzyme was initially prepared from rat intestinal acetone powder (Sigma-Aldrich Company Ltd, Dorset, UK) in a 9-fold volume of citrate buffer (pH 5.6) and supernatant obtained as the crude enzyme solution for use in assays. The control assay contained 300 µl of maltose at 10 mg ml⁻¹ and 150 µl of phosphate-buffered saline (PBS) buffer (pH 7.4). Seaweed extracts were assayed at 12.5 mg ml⁻¹ in the 150 µl volume and reactions were started by the addition of 10 µl of rat intestinal alpha-glucosidase enzyme. The solutions were incubated at 37°C for 75 min and analysed for glucose content every 15 min on a PGM7 Micro-Stat Analyser (Analoxy Instruments Ltd, London, UK). Acarbose (Sigma-Aldrich Company Ltd) was dissolved at 1 mg ml⁻¹ and used as a positive control.

**SGLT-2 assay**

Determination of SGLT-2 inhibition was carried out according to the protocol of Castaneda and Kinne (2005) involving a [¹⁴C]AMG assay (NEN, Bad Homburg, Germany) which specifically measures SGLT-2 mediated glucose uptake (Castaneda & Kinne, 2005). Briefly, Chinese hamster ovary cells transfected with human kidney SGLT-2 were seeded into 96-well plates. After overnight attachment, culture medium was removed and plates washed three times with Krebs-Ringer-Henseleit (KRH) solution containing 120 mM NaCl, 4.7 mM KCl, 1.2 mM MgCl₂, 2.2 mM CaCl₂, 10 mM HEPES (pH 7.4 with Tris). Transport buffer containing KRH-Na⁺ with [¹⁴C]AMG (0.1 µCi µl⁻¹) was added to each well and incubated for 1 h at 20°C. At the end of the uptake period the transport buffer was removed and uptake of [¹⁴C]AMG stopped by addition of ice-cold stop buffer (KRH-Na⁺ with 0.5 mM phlorizin (positive control) or seaweed sample at 12.5 mg ml⁻¹). Wells were washed three times with stop buffer and then solubilized by adding ATPlite substrate solution (Perkin-Elmer, Boston, USA). Luminescence of ATP was measured using a MicroBeta Trilux (Perkin-Elmer, Boston, USA). After 24 h a scintillation counter was used to determine radioactive [¹⁴C]AMG. The mean counts per minute were calculated and converted to picomoles and percentage inhibition calculated by comparison of negative control.

**DPP-4 assay**

Seaweed samples were analysed for DPP-4 inhibition fluorometrically using a method described by Fujiwara and Tsuru (1978) for measurement of free AMC (7-amino-4-methyl-coumarin) liberated from the DPP-4 substrate, Gly-Pro-AMC. Using half-volume 96-well plates with opaque walls and transparent bottoms, 20 µl of seaweed extracts dissolved in 100 mM HEPES (4-(2-Hydroxyethyl)-1-piperazine ethanesulfonic acid)
buffer and 30 µl of Gly-Pro-AMC (1 mM; Bachem AG, Bubendorf, Switzerland) were added to each well. The reaction was initiated by the addition of 20 µl of bovine serum and the plate was incubated at 37°C with agitation (microplate shaker) for 1 h. 100 µl of 3 mM acetic acid was added to halt the reaction and plates were immediately measured using a desktop fluorometer (Tecan UK Ltd, Reading, UK) at excitation and emission wavelengths of 351 and 430 nm, respectively. Berberine (Fluorochem, Derbyshire, UK), an established natural DPP-4 inhibitor (Al-Masri et al., 2009), was dissolved at 1 mg ml⁻¹ and used as a positive control.

**GLP-1 secretion, accumulation and cellular content in STC-1 cells**

Cell culture STC-1 cells were cultured in Dulbecco’s Modified Eagle Medium (DMEM) containing 4.5 g l⁻¹ D-glucose with L-glutamine, without sodium pyruvate (Gibco, Paisley, UK) and supplemented with 17.5% foetal bovine serum, 100 U ml⁻¹ penicillin and 100 µg ml⁻¹ streptomycin. Cells were incubated in a 5% CO₂ humidified atmosphere at 37°C and used between passage numbers 20–50 when 70–90% confluence had been reached (Hand et al., 2013).

Cell secretion and accumulation studies

STC-1 cells were seeded in 12 well plates (2 x 10⁶ cells per well) with 1.5 ml DMEM and incubated overnight at 37°C in a 5% CO₂ humidified atmosphere to allow attachment (Hand, Bruen, O’Halloran, Giblin, & Green, 2010). Media were removed and cells were washed twice with HEPES buffer (20 mM HEPES, 10 mM glucose, 140 mM NaCl, 4.5 mM KCl, 1.2 mM CaCl₂, 1.2 mM MgCl₂) and pre-incubated in the same HEPES buffer for 1 h. After removal of buffer, seaweed extracts reconstituted in buffer were added to cells in triplicate for 3 h. After the incubation period the supernatant was removed, centrifuged at 1000 g for 10 min to remove cellular debris and stored at −20°C prior to analysis.

Cellular content studies

To determine cellular GLP-1 content, cells were washed twice with HEPES buffer after sample incubations and incubated overnight at 4°C in an acid/ethanol solution (1.5% hydrochloric acid (v/v): 75% ethanol (v/v): 23.5% water (v/v)) to lyse the cells. The incubation solution was removed and centrifuged (900 g for 5 min) to remove cellular debris. The supernatant was collected and solvent evaporated using a Speedvac sample concentrator (Genevac, Ipswich, UK). Samples were reconstituted in PBST 0.1% BSA and stored at −80°C (for < 4 weeks) prior to analysis.

**Measurement of GLP-1 by radioimmunoassay**

GLP-1 was determined by means of an in-house fully optimized radioimmunoassay using a polyclonal rabbit antibody for GLP-1(7–36)amide with no cross-reactivity for glucagon or GIP (sensitivity = 59 ± 26.8 pM, r² = 0.99). In brief, PBST (phosphate-buffered saline Tween) buffer with 0.1% BSA, seaweed extracts and GLP-1 standards (GLP-1(7–36)amide, American Peptide Company, California, USA) was incubated with primary antibody (rabbit anti-GLP-1, made in-house) in plastic tubes overnight at 4°C. Freshly labelled GLP-1 ¹²⁵I (PerkinElmer, LAS, UK Ltd) diluted to approximately 10 000–15 000 cpd was added to each tube, and the mixture vortexed and incubated at 4°C for a further 48 h. Secondary antibody was added (anti-rabbit IgG Sac-Cel, IDS, Boldon, UK) and left for 30 min at room temperature prior to addition of 1 ml of deionized water. Tubes were centrifuged at 3000 g for 20 min at 4°C and the supernatant aspirated off. Counts for radioimmunoassay tubes were measured using a Packard Cobra ll Model 5002 gamma counter (PerkinElmer LAS, Beaconsfield, UK).

**Glucose tolerance tests in mice**

C57/BL6 mice (5–10 weeks old) were obtained from Harlan® (Harlan Laboratories, Wyton, UK) and housed at 23 ± 1°C with 50% humidity and 12 h light/12 h dark cycle. Mice had free access to food (Teklad standard rodent diet; Harlan, Wyton, UK) and water. Prior to experimentation mice were randomly divided into groups. Control animals were orally gavaged with saline containing 2 g kg⁻¹ of glucose alone. Treatment groups received glucose combined with 500 mg kg⁻¹ of seaweed. Blood glucose levels were measured at time points 0, 15, 30, 60 and 105 min using a glucometer (FreeStyle Freedom Lite Blood Glucose Monitoring System, Abbott, USA). Mice used were housed throughout these studies under constant climatic conditions. All experimental procedures were performed in accordance with the Guidance on the Operation of the Animals (Scientific Procedures) Act, 1986 (UK) and approved by the Queen’s University Belfast Animal Welfare and Ethical Review Body.

**Statistical analysis**

All data are expressed as mean ± standard error mean (SEM). For α-glucosidase and in vivo tolerance tests data were subject to Area Under Curve (AUC) analysis. For DPP-4 and SGLT-2 studies inhibition was calculated as a percentage of the negative control. GLP-1 concentrations were determined by interpolation from a standard curve. All statistical analyses were carried out using a one-way ANOVA with Tukey’s Multiple Comparison Test to
compare differences between groups with the exception of in vivo tolerance tests where a two-way ANOVA was carried out to compare different time points (*p < 0.05, **p < 0.01, ***p < 0.001). All statistical analyses were performed using GraphPad Prism 5.0 software (GraphPad, California, USA).

Results

**Alpha-glucosidase inhibition**

Only brown seaweed extracts caused potent inhibition of alpha-glucosidase (Figure 1(a)). Both water and ethanol extracts of *F. vesiculosus* (88.3 ± 1.5% and 60.2 ± 0.3%, respectively) and *A. nodosum* (89.5 ± 0.4% and 82.3 ± 0.3%, respectively) significantly inhibited enzyme activity (p < 0.001), whilst the water extract of *A. esculenta* and ethanol extract of *H. elongata* also were effective inhibitors (82.8 ± 3.6% and 13.9 ± 0.2%, respectively; p < 0.001). *F. vesiculosus* extracts were the most potent at inhibiting alpha-glucosidase and acted in a concentration-dependent manner (Figure 1(b)).

**SGLT-2 inhibition**

None of the seaweeds tested at 12.5 mg ml⁻¹ were effective blockers of SGLT-2 (Figure 2). The inhibitor phlorizin was successfully used as the positive control (Figure 2).

**DPP-4 inhibition**

All water extracts of red and brown seaweeds tested displayed significant DPP-4 inhibitory activity ranging from 41.2 to 90.1% inhibition (p < 0.001) (Figure 3(a)). Furthermore, ethanol extracts of *A. esculenta*, *F. vesiculosus* and *P. palmata* significantly inhibited DPP-4 activity demonstrating 91.3 ± %, 78.8% and 81.0% inhibition, respectively (p < 0.001). As with

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**Figure 1.** Screening of seaweed extracts for alpha-glucosidase inhibitory activity. a) % alpha-glucosidase inhibition of aqueous and ethanol extracts of seaweeds at 12.5 mg ml⁻¹; b) inhibitory activity of the most promising seaweed extracts at concentrations ranging from 0–12.5 mg ml⁻¹. Data represent mean ± SEM. Data analysed using Area Under Curve (AUC) analysis with inhibition calculated as a percentage of negative control. Acarbose (1 mg ml⁻¹) was used as positive control (*p < 0.05, **p < 0.01, ***p < 0.001; n = 3) (H₂O indicates water extract, EtOH indicates ethanol extract).
alpha-glucosidase activity, the most promising seaweed extracts exerted inhibition in a concentration-dependent manner (Figure 3(b)).

**GLP-1 secretion, accumulation and cellular content in STC-1 cells**

Red seaweed species were very potent at increasing acute GLP-1 secretion from STC-1 cells at concentrations of 25 mg ml\(^{-1}\) (Figure 4(a)). The water extract of *P. linearis* and ethanol extract of *U. rigida* increased GLP-1 secretion by 2.3-fold and 1.9-fold, respectively, during a 3 h acute exposure. Over a 3-day period ethanol extracts of *A. esculenta* and *U. rigida* were the best GLP-1 secretagogues, increasing secretion by around 41.2-fold to 52.3-fold, while the water extract of *L. digitata* also significantly increased GLP-1 levels over 3 days by 25.4-fold (Figure 4(b)). Water extracts of *C. fragile*, *L. digitata* and *A. nodosum* and ethanol extracts of *U. rigida* and *A. esculenta* were able to increase the cellular content of GLP-1 significantly (Figure 4(c)).

**Oral glucose tolerance tests in vivo**

In normal mice during oral glucose tolerance tests the water extract of *C. crispus* and ethanol extract of *P. palmata* at 500 mg kg\(^{-1}\) concentration significantly reduced the rise in blood glucose levels at 15 min and 30 min, respectively (*p < 0.01* and *p < 0.05*, respectively), however blood glucose level returned to that of the control animals after this time (Figure 5). Only the water extract of *P. linearis* at 500 mg ml\(^{-1}\) significantly reduced the overall blood glucose levels when orally gavaged with glucose (*p < 0.05*), and in fact its response was significantly lower than the control by 30 min. This indicates the potential of these extracts for acutely reducing postprandial blood glucose.

**Discussion**

These screening studies demonstrate that extracts from common edible Irish seaweeds exhibit a range of potential anti-diabetic effects and could potentially form the basis for future development of novel treatments or prophylactics for T2DM. Our results confirm previous work demonstrating that water extracts of *Ascophyllum nodosum* potently inhibit alpha-glucosidase activity at concentrations as low as 12.5 mg ml\(^{-1}\) (Apostolidis & Lee, 2010). This study is in accordance with the finding that *A. nodosum* and *Alaria esculenta* have potent inhibitory activity on alpha-glucosidase (Nwosu et al., 2011). Our findings are also supported by a study which combined *Ascophyllum nodosum* and *Fucus vesiculosus* extracts and achieved alpha-glucosidase inhibition of nearly 100% (Roy et al., 2011). Extraction and assay methodologies differ substantially from study to study making it difficult to closely compare the inhibitory activities of each seaweed species without identification of the bioactive secondary metabolite. However, it can be concluded that brown seaweed species have future potential for managing T2DM by means of inhibiting alpha-glucosidase. One drug currently used for managing T2DM is acarbose, which is effective in inhibiting alpha-glucosidase but has several undesirable side-effects such as excessive gas, abdominal distention and flatulence (Fujisawa, Ikegami, Inoue, Kawabata, & Oghihara, 2005). We suggest that natural products consumed with or as part of a meal potentially could offer...
lesser side-effects and similar efficacy. To date, few results of human clinical trials have been reported (https://clinicaltrials.gov/ct2/show/NCT03075943).

Sakai et al. (2019) investigated the results of inclusion of fucoidan in the diet.

The glucose transporter SGLT-2 is known to be inhibited by plant flavonoids isolated from Cynodon dactylon (Annapurna et al., 2013), and also by two cyclic diarylheptanoids isolated from the bark of Acer nikoense (Morita et al., 2010). The potential development of drugs from natural products in this therapeutic area has been reviewed recently (Blaschek, 2017; Choi, 2016). To our knowledge ours is the first study to investigate the effect of seaweed on SGLT-2 inhibition, although none of the seaweeds tested were effective.

More promisingly, our study demonstrated that seaweeds have a significant ability to inhibit DPP-4 activity. Palmaria palmata has previously been shown to cause potent DPP-4 inhibition, up to 81% after solid-phase fractionation (Harnedy et al., 2015). Our results supported previous findings that brown seaweeds tend to have higher inhibitory activity than red or green seaweed species (Chin et al., 2014; Harnedy et al., 2015), indicating they have specific compounds able to bind and inhibit DPP-4. These compounds may be structurally similar to those in berberine, where they readily interact with the binding pocket of DPP-4 (Al-Masri et al., 2009). However, more studies will need to be carried out to isolate the compounds responsible and to determine their mechanism of DPP-4 inhibition.

A number of plant sources and food constituents are known to stimulate GLP-1 secretion (Rafferty et al., 2011). The finding that extracts from red, brown and green seaweeds caused increased GLP-1 synthesis and secretion by STC-1 cells potentially conflicts with another study which demonstrated that a 10-day treatment with sodium alginate from L. digitata did not alter
Figure 4. Effects of seaweed extracts on glucagon-like peptide-1 (GLP-1) secretion, accumulation and cellular content. a) Acute (3 h) GLP-1 secretion elicited by aqueous and ethanol seaweed extracts. b) Accumulation of GLP-1 in cell culture medium and c) cellular GLP-1 content of STC-1 cells following 3-day exposure to aqueous and ethanol seaweed extracts. All incubations were performed at a sample concentrations of 25 mg ml⁻¹. Bars represent mean ± SEM. (*p < 0.05, **p < 0.01, ***p < 0.001; n = 4).
GLP-1 levels in overweight and obese subjects (Odunsi et al., 2010). Similarly, Sakai et al. (2019) found that consumption of fucoidan resulted in a decrease of the baseline GLP-1 level, possibly as a result of altered gastrointestinal function in patients. For this reason, it is likely that molecules other than sodium alginate, such as phenolic compounds in L. digitata, are responsible (Heffernan, Smyth, Soler-Villa, Fitzgerald, & Brunton, 2015). For example, natural phenolic compounds such as resveratrol, produced by various plants, increase both portal vein GLP-1 and intestinal content of GLP-1 (Dao et al., 2011). Our study found that P. palmata, whether extracted in water or ethanol, resulted in significant GLP-1 secretion. Water and ethanol extracts of other red algae, nori (dried Porphyra/Pyropia species), were previously reported to possess GLP-1 secretory activity (Schultz Moreira et al., 2013). It is a promising finding that seaweed extracts not only caused GLP-1 secretion from STC-1 cells, but they were also able to increase GLP-1 synthesis within the cell (as indicated by increased cellular GLP-1 content). Our results open up the future possibility of using a seaweed species with dual L-cell activities, e.g. U. rigida which increases both GLP-1 synthesis and GLP-1 secretion, although this requires further investigation.

The mechanisms whereby P. linearis extracts generate lower glucose excursions in vivo indicated by our in vitro data may result from an increase in GLP-1 secretion and/or DPP-4 inhibition, although it should be noted that there are other physiological mechanisms by which glucose lowering can occur. Porphyran, a soluble dietary fibre present in Porphyra, has been shown to improve glucose metabolism in KK-Ay mice via upregulation of adiponectin levels (Kitano et al., 2012), while several studies have shown the ability of seaweed to reduce postprandial blood glucose levels and improve glucose intolerance, particularly in

Figure 5. In vivo effects of seaweed extracts on blood glucose following oral glucose challenge. a) and c) Each seaweed extract (500 mg kg⁻¹) was orally gavaged with glucose (2 g kg⁻¹) to normal mice and glucose responses were monitored at 0, 15, 30, 60 and 105 min. b) and d) Incremental areas under plasma glucose curves (ΔAUC0-105) were calculated with baseline subtraction. Bars and points represent mean ± SEM. Line graph data were compared with saline treated mice and analysed by two-way ANOVA with a Bonferroni post-test. Bar graphs are AUC data compared by one-way ANOVA (*p < 0.05, **p < 0.01, ***p < 0.001; n = 4–5) (H₂O – water extract, EtOH – ethanol extract).
animal models of T2DM (Iwai, 2008; Kang et al., 2013; Motshakeri, Ebrahimi, Goh, Matanjun, & Mohmed, 2013; Park, Kim, Kim, Kim, & Kim, 2009; Tas, Celikler, Ziyanok-Ayvalik, Sarandol, & Dirican, 2011: Xu et al., 2012). Any of these mechanisms could be responsible for the observed glucose-lowering effect caused by P. linearis.

In conclusion, it is clear that common edible European seaweeds have anti-diabetic properties. There is a need to identify the compound(s) responsible for these effects and to maximize their yield by studying the composition of the seaweed, the extraction time/temperature/solvents used, and also to assess the effect of harvesting location and season of collection. These studies are supportive of the notion that edible seaweeds (such as those studied here) are beneficial to human health if consumed as part of the normal diet, and may promote normoglycaemia (Lee, Kim, Chang, & Nam, 2010). Much work is still needed, including the isolation and identification of the bioactive secondary metabolites and confirmation of their in vitro and in vivo activity but there is huge potential for seaweeds as natural therapeutics for treatment or management of T2DM.

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Disclosure statement

The authors declare no conflict of interest.

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Author contributions

DC: conducted in vitro and in vivo studies; drafted and edited manuscript; VS, CF, ER, FC: conducted and analysed in vitro studies; AW: supervision of radioimmunoassay methods; BFG: conceived studies and provided PhD supervision of DC; CAM and AI: collected and identified seaweed species; BDG: conceived studies, drafted and edited manuscript.

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References

Al-Masri, I. M., Mohammad, M. K., & Tahaa, M. O. (2009). Inhibition of dipeptidyl peptidase IV (DPP IV) is one of the mechanisms explaining the hypoglycemic effect of berberine. Journal of Enzyme Inhibition and Medicinal Chemistry, 24, 1061–1066.

Annapurna, H. V., Apoorva, B., Ravichandran, N., Arun, K. P., Brindha, P., Swaminathan, S., … Nagarajan, A. (2013). Isolation and in silico evaluation of antidiabetic molecules of Cynodon dactylon (L.). Journal of Molecular Graphics and Modelling, 39, 87–97.

Apostolidis, E., & Lee, C. M. (2010). In vitro potential of Ascorphyllum nodosum phenolic antioxidant-mediated α-glucosidase and α-amylase inhibition. Journal of Food Science, 75, H97–H102.

Blaschek, W. (2017). Natural products as lead compounds for sodium glucose cotransporter (sglt) inhibitors. Planta Medica, 83, 985–993.

Brodie, J., Maggs, C. A., & John, D. M., Eds. (2007). Green seaweeds of Britain and Ireland (pp. 242). London: British Phycolological Society.

Castaneda, F., & Kinne, R. (2005). A 96-well automated method to study inhibitors of human sodium-dependent D-glucose transport. Molecular and Cellular Biochemistry, 280, 91–98.

Chin, Y. X., Lim, P. E., Maggs, C. A., Phang, S. M., Sharifuddin, Y., & Green, B. D. (2014). Anti-diabetic potential of selected Malaysian seaweeds. Journal of Applied Phycology, 27, 2137–2148.

Choi, C. I. (2016). Sodium-glucose cotransporter 2 (srl2) inhibitors from natural products: Discovery of next-generation antihyperglycemic agents. Molecules, 21, E1136.

Cotas, J., Leandro, A., Pacheco, D., Gonçalves, A. M. M., & Pereira, L. (2020). A comprehensive review of the nutra-ceutical and therapeutic applications of red seaweeds (Rhodophyta). LIFE, 10, 19.

Dao, T. A., Waget, A., Klopp, P., Serino, M., Vachoux, C., Pechere, L., … Seree, E. (2011). Resveratrol increases glucose induced GLP-1 secretion in mice: A mechanism which contributes to the glycemic control. Plos One, 6, e20700.

Ehrenkrantz, J., Lewis, N., Kahn, C., & Roth, J. (2005). Phlorizin: A review. Diabetes/Metabolism Research and Reviews, 21, 31–38.

Fujisawa, T., Ikegami, H., Inoue, K., Kawabata, Y., & Oghara, T. (2005). Effect of two alpha-glucosidase inhibitors, voglibose and acarbose, on postprandial hyperglycemia correlates with subjective abdominal symptoms. Metabolism, 54, 387–390.

Fujiwara, K., & Tsuru, D. (1978). New chromogenic and fluorogenic substrates for pyrrolidonyl peptidase. Journal of Biochemistry, 83, 1145–1149.

Gillespie, A. L., Pan, X., Marco-Ramell, A., Meharg, C., & Green, B. D. (2017). Detailed characterisation of STC-1 cells and the pGIP/Neo sub-clone suggests the incretin hormones are translationally regulated. Peptides, 96, 20–30.

González-Abuin, N., Martínez-Micela, N., Blay, M., Green, B. D., Pinent, M., & Ardévol, A. (2014). Grape-seed procyanidins modulate cellular membrane potential and nutrient-induced GLP-1 secretion in STC-1 cells. American Journal of Physiology-Cell Physiology, 306, C485–92.
Gonzalez-Abuin, N., Martinez-Micaelo, N., Blay, M., Pujadas, G., Garcia-Vallve, S., Pinent, M., & Ardevol, A. (2012). Grape seed-derived procyanidins decrease dipeptidyl-peptidase 4 activity and expression. *Journal of Agricultural and Food Chemistry*, 60, 9055–9061.

Green, B. D., Gault, V. A., Mooney, M. H., Irwin, N., Bailey, C. J., Harriott, P., … O’Harte, F. P. (2003). Novel dipeptidyl peptidase IV resistant analogues of glucagon-like peptide-1(7-36)amide have preserved biological activities in vitro conferring improved glucose-lowering action in vivo. *Journal of Molecular Endocrinology*, 31, 529–540.

Green, B. D., Irwin, N., Gault, V. A., FPM, O., & Flatt, P. R. (2005). Review: Development and therapeutic potential of incretin hormone analogues for type 2 diabetes. *The British Journal of Diabetes & Vascular Disease*, 5, 134–140.

Gunathilaka, T. L., Samarakoona, K., Ranasinge, P., & Peiris, L. D. C. (2020). Antidiabetic potential of marine brown algae—a mini review. *Journal of Diabetes Research*, 2020, 1–13.

Gutniak, M., Orskov, C., Holst, J., Ahren, B., & Efendic, S. (1992). Antidiabetogenic effect of glucagon-like peptide-1 (7-36)amide in normal subjects and patients with diabetes mellitus. *New England Journal of Medicine*, 326, 1316–1322.

Hand, K. V., Bruen, C. M., O’Halloran, F., Giblin, L., & Green, B. D. (2010). Acute and chronic effects of dietary fatty acids on cholecytokinin expression, storage and secretion in enterodocrine STC-1 cells. *Molecular Nutrition & Food Research*, 54, S93–S103.

Hand, K. V., Bruen, C. M., O’Halloran, F., Panwar, H., Calderwood, D., Giblin, L., & Green, B. D. (2013). Examining acute and chronic effects of short- and long-chain fatty acids on peptide YY (PYY) gene expression, cellular storage and secretion in STC-1 cells. *European Journal of Nutrition*, 52, 1303–1313.

Harnedy, P. A., FitzGerald, R. J., & FitzGerald, R. J. (2015). Purification and identification of dipeptidyl peptidase (DPP) IV inhibitory peptides from the macroalga *Palmaria palmata*. *Food Chemistry*, 172, 400–406.

Heffernan, N., Smyth, T. J., Soler-Villa, A., FitzGerald, R. J., & Bruton, N. P. (2015). Phenolic content and antioxidant activity of fractions obtained from selected Irish macroalgae species (*Laminaria digitata, Fucus serratus, Gracilaria gracilis* and *Codium fragile*). *Journal of Applied Phycolgy*, 27, 519–530.

Hughes, J. R., Maggs, C. A., Mineur, F., Jarvis, C., Miller, K. A., Shabaka, S. H., & Gabrielson, P. W. (2019). Genetic analysis of the Linnaean *Ulva lactuca* (Ulvales, Chlorophyta) holotype and related type specimens reveals name misapplications, unexpected origins, and new synonyms. *Journal of Phycology*, 55, 503–508.

Iwai, K. (2008). Antidiabetic and antioxidant effects of polyphenols in brown Alga *Ecklonia stolonifera* in genetically diabetic KK-Ay mice. *Plant Foods for Human Nutrition*, 63, 163–169.

Kang, M. C., Wijesinghe, W. A., Lee, S. H., Kang, S. M., Ko, S. C., Yang, X., … Lee, D. H. (2013). Dieckol isolated from brown seaweed *Ecklonia cava* attenuates type II diabetes in db/db/db mouse model. *Food and Chemical Toxicology*, 53, 294–298.

Kawamura-Konishi, Y., Watanabe, N., Saito, M., Nakajima, N., Sakaki, T., Katayama, T., & Enomoto, T. (2012). Isolation of a new phlorotannin, a potent inhibitor of carbohydrate-hydrolyzing enzymes, from the brown alga *Sargassum patens*. *Journal of Agricultural and Food Chemistry*, 60, 5565–5570.

Kitano, Y., Murazumi, K., Duan, J., Kurose, K., Kobayashi, S., Sugawara, T., & Hirata, T. (2012). Effect of dietary porphyran from the red alga, *Porphyra yezoensis*, on glucose metabolism in diabetic KK-ay mice. *Journal of Nutritional Science and Vitaminology*, 58, 14–19.

Krupnik, N., Paz, G., Douek, J., Lewinsohn, E., Israel, A., Carmel, N., … Maggs, C. A. (2018). Native, invasive and cryptogenic *Ulva* species from the Israeli Mediterranean Sea: Risk and potential. *Mediterranean Marine Science*, 19, 132–146.

Lauritano, C., & Ianora, A. (2016). Marine organisms with anti-diabetes properties. *Marine Drugs*, 14, 220.

Lee, H. J., Kim, H., Chang, L. V., & Nam, C. M. (2010). Algae consumption and risk of type 2 diabetes: Korean national health and nutrition examination survey in 2005. *Journal of Nutritional Science and Vitaminology*, 56, 13–18.

Lee, S., Karadeniz, F., Kim, M., Kim, S., & Kim, S.-K. (2009). α-Glucosidase and α-amylase inhibitory activities of phloroglucinol derivatives from edible marine brown alga, *Ecklonia cava*. *Journal of the Science of Food and Agriculture*, 89, 1552–1558.

Lee, S., Min, K., Han, J., Lee, D., Park, D., Jung, W., … Jeon, Y. (2012a). 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.

Lee, S., Park, M., Han, J., Jeong, Y., Kim, M., & Jeon, Y. (2012b). Bioactive compounds extracted from *Gamtae (Ecklonia cava)* by using enzymatic hydrolysis, a potent α-glucosidase and α-amylase inhibitor, alleviates postprandial hyperglycemia in diabetic mice. *Food Science and Biotechnology*, 21, 1149–1155.

Li, X., Niu, R., Fan, X., & Han, L. (2005). Macroalgae as a source of alpha-glucosidase inhibitors. *Journal of Oceanology and Limnology*, 23, 354–356.

Lopes, G., Andrade, P. B., & Valenta, P. (2017). Phlorotannins: Towards new pharmacological interventions for diabetes mellitus type 2. *Molecules*, 22, UNSP 56.

Marguet, D., Baggio, L., Kobayashi, T., Bernard, A., Pierres, M., Nielsen, P., … Wagtmann, N. (2000). Enhanced insulin secretion and improved glucose tolerance in mice lacking CD26. *Proceedings of the National Academy of Sciences*, 97, 6874–6879.

McCarthy, T., Green, B. D., Calderwood, D., Gillespie, A., Cryan, J. F., & Giblin, L. (2015). STC-1 Cells. In K. Verhoeckx, P. Cotter, I. López-Expósito, C. Kleiveland, T. Lea, A. Mackie, … H. Wichers (Eds.), *The impact of food bioactives on health: In vitro and ex vivo models. Part IV* (pp. 211–220). Springer.

Morita, H., Deguchi, J., Motegi, Y., Sato, S., Aoyama, C., Takeo, J., … Hirasawa, Y. (2010). Cyclic diarylheptanoids as nα-glucose cotransporter (SGLT) inhibitors from *Acer nikoense*. *Bioorganic & Medicinal Chemistry Letters*, 20, 1070–1074.

Motshakeri, M., Ebrahimi, M., Goh, Y. M., Matanjan, P., & Mohamed, S. (2013). *Sargassum polycystum* reduces hyperglycaemia, dyslipidaemia and oxidative stress via increasing insulin sensitivity in a rat model of type 2 diabetes. *Journal of the Science of Food and Agriculture*, 93, 1772–1778.
Roy, M. H. A., & Tonnejick, L. (2017). GLP-1 and the kidney: From physiology to pharmacology and outcomes in diabetes. Nature Reviews Nephrology, 13, 605–628.

Nguyen, V. B., Nguyen, T. H., Nguyen, A. D., Le, T.; Kuo, Y. H., Wang, S. L., & Wang, S.-L. (2019). Bioprocessing shrimp shells for rat intestinal alpha-glucosidase inhibitor and its effect on reducing blood glucose in a mouse model. Research on Chemical Intermediates, 45, 4829–4846.

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

Odunsi, S. T., Vazquez-Roque, M. I., Papathanasopoulos, C. M., Clark, A., Wodrich, M. M., & Lempe, L. (2010). Effect of alginate on satiation, appetite, gastric function, and selected gut satiety hormones in overweight and obesity. Obesity, 18, 1579–1584.

Park, M., Kim, E., Kim, M., Kim, K., & Kim, H. (2009). Dietary supplementation of sea tangle (Laminaria japonica) improves blood glucose and lipid metabolism in the streptozotocin-induced diabetic rats. Food Science and Biotechnology, 18, 712–716.

Provan, J., Glendinning, K., Kelly, R., & Maggs, C. A. (2013). Levels and patterns of population genetic diversity in the red seaweed Chondrus crispus (Florideophyceae): A direct comparison of single nucleotide polymorphisms and microsatellites. Biological Journal of the Linnean Society, 108, 251–262.

Provan, J., Murphy, S., & Maggs, C. A. (2005b). Tracking the invasive history of the green alga Codium fragile subsp. tomentosoides. Molecular Ecology, 14, 1084–1091.

Provan, J., Wattier, R. A., & Maggs, C. A. (2005a). Phylogeographic analysis of the red seaweed Palmaria palmata reveals a Pleistocene marine glacial refugium in the English Channel. Molecular Ecology, 14, 793–803.

Rafferty, E. P., Wylie, A. R., Elliott, C. T., Chevallier, O. P., Grieve, D. J., & Green, B. D. (2011). In vitro and in vivo effects of natural putative secretagogues of glucagon-like peptide-1 (GLP-1). Scientia Pharmaceutica, 79, 615–621.

Rosenstock, J., Aggarwal, N., Polidori, D., Zhao, Y., Arbit, D., Usiskin, K., … Canovatchel, W. (2012). Dose-ranging effects of canagliflozin, a sodium-glucose cotransporter inhibitor, as add-on to metformin in subjects with type 2 diabetes. Diabetes Care, 35, 1232–1238.

Roy, M., Anguenot, R., Fillon, C., Beaulieu, M., Bérubé, J., & Richard, D. (2011). Effect of a commercially-available algal phlorotannins extract on digestive enzymes and carbohydrate absorption in vivo. Food Research International, 44, 3026–3029.

Sakai, C., Abe, S., Kouzuki, M., Shimohiro, H., Ota, Y., Sakinada, H., … Hanaki, K. (2019). A randomized placebo-controlled trial of an oral preparation of high molecular weight fucoidan in patients with Type 2 diabetes with evaluation of taste sensitivity. Tonaga Acta Medica, 62, 14–23.

Schultz Moreira, A. R., Garcia Martin, A., Bastida, S., Jiménez-Escrig, A., Rupérez, P., Green, B. D., … Benedí, J. (2014). Effects of Undaria pinnatifida, Himanthalia elongata and Porphyra umbilicalis extracts on in vitro α-glucosidase activity and glucose diffusion. Nutricion Hospitalaria: Organo Oficial De La Sociedad Espanola De Nutricion Parenteral Y Enteral, 29, 1434–1446.

Schultz Moreira, A. R., Rafferty, E., Green, B. D., Garcia Martin, A., Benedí, J., Bastida, S., & Sanchez-Muniz, F. I. (2013). In vitro modulation of GLP-1 activity by different aqueous and organic extracts of edible seaweeds. Annals of Nutrition and Metabolism, 63, 1674.

Shannon, E., & Abu-Ghannam, N. (2019). Seaweeds as nutraceuticals for health and nutrition. Phycolgia, 58, 563–577.

Sørensen, L. E., Jeppesen, P. B., Christiansen, C. B., Hermansen, K., & Gregersen, S. (2019). Nordic seaweed and diabetes prevention: Exploratory studies in KK-Ay mice. Nutrients, 11, 1435.

Tas, S., Celikler, S., Ziyanok-Ayvalik, S., Sarandol, E., & Dirican, M. (2011). Ulva rigida improves carbohydrate metabolism, hyperlipidemia and oxidative stress in streptozotocin-induced diabetic rats. Cell Biochemistry and Function, 29, 108–113.

Williams, R., Karuranga, S., Malanda, B., Ogurtsova, K., Zhang, P., Colagiuri, S., … Colagiuri, S. (2020). Global and regional estimates and projections of diabetes-related health expenditure: Results from the international diabetes federation diabetes atlas, 9th edition. Diabetes Research and Clinical Practice, 162, 108072.

World Health Organization. (2016). Global report on diabetes. Geneva 27, Switzerland: WHO.

Wright, E. M., Hirayama, B. A., & Loo, D. F. (2007). Active sugar transport in health and disease. Journal of Internal Medicine, 261, 32–43.

Xu, H., Kitajima, C., Ito, H., Miyazaki, T., Baba, M., Okuyama, T., & Okada, Y. (2012). Antidiabetic effect of polyphenols from brown alga Ecklonia kurome in genetically diabetic KK-Ay mice. Pharmaceutical Biology, 50, 393–400.