Impact of a (poly)phenol-rich extract from the brown algae Ascophyllum nodosum on DNA damage and antioxidant activity in an overweight or obese population: a randomized controlled trial


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Impact of a (poly)phenol-rich extract from the brown algae *Ascophyllum nodosum* on DNA damage and antioxidant activity in an overweight/obese population

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Running head: Seaweed phenolics, *Ascophyllum nodosum*, inflammation, DNA damage, C-reactive protein (CRP)

Abbreviations used: SPE, seaweed (poly)phenol extract; CRP, C-reactive protein; CVD, cardiovascular disease; ROS, reactive oxygen species; TNF-α, tumour necrosis factor alpha; COX, cyclooxygenase; UUREC, University of Ulster Research Ethics Committee; WISP, Weighed Intake Software Program; TF, tissue factor; TOC, Total oxidative capacity; TMB, tetramethylbenzidine; IL, interleukin; IFN, interferon; TNF, tumour necrosis factor; UHPLC-HRMS: ultra-high performance liquid chromatography-high resolution-mass spectrometry; VIP: variable of importance in projection; OPLS-DA: Orthogonal Partial Least Square Discriminant Analysis.

This trial was registered at clinicaltrials.gov as NCT02295878.

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ABSTRACT

**Background:** Epidemiological evidence suggests a diet rich in (poly)phenols has beneficial effects on many chronic diseases. A rich source of (poly)phenols can be found in brown seaweed.

**Objective:** The aim of this study was to investigate the bioavailability and effect of a seaweed (poly)phenol extract from *Ascophyllum nodosum* on DNA damage, oxidative stress, and inflammation *in vivo.*

**Design:** A randomised double-blind placebo-controlled crossover trial was conducted in 80 participants aged 30-65 years with a BMI ≥25kg/m². The participants consumed either a 400 mg capsule containing 100 mg of seaweed (poly)phenol and 300 mg maltodextrin or a 400 mg maltodextrin placebo control capsule daily for an 8-week period. Bioactivity was assessed with a panel of blood-based markers including lymphocyte DNA damage, plasma oxidant capacity, C-reactive protein and inflammatory cytokines. To explore the bioavailability of seaweed phenolics, an untargeted metabolomics analysis of urine and plasma samples following seaweed consumption was determined by UHPLC-HR-MS.

**Results:** Consumption of the seaweed (poly)phenols resulted in a modest decrease DNA damage but only in a subset of the total population who were obese. There were no significant changes in CRP, antioxidant status, inflammatory cytokines or isoprostanes. We identified phlorotannin metabolites including pyrogallol/phloroglucinol-sulfate, hydroxytrifurahol A-glucuronide, dioxinodehydroeckol-glucuronide, diphlorethol sulfates, C-O-C dimers of phloroglucinol sulfate, C-O-C dimers of phloroglucinol and diphlorethol sulfate are considered potential biomarkers of seaweed consumption.

**Conclusion:** To the best of our knowledge, our work represents the first comprehensive study in human participants investigating the bioactivity and bioavailability of seaweed (poly)phenolics. There was a modest improvement in DNA damage but only in the obese subset
of the total population, and several biomarkers of seaweed consumption, further studies involving stratification according to weight are required to elucidate these findings.

Key words: Seaweed, Phenolic compounds, Inflammation, DNA damage, Oxidative stress, bioavailability
INTRODUCTION

Dietary patterns rich in plant-derived foods protect against chronic degenerative diseases, including cardiovascular disease (CVD), effects attributable in part to highly bioactive (poly)phenolic compounds contained therein (1-3). Fruits and vegetables are a well-known source of (poly)phenols but a less familiar source rich in (poly)phenolic compounds is brown algae which uniquely contains phlorotannins (4) for example *Ascophyllum nodosum*, a brown algal species common to the British Isles, that is rich in phlorotannin (5). Phlorotannins are oligomers of phloroglucinol whose concentration in seaweed is affected by numerous factors including plant size and age, water salinity, nutrient and heavy metal content, and light intensity changes (6-8).

Phlorotannins, and more commonly brown seaweed extracts, beneficially effect a range of biological processes including modulation of inflammation; reduction of oxidative stress and improvement in cardiovascular function (9-11). However, the evidence base depends heavily upon cell and small animal models with few studies involving humans (12-13). Moreover, species relevance also becomes an issue; in Southeast Asia, *Ecklonia* and *Eisenia* are considered commercially important seaweed species, while from a European perspective *Ascophyllum nodosum* is of interest as it is one of only a few commercially sustainable seaweed species.

Phlorotannin-rich extracts from brown seaweeds have been shown to be effective in controlling inflammation *via* a number of pathways including inhibition of pro-inflammatory cytokines including tumour necrosis factor (TNF)-α and interleukins (IL)-1β and IL-6 (14). Extracts from *Ascophyllum nodosum* and other Fucoid species have shown efficacy in mitigating the effects of oxidative stress by playing an inhibitory role in the generation of reactive oxygen species (ROS); in preventing DNA damage and also in stimulating the production of glutathione in affected cells (15-19). Our initial *in vitro* (15) and acute *in vivo*
(20) studies on the *Ascophyllum nodosum* extract(s) demonstrated antioxidant and anti-inflammatory activity, suggesting that the potential antioxidant and anti-inflammatory benefits from longer term consumption of *Ascophyllum nodosum*-derived (poly)phenols was worthy of further investigation *in vivo*.

A few human and *in vitro* studies have been conducted in relation to the bioactivity of phlorotannin rich extracts from Fucoid species. Clinical studies have been used to evaluate the safe consumption of some of these extracts including a (poly)phenol-enriched extract of the brown seaweeds *Ascophyllum nodosum* and *Fucus vesiculosus* and its effects on glycaemic response to sucrose (21). However, to the best of our knowledge, the current investigation is the first clinical study aimed to specifically address the effect of a phlorotannin-rich extract from *Ascophyllum nodosum* on oxidative damage to DNA, plasma antioxidant capacity, inflammatory responses and chronic, low level inflammation *in vivo*.

**PARTICIPANTS AND METHODS**

**Seaweed material**

Fresh *Ascophyllum nodosum* was supplied by The Hebridean Seaweed Company, Isle of Lewis, Scotland in March 2011. The seaweed biomass was harvested by hand to ensure quality, cleaned of any contaminating sand and fouling organisms and then shipped refrigerated to the processing facility (CEVA) in France where it was immediately chopped and frozen.

**Preparation of food-grade seaweed extracts and capsule**

A novel (poly)phenol-rich seaweed extract from *Ascophyllum nodosum* was produced by CEVA (France) using food grade solvent based (ethanol:water, 60:40) extraction system that was specifically developed for use with fresh or frozen *Ascophyllum nodosum*. Approximately half of the produced extract was then fractionated using tangential flow ultra-filtration to produce further extracts of varying molecular weight range and with varying (poly)phenol
content. A standardised blended (poly)phenol-rich \textit{Ascophyllum nodosum} extract was formulated by CEVA (Table 1) using 175 mg of extract and 50 mg of high molecular weight fraction (>10 kDa cut off) for use in the current study, so as to maximise the seaweed (poly)phenol content (>100 mg per day) available from the extraction of fresh or frozen \textit{Ascophyllum nodosum} against the need to minimise the level of iodine to within accepted regulatory guidelines (<500 μg per day), potential for heavy metal contamination was also assessed (Table 1). Maltodextrin (175 mg) was added to the capsule formulation as an excipient. Blending was carried out at the food grade CEVA facilities in France. Samples of 400 mg of the \textit{Ascophyllum} (poly)phenol rich blend (SPE) or a placebo which contained 400 mg maltodextrin of and no seaweed (poly)phenols were packed into identical white, opaque, vegetarian capsules by Irish Seaweeds, Belfast, UK and identically sized and match capsules used for the clinical study. The food grade seaweed capsule was characterized by NP-HPLC and LC-MS analysis and has been reported previously (20) (see supplemental figure 2 & 3). Phlorotannins were quantified using the Folin-Ciocalteau Method (22) using phloroglucinol as the standard. In brief, 1 mL of suitability diluted sample was reacted with 1.00 mL of 40% Folin Ciocalteu reagent for 5 min, and then made alkaline with the addition of 1.00 ml of 100 g/L Na$_2$CO$_3$. Absorbance was read at 730 nm after the solution had developed for 1 h at room temperature. Phloroglucinol dihydrate (0-30 mg/L) was used as the standard and was treated in the same way as samples.

**Ethics and participants**

Ethical approval was received from the Ulster University Research Ethics Committee (UUREC). All participants gave written informed consent. Participants were recruited between May 2011 and August 2011 from Ulster University and the surrounding area. The intervention
study ran between August 2011 and February 2012. The study was registered at clinicaltrials.gov as NCT02295878.

The study was conducted in 80 participants (age range 30-65 years). All participants were apparently healthy, non-smoking, BMI $\geq 25$ kg/m$^2$, omnivores, who did not habitually use vitamin or mineral supplements, as determined using a pre-screening health and lifestyle questionnaire. Pregnant and lactating women, vegetarians and vegans and lactose intolerant individuals were excluded from the study, as were those with chronic medical complications such as diabetes; cardiovascular diseases; autoimmune/inflammatory disorders or those who had chronic medication use including anti-inflammatory agents.

**Study design**

The study was a 24-week randomised, double-blind, placebo-controlled crossover trial. After obtaining consent, participants were randomly assigned, in blocks of four using a random-number generator (www.randomization.com), to either the intervention or the control. In total, eighty participants were randomised to 2 groups of 40, each starting on either a 400 mg seaweed (poly)phenol extract (SPE) capsule containing 100mg of (poly)phenols or a 400 mg maltodextrin placebo control capsule (Avebe MD14P) daily for an 8-week period. The participants were supplied with all capsules in weekly labelled capsule boxes at the beginning of each phase, which was interspersed by an 8-week washout phase. During the washout phase, the participants were asked to maintain their habitual diet. Participants were asked to bring any unconsumed capsules to their study appointment at the end of each treatment phase. Participants were also contacted weekly by the study researcher to encourage compliance and to discuss any difficulties they were experiencing.
Blood and urine sample collection

Fasting blood samples were collected before and after each phase (week 0, week 8, week 16, and week 24) by venepuncture into EDTA, serum or sodium heparin-containing tubes, as required. All blood samples were processed on ice. Lymphocytes were isolated by using Histopaque-1077, according to the manufacturer’s instructions (Sigma Diagnostics, St Louis, MO), and plasma samples were prepared by centrifugation at 1000 x g for 10 min at 4ºC. Serum samples were allowed to clot for 30 min at room temperature and then were centrifuged at 2000 x g for 10 min at 4ºC. Whole blood from sodium-heparin treated tubes was prepared according to the manufacturer’s instructions (BD Bioscience Fast Immune Cytokine System) and 24-hour urine samples were prepared by centrifugation at 1000 x g for 10 min at 4ºC. Whole blood, plasma, serum and urine samples were immediately stored at -80ºC, whereas lymphocytes were stored frozen in liquid nitrogen. All biological measurements were carried out at the end of the intervention in batches containing equal numbers of active and control phase samples in each batch, and the researchers were blinded to these samples during analyses. A 24hr urine collection occurred at each time point, volume and pH was measured. The urine sample was mixed and 2x 14ml aliquots removed, centrifuged at 3000rpm for 10 minutes, supernatant was stored -80ºC until use.

Questionnaire assessments

All participants completed a health and lifestyle questionnaire assessing their alcohol intake and physical activity levels, as well as a validated 4-day food diary at the mid-point during each treatment phase (active/placebo) of the study. Data on type of food and corresponding weight was entered into a food analysis database (WISP, Weighed Intake Software Program; Tinuviel Software, Warrington, U.K.) by two independent researchers and the dietary composition calculated.
DNA damage in peripheral blood mononuclear cells

Peripheral blood lymphocytes, previously isolated and stored in liquid nitrogen, were thawed and screened for basal single strand breaks (SBs) in DNA using the single cell gel electrophoresis (Comet) assay (23) and adapted by Gill *et al.* (24). Spontaneous DNA SBs are associated with an altered cell function spontaneous DNA SBs are considered appropriate for the substantiation of health claims in the context of protection against generic DNA damage (25). In addition, resistance to induced DNA damage (SB) was measured in lymphocytes subjected to increased oxidative insult *ex vivo* by pre-treating lymphocytes with 150 µmol H$_2$O$_2$/L for 5 min at 4°C, before the measurement of SBs. The mean (percentage DNA in tail) was calculated from 50 cells per gel (each sample in triplicate) and the mean of each set of data were used in the statistical analysis.

Plasma total oxidative capacity

Total oxidative capacity (TOC) measures total peroxide levels in plasma, by the reaction of endogenous peroxides with peroxidases, using tetramethylbenzidine (TMB) as the chromogenic substrate (26). The blue colour of TMB turns to yellow after addition of the stop solution and can be measured photometrically at 450 nm. For the assay protocol, 10 µl of standard (freshly-prepared hydrogen peroxide, 0-1 mmol/L) and samples were incubated with 200 µl of the reaction mixture consisting of a reaction buffer (phosphate-citrate buffer 0.05M, pH 5.0), TMB solution (1 mg/ml), and peroxidase (>2500U/ml) in a proportion of 100:10:1 in uncoated microtiter plates and incubated at room temperature for 15 min. 50 µl of stop solution (2M H$_2$SO$_4$) were added into all wells and the absorbance at 450 nm was measured using a microplate reader (GENIOS Tecan). Hydrogen peroxide standard solutions (0-1 mmol/L) for calibration curve were freshly prepared before use.
Lipid profile and serum C-reactive protein

Plasma total cholesterol, HDL cholesterol and triglycerides were measured on an Instrument Laboratory (ILAB) 600 (Warrington, UK) autoanalyzer using commercial kits (Roche diagnostics, Lewis, UK) according to kit manufacturer’s protocols. Plasma LDL cholesterol was calculated using the Friedewald formula (27).

C-reactive protein, an acute phase protein synthesized by the liver in response to inflammatory stimuli, especially the cytokine interleukin (IL)-6, was determined on an ILAB 600 autoanalyser using a quantex CRP Ultra-Sensitive commercial kit (0.4-18.3 µg/dl) in accordance with manufacturer’s instructions.

Measurement of inflammatory markers

Intracellular cytokine levels in lymphocyte and monocyte populations and tissue factor (TF) expression were assessed using a whole blood labelling method that utilises flow-cytometry (Fast Immune Cytokine System, BD Biosciences) in accordance with manufacturer’s instructions for all participants at all time-points. The method was used to measure intracellular IL-1β, IL-2, IL-6, IL-10, IL-12, interferon (IFN)-γ and tumour necrosis factor (TNF)-α expression in mononuclear cells. Briefly, whole blood was incubated with either lipopolysaccharide or phorbol 12-myristate 13-acetate to activate monocytes and lymphocytes, respectively. Cells were labelled with the appropriate cell surface antibody and cytokine-specific antibody and analysed on a Gallios flow cytometer (Beckman Coulter). The number and percentage of each cell type expressing the cytokine, as well as the mean channel fluorescence was recorded. The cytokine profiles were examined by ratio of TNF-α to IL-10, IL-1β to IL-10, IL-6 to IL-10 and CRP to IL-10, according to Laird et al. (29).
Isoprostanes have been established as chemically stable, highly specific and reliable biomarkers of *in vivo* oxidative stress, and were measured in frozen serum samples using a commercial 8-Isoprostane EIA Kit (Item no. 516351, Cayman Chemicals). This assay is based on the competition between 8-isoprostane and an 8-isoprostane acetylcholinesterase (Ache) conjugate (8-Isoprostane Tracer) for a limited number of 8-isoprostane-specific rabbit antiserum binding sites consequently 8-isoprostane concentrations are measured as a function of turbidity (absorbance).

**Processing of urine and plasma.**

Urine samples were defrosted, vortexed, centrifuged at 16110 g for 10 min at 5 °C, and passed through 0.45 μm filter discs prior to the analysis of 50 μL aliquots by UHPLC-HR-MS. The extraction of metabolites from the plasma samples has been carried out as described previously (28). Briefly, plasma samples were defrosted, vortexed and 400 μL aliquots were mixed with 10 μL of ascorbic acid (10%, v/v), and 980 μL of 1% formic acid in acetonitrile. One μg of rutin was added to the samples as internal standard for plasma extraction efficiency. The samples were then vortexed for 1 min and ultrasonicated for 10 min. After centrifugation at 16,110 g for 15 min, supernatants were reduced to dryness in vacuo using a concentrator plus (Eppendorf, Hamburg, Germany) and resuspended in 150 μL of distilled water containing 1% formic acid and 50 μL of methanol, which was then centrifuged at 16,100 g for 10 min and 10 μL aliquots of the supernatant analysed by UHPLC-HRMS. The recoveries values of the internal standard were of 79 ± 16% (n=78).

**Non-targeted analysis of urine and plasma by UHPLC-HR-MS.**

Aliquots of selected plasma and urine samples were analysed using a Dionex Ultimate 3000 RS UHPLC system comprising of a UHPLC pump, a PDA detector scanning from 200 to 600
nm, and an autosampler operating at 4 °C (Thermo Scientific). The HPLC conditions were previously described by Corona et al., (20) with some modifications. Briefly, reverse phase separations were carried out using a 100 x 2.1 mm i.d. 1.8 µm Zorbax SB C18 (Agilent) maintained at 25 °C and eluted at a flow rate of 0.2 mL/min with a 50 min gradient of 3-70% of 0.1% acidic methanol in 0.1% aqueous formic acid. After passing through the flow cell of the PDA detector the column eluate was directed to an ExactiveTM Orbitrap mass spectrometer fitted with a heated electrospray ionization probe (Thermo Scientific) operating in negative ionization mode. Analyses were based on scanning from 100 to 1000 m/z, with in-source collision-induced dissociation at 25.0 eV. The capillary temperature was 350 °C, the heater temperature was 150 °C, the sheath gas and the auxillary gas flow rate were both 25 and 5 units, respectively, and the sweep gas was 4 and the spray voltage was 3.00 kv. Data acquisition and processing were carried out using Xcalibur 3.0 software.

Untargeted analysis of the selected urine and plasma samples was performed using mass spectral data from the orbitrap analysis applied to the Compound Discoverer software (version 2, Thermo Fisher Scientific Inc.). The Compound Discover application processes the raw data in called processing workflows that can be defined on the basis of the nature of the experimental setup. In our case, the workflow selected was 'untargeted metabolomics workflow' that includes retention time alignment, component detection, grouping, elemental composition prediction, gap filling, hide chemical background (using blanks), ID using mzCloud and ChemSpider and differential analysis. The parameters were adjusted to our experimental conditions. Samples were grouped and labelled according to our experimental design, either before or after supplementation of seaweed capsule, 4 The output, as peak areas for the detected peaks was used to develop a multivariate data analysis by Orthogonal Partial Least Square Discriminant Analysis (OPLS-DA). Analysis of seaweed phenolics in blood and urine was also undertaken
by HPLC-DAD analysis (reported in Supplemental Tables 4 & 5) in a manner consistent with previous studies (15,20).

**Power calculations and statistical analyses**

Power calculations were performed for the primary endpoint of the change in DNA damage in peripheral blood mononuclear cells. Based on data from a previous study (24), 72.6 participants were needed to detect a 25% change in DNA damage in lymphocytes ($\alpha 0.05$).

All values are expressed as mean ± SD, unless otherwise specified. The mean values are reported for all participants (n=78) during both treatment phases (SPE, Maltodextrin). Significant associations ($P>0.05$) between outcome variables including DNA damage and possible cofounders (age, gender, BMI) were identified at baseline using bivariate correlations or independent t-tests, where appropriate. Therefore, data was also analysed by stratification of increasing risk, including overweight participants (n=42) and obese participants (n=36). All biochemical analysis was conducted in duplicates, unless otherwise stated, and the mean values taken as the final result. For all markers, the results are presented as treatment effects. This was undertaken by calculating individual differences between pre- and post-values for both control and treatment phases for each subject. Paired T-tests were then carried out on the difference scores (post-treatment value minus pre-treatment values) for both treatment phases (SPE and Maltodextrin). Significance level was set at $P<0.05$. All statistical analyses were performed using the Statistical Package for the Social Sciences (SPSS) for Windows version 22.0 (SPSS Inc., Chicago, IL, USA).

**Multivariate Data Analysis**

Data were obtained as peak areas from the Compound Discover automatic integration software and consisted of 2194 and 3289 potential metabolites (or features) in urine and plasma samples,
respectively. Relative peak areas of the metabolites (normalized by the total urine excretion of each subject) obtained by UPLC-HRMS were imported into MATLAB R2015b (Mathworks, USA). PLS toolbox v.8.5 (Eigenvector, USA) and homemade scripts were used. Principal component analysis (PCA) and hierarchical cluster analysis (HCA) are the most widely used tools to explore similarities and patterns among samples where data grouping are unclear. Moreover, the orthogonal partial least squares discriminate analysis (OPLS-DA) method was performed as a typical supervised multivariate methodology used in metabolomics studies (30,31). Several data pre-processing transformations were performed and evaluated, such as Probabilistic Quote Normalization (PQN), Log transformation, mean centering, pareto scaling and auto-scaling. In our case, PQN and autoscaling were selected as pre-processing techniques to reach the lowest root mean square error (RMSE) in an iterative process. A re-sampling method, cross-validation k-fold cross validation (k=5) was also used to evaluate the number of latent variables (according to the lowest RMSE) and the prediction ability of our models. To reduce the impact of the random split of CV-participants, the mean values of the estimated results were obtained after 20 random 5-fold CV. Urine models provided successful classification results at the cross validation step and achieved good prediction parameters that can be explained by the area under the curve values (1.00 and 0.895). Based on the plasma data set and classes selected a discriminant model could not be developed. Moreover, the Variable Importance in Projection (VIP) is the widely known metric that is used to identify potential markers in metabolomics studies (32). VIP is a weighted sum of squares of the PLS weight which indicates the importance of the variable to the whole model. The cut off VIP value selected in our study was 2. The potential markers were extracted from the two models to compare the results obtained.

RESULTS
Baseline characteristics

Eighty participants (males n=39, females n=41) were enrolled on this 24-week randomised, double-blind, placebo-controlled crossover trial. The intervention was conducted as per the protocol and there were no adverse events associated with the intervention. The study population had a mean group age of 42.7±7.1 years and a mean BMI of 30.2±3.9 kg/m². The study had an overall compliance of 97% with 78 participants completing the 24-week study; two participants withdrew from the study at the midpoint for personal reasons and compliance was not significantly different by treatment group or time period (P>0.05) (Figure 1). There were no significant differences between the participants in age and physical characteristics at the beginning of either treatment phase (Table 2).

Habitual dietary intake

Dietary analysis of habitual intake (midpoint) during both treatment phases (SPE, Maltodextrin) of the crossover trial is described in Table 3. There were no significant differences between treatment phases for any of the macronutrients or micronutrients analysed indicating that the seaweed phenolic extract did not affect the habitual food consumption patterns in the study population.

Effects of seaweed (poly)phenol extract on DNA damage

The basal levels of DNA damage observed in the study were consistent with previous studies with a mean group average of 6.72 ± 2.48% tail DNA (data not shown). In response to an oxidative challenge with 150 µM H₂O₂, DNA damage was increased to an average of approximately 34 ± 7% tail DNA in both the placebo and SPE phase in all participants as reported in Table 4. The 8-week intervention with a seaweed phenolic extract resulted in a significant reduction in basal DNA damage, as measured by the Comet assay, in obese
participants only (BMI >30 kg/m²), with a significant reduction (P=0.044) in basal DNA damage observed (Table 4). A significant reduction was not observed in the total population (n=78) nor in the overweight participants (n=42), either in terms of a challenge response or in basal levels. Additionally, consumption of seaweed phenolic extract also significantly reduced (P=0.009) basal DNA in males only with a mean group change value of -0.8 ± 2.5 % tail DNA (SPE) compared to 0.9 ±2.8% tail DNA in the control. No significant effects where observed for females.

**Total oxidative capacity of seaweed phenolics**

Figure 2 shows the total oxidative capacity (peroxide levels) in plasma samples from all participants (n=78), in overweight participants (n=42) and in obese participants (n=36) measured after the placebo and seaweed capsule intervention. There were no significant changes from baseline after either treatment phase (SPE, Maltodextrin) in all participants (n=78) nor in overweight (n=42) or obese (n=36) sub groups. However the consumption of seaweed phenolic extract also significantly reduced (P=0.018) TOC in females only with a mean group change value of -7.44 ± 29.37 (SPE) compared to 4.33 ± 22.36 in the control. No significant effects where observed for males.

**Effects of seaweed (poly)phenol extract on blood lipids and CRP**

Table 4 shows the pre- and post- values, as well as the percentage change, for both the placebo phase and the SPE phase for each blood lipid biomarker and CRP. An 8-week supplementation with seaweed (poly)phenol extract did not significantly affect any cardiovascular risk marker.

**Effects of seaweed (poly)phenol extract on inflammatory markers**

F₂α-isoprostane levels at baseline were 392 ± 219 pg/ml (data not shown). A high degree of inter-individual variation was observed, and the 8-week SPE intervention did not exhibit a
significant effect on this marker of oxidative stress (Table 4). There were no significant
differences for any of the inflammatory cytokines measured in either treatment phase (SPE,
Maltodextrin) for all of the participants (n=78), in the overweight participants (n=42) or in the
obese participants (n=36) (Figure 4). Nor were significant effects observed in the cytokine
profiles when examined by ratio of TNF-α to IL-10, IL-1β to IL-10, IL-6 to IL-10 and CRP to
IL-10, according to Laird et al. (29)

Bioavailability of seaweed phenolics, untargeted Analysis

Initially an unsupervised PCA model was carried out (Figure 4A) on the urine data from all
78 participants. The first component of the PC1 vs PC2 scores plot obtained from the data set
explained 23.14% of the total variance showing a clear trend among some of the participants
compared to the rest. Moreover, a hierarchical cluster analysis was performed to verify this
natural aggregation pattern among the 78 participants suggesting, two clusters among all the
participants (Figure 4B) which share, accounting for the inter-individual differences associated
with differences in the urinary metabolite profiles. For instance, recent studies suggest that
individuals can be clustered into distinct groups based on their gut microbiome composition,
functional metabolism (33) or individual responses to obtain markers of a specific treatment,
minimizing the inter-individual data that are not the target of the study. In order to elucidate
the observed variability between these two groups, two supervised models were performed,
one with the urinary profiles from all the participants before the seaweed consumption and
another one with the urinary profiles from all the participants after seaweed consumption. Both
supervised models verified differences between the two groups of individuals (Group 1 and
Group 2) and provided successful classification results at the cross validation step and achieved
in the pre-treatment and post-treatment samples 95% and 98% sensitivity (participants of the
class of interest correctly assigned to their class) and 100% and 100% specificity (participants
not belonging to the class of interest were correctly not assigned to that class), respectively
It is noteworthy that even stratifying for BMI category, the multivariate analysis of the urine profiles did not show clear differentiation of the metabolome between obese or overweight individuals.

By using the preliminary information provided by PCA and HCA related to the stratification of the individuals into groups which share a common excretion profile before and after supplementation with seaweed capsules, two data sets were analysed. The individuals were stratified into two groups; Group 1 including 70 participants; and Group 2 including 8 participants (S58, S60, S61, S71, S76, S78, S79, S83, all being overweight individuals). Two supervised OPLS-DA models were built to discriminate according to the seaweed treatment in both models (Figure 5). In our study we used two latent variables (LV) and the sensitivity and specificity values were set to 100% at the calibration step recognition ability of the two models. These analyses showed that the human urine metabolome in both groups of participants was modified after the 8-week supplementation with seaweed (poly)phenol extract compared to baseline urine metabolic profiles. Using the loadings plot and the variable importance in projection values (VIP) we ascertained important contributors between the modelled classes and therefore identified the compounds responsible for the difference in the urine metabolic profile before and after 8-weeks seaweed (poly)phenol extract consumption in both groups. VIP is the widely known metric that is used to identify potential markers (metabolites) in metabolomics studies. Further, the urine scores plot allowed the identification of those metabolites which appeared after the seaweed (poly)phenol extract intake (Figure 5) in both groups of participants.

The main contributors to metabolome differentiation in the urine before and after seaweed consumption in both groups of participants are described in Supplemental Table 1 and Table 2 (Supplemental Material). Positive loading values (x axis) and higher VIP values (VIP>2.0)
were detected in urine after seaweed consumption and were the responsible of the observed differences between the participants classes (post ingestion vs non ingestion of seaweed (poly)phenol extract). Urine metabolites tentatively identified as pyrogallol/phloroglucinol sulfate, hydroxytrifurahol A-sulfate and dioxinodehydroeckol glucuronide are considered clear biomarkers of seaweed consumption in Group 1 while C-O-C dimer of phloroglucinol-sulfate, C-O-C-dimer of phloroglucinol, fucophloroethol-glucuronide, diphlorethol sulfates and dioxinodehydroeckol glucuronide are those biomarkers of seaweed consumption in Group 2. All of them have shown high values in the variable importance in the projection (values > 2) (Table 5).

Based upon the identified seaweed (poly)phenol metabolites, the total amount of seaweed metabolites excreted in urine varied noticeably between participant ranging from 0.001 to 4.140 mmoles, with a group average of 1.29 +/- 0.88 mmoles. 25% of the population (19/78) appeared to be low excretors with urinary seaweed metabolites excretion less than 0.5 mmoles, 55% of the population (43/78) were medium excretors with an urinary seaweed excretion between 0.5 and 2 mmoles, while 20% of the population (16/78) where high excretors with total seaweed metabolites concentrations >2 mmoles (see Supplemental Table 6).

The plasma metabolome demonstrated no clear differentiation for participants in either treatment phase (data not shown), nor with exploration of the data by PCA or HCA. Consequently no exposure biomarkers could be defined for plasma samples taking into account all the participants (n=78).

**DISCUSSION**

The association of seaweed consumption with reduced risk factors for CVD has been tested largely within *in vitro* or animal models, and only limited human data exists to substantiate the
proposed beneficial properties of seaweeds (12,13,34). To the best of our knowledge this trial is the first comprehensive study to investigate the *in vivo* bioactivity and bioavailability of seaweed (poly)phenolics on biomarkers of inflammation and oxidative stress in overweight and obese individuals. Participant retention rates were high (98%) and self-reported SPE capsule intake (97%) indicated that the intervention was implemented successfully in this group of participants. There was, however, no significant difference between the intervention (SPE capsule) and control (placebo capsule) phases for markers of oxidative stress, antioxidant status or inflammation in the population as a whole (n=78). Subset analysis, stratifying for BMI category, showed a significant albeit modest difference in DNA damage in the obese population (n=36). This is consistent with Park *et al.* (35) who tested astaxanthin, a natural carotenoid in the form of microalgae *Haematococcus pluvialis* in 14 healthy females (2 mg/d extract for 8 weeks) demonstrating a significant reduction in the oxidative damage marker plasma 8-hydroxy-2’-deoxyguanosine. Obese individuals are associated with increased DNA damage, higher oxidant status and increased oxidative damage to macromolecules a group and are at higher risk of chronic disease (36-39). In our study, an overweight/obese population (age 30-65 years and a BMI >25 kg/m²) was shown to have significantly reduced basal levels of lymphocyte DNA damage (by 23%) but only in the obese population subset (n=36) following supplementation. Both seaweed-based intervention studies support data from *in vitro* studies indicating anti-genotoxic activity for seaweed extracts on a range of cell lines (40,41) including the SPE extract used in the study (15). Although a mechanism was not determined for the observed reduction in DNA damage in the obese group activate Nrf2-mediated cytoprotection may be involved, as seaweed (poly)phenols such as eckol are anti-genotoxic (42) and can activate Nrf2-mediated HO-1 induction (43,44) consistent with effects observed for (poly)phenols from terrestrial sources (45,46). Park *et al.* (35) also observed a significant reduction in plasma CRP levels, an acute marker of inflammation. Although in our study we
observed a 28% decrease in CRP levels in response to seaweed extract consumption, this change was not significant. However, a similar study investigating the effect of consuming *Palmaria palmata* (5 g/day) incorporated into bread found that it significantly increased CRP by 16%, suggesting that *P. palmata* stimulates inflammation rather than reducing it (47). Park et al. (35) reported that there was no difference in TNF and IL-2 concentrations, but plasma IFN-γ and IL-6 increased on week 8 in participants given 8mg astaxanthin. Within this study, we similarly observed no significant changes in IL-2 or TNF, nor did we see an alteration in IFN-γ or IL-6. The seaweed extract tested did not affect immune function (cell mediated and humoral immune responses as tested by the cytokine markers) following supplementation.

In order to evaluate the seaweed molecules responsible for its modest beneficial effects in vivo and the potential biological markers linked to seaweed consumption, an untargeted approach comparing the metabolic profiles between urine and plasma samples of 78 individuals who consume seaweed capsules have been carried out. As a first step, we have determined differences in the urine metabolic profiles between participants being able to stratify the individuals into groups which share common excretion metabolite profile before and after seaweed capsule supplementation. Urinary profiles of these two groups were statistically different between each other and the metabolites responsible for this discrimination have been selected as potential biomarkers of seaweed consumption (Table 5). Subsequently, the quantification of these potential biomarkers within all participants revealed substantial inter-individual variation in the concentration of seaweed metabolites excreted in urine by the participants ranging from 0.001 to 4.140 mmoles. Besides, the outcomes of the comprehensive multivariate analysis of the metabolite profiles shows, as expected, person-to-person variation in the 0-24h urinary excretion of seaweed (poly)phenols, probably due to differences in gut microbiota and no strict living conditions of the participants. As a consequence of these inter
individual differences, the 78 participants involved in this study can be grouped by their
dissimilarity to metabolize seaweed (poly)phenols in two well established groups. For instance,
group 1 of individuals is characterized by greater excretion of seaweed derived metabolites
such as pyrogallol/phloroglucinol sulfate, hydroxytrifurahol A-sulfate and
dioxinodehydroeckol; while participants belonging to group 2 C-O-C dimer of phloroglucinol-
sulfate, C-O-C dimer of phloroglucinol, fucophloroethol-glucuronide, diphlorethol sulfates and
dioxinodehydroeckol glucuronide in low concentrations. This inter-individual variation in the
absorption of seaweed polyphenols has been observed in the bioavailability of dietary
(poly)phenols such as orange juice, cranberry and pomegranate (48-50).

The results from the non-targeted approach confirmed the presence of seaweed phenolic
metabolites, and can be divided into a) phase II sulphated and glucuronidated metabolites
related to the targeted components described earlier by Corona et al. (20) (supplemental Tables
4,5); arguably formed in the liver; b) an extended list of unknown compounds which may be
potential breakdown products or metabolites of the original seaweed (poly)phenols catabolised
by the colonic bacteria but further studies would be required to confirm the identity of these
unknown compounds. Poor absorption of the high molecular weight phlorotannins in the upper
gastrointestinal tract following acute consumption of our seaweed (poly)phenolic extract as
reported (20) likely results in them reaching the colon and becoming subject to microbial
fermentation to lower molecular weight derivatives; recently confirmed by in vitro gut
modelling of SPE by colonic microbiota (15). The urinary metabolite profiles of seaweed
phenolics from the low and high excretors clearly indicated a high inter-individual variation in
metabolism similar to that observed in terrestrial plant phenolics, and no single common
metabolite or pattern could be detected following seaweed consumption. It is possible that
inter-individual variation in gut microbiota underlie these metabolic changes and were
responsible for the observed differences. That said, the study of bioavailability of seaweed
polyphenolics remains challenging due to the high range of molecular weight compounds present, and their characterisation is complicated further by the lack of commercially available standards. Other limitations of the present study include a relatively short-term intervention, results cannot therefore be extrapolated to long-term chronic consumption. Participants in this study may not be representative of the general overweight population of Northern Ireland as they were mostly recruited from the University of Ulster staff and local residents. While lymphocyte DNA damage (spontaneous DNA SBs) is considered appropriate for the substantiation of EFSA health claims in the context of protection against generic DNA damage, a lesion specific enzyme such as (ENDO III) would have been required for a claim related to oxidative DNA damage (51). Finally, the study population number may have been too small to yield significant results in intercellular cytokines. These factors should be considered for the design of future studies in this area.

To conclude, to the best of our knowledge, this work represents the first comprehensive study involving 78 human participants investigating the bioavailability of seaweed (poly)phenolics. Consumption of SPE, decreased DNA damage albeit to a modest extent in obese individuals only; with no clear effects on clinical markers of inflammation. While untargeted analysis identified novel urinary biomarkers of seaweed consumption and highlighted a high degree of inter-person variation in the metabolism of seaweed phenolics. Future studies that address the ingestion of seaweed phenolics will need to consider and adjust for these parameters, including the importance of establishing an individual’s capacity for metabolising (poly)phenol (52-54).
ACKNOWLEDGEMENTS

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CIRG, IRR, JMWW, JS and PY designed research; FRB, CS, SH, RC and CIRG conducted research; FRB, CS, TM, KMF, MI, KT, KP GPC, FJC, JMMR, LKP and GC analysed data; FRB, LKP, GC, NGT, GPC and CIRG wrote the paper; CIRG had primary responsibility for final content. All authors read and approved the final manuscript. None of the authors had any financial or personal conflict of interest.
References


30


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mediated through the ERK/Nrf2 pathway and protects against oxidative stress. Free Radic Biol Med. 2011 Dec 1;51(11):2073-81.


Table 1. Key components of phlorotannin rich basic seaweed extract, High Molecular Weight (HMW) seaweed extract fraction, and blend used for intervention capsules (SPE).

<table>
<thead>
<tr>
<th>Extract component</th>
<th>Basic seaweed extract</th>
<th>HMW seaweed extract fraction</th>
<th>Blend (SPE capsule)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phlorotannin</td>
<td>61.25</td>
<td>46.05</td>
<td>107.3</td>
</tr>
<tr>
<td>Iodine</td>
<td>0.48</td>
<td>0.02</td>
<td>0.5</td>
</tr>
<tr>
<td>Maltodextrin(^1)</td>
<td>0</td>
<td>0</td>
<td>175</td>
</tr>
<tr>
<td>Minerals</td>
<td>39.38</td>
<td>13</td>
<td>52.38</td>
</tr>
<tr>
<td>Fucoxanthin</td>
<td>0</td>
<td>36.5</td>
<td>36.5</td>
</tr>
<tr>
<td>Laminarin as glucose</td>
<td>10.68</td>
<td>1.75</td>
<td>12.43</td>
</tr>
<tr>
<td>Fucoidan as fucose</td>
<td>0</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Mannitol</td>
<td>29.23</td>
<td>5.9</td>
<td>35.13</td>
</tr>
<tr>
<td>Inorganic arsenic</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Cadmium (LD 0.15mg/kg)</td>
<td>&lt;LD</td>
<td>&lt;LD</td>
<td>&lt;LD</td>
</tr>
<tr>
<td>Mercury (LD 0.016mg/kg)</td>
<td>&lt;LD</td>
<td>&lt;LD</td>
<td>&lt;LD</td>
</tr>
<tr>
<td>Lead (LD 1.1mg/kg)</td>
<td>&lt;LD</td>
<td>&lt;LD</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Tin (LD 1.7mg/kg)</td>
<td>&lt;LD</td>
<td>&lt;LD</td>
<td>&lt;LD</td>
</tr>
</tbody>
</table>

\(^1\) maltodextrin was added to the capsule formulation as an excipient.
Table 2. Baseline characteristics of the study population (n=80).

<table>
<thead>
<tr>
<th>Variable</th>
<th>Placebo treatment phase (n=80)</th>
<th>SPE treatment phase (n=80)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (y)</td>
<td>42.8 ± 7.2</td>
<td>42.9 ± 7.1</td>
</tr>
<tr>
<td>Gender (M/W)</td>
<td>20/20</td>
<td>19/21</td>
</tr>
<tr>
<td>Height (m)</td>
<td>1.71 ± 0.08</td>
<td>1.72 ± 0.10</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>88.9 ± 14.1</td>
<td>89.1 ± 17.3</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>30.3 ± 3.5</td>
<td>30.0 ± 4.4</td>
</tr>
</tbody>
</table>

No significant differences P>0.05, Paired T Test.
Table 3. Habitual nutrient intake of subjects during intervention study

<table>
<thead>
<tr>
<th>Variable intake</th>
<th>Placebo treatment phase (n=77)</th>
<th>SPE treatment phase (n=77)</th>
<th>% Change</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Energy (kcal/d)</td>
<td>1949 ± 590</td>
<td>2057 ± 684</td>
<td>5</td>
<td>0.110</td>
</tr>
<tr>
<td>Protein (g/d)</td>
<td>79.4 ± 28.6</td>
<td>79.9 ± 25.3</td>
<td>0.5</td>
<td>0.782</td>
</tr>
<tr>
<td>Carbohydrate (g/d)</td>
<td>220.6 ± 72.1</td>
<td>231.3 ± 84.2</td>
<td>5</td>
<td>0.278</td>
</tr>
<tr>
<td>Total fat (g/d)</td>
<td>78.6 ± 26.5</td>
<td>85.2 ± 34.8</td>
<td>8</td>
<td>0.058</td>
</tr>
<tr>
<td>Saturated fat (g/d)</td>
<td>29.0 ± 10.2</td>
<td>30.7 ± 15.3</td>
<td>6</td>
<td>0.417</td>
</tr>
<tr>
<td>Monounsaturated fat (g/d)</td>
<td>24.4 ± 9.3</td>
<td>26.8 ± 12.4</td>
<td>9</td>
<td>0.112</td>
</tr>
<tr>
<td>Polyunsaturated fat (g/d)</td>
<td>12.1 ± 5.4</td>
<td>13.5 ± 6.4</td>
<td>10</td>
<td>0.059</td>
</tr>
<tr>
<td>Fibre (g/d)</td>
<td>12.9 ± 6.2</td>
<td>13.3 ± 4.8</td>
<td>3</td>
<td>0.259</td>
</tr>
<tr>
<td>Vitamin C (mg/d)</td>
<td>71.8 ± 50.5</td>
<td>69.4 ± 47.1</td>
<td>-3</td>
<td>0.909</td>
</tr>
<tr>
<td>Vitamin E (mg/d)</td>
<td>7.17 ± 3.7</td>
<td>7.67 ± 4.0</td>
<td>7</td>
<td>0.279</td>
</tr>
<tr>
<td>Folate (µg/d)</td>
<td>223.9 ± 98.3</td>
<td>228.2 ± 85.5</td>
<td>2</td>
<td>0.370</td>
</tr>
<tr>
<td>Carotene (µg/d)</td>
<td>2821 ± 1900</td>
<td>2553 ± 1706</td>
<td>-11</td>
<td>0.185</td>
</tr>
</tbody>
</table>

1S66 did not complete a food diary in either phase. 2 Calculated from SPE phase – Placebo phase. 3 Mean treatment group values were not significantly different between phases, P>0.05 (Wilcoxin signed rank test). 4 Mean ± SD. 5 Calculated using the Englyst method.
Table 4. Effects of seaweed polyphenol extract on lymphocyte DNA damage, CRP, blood lipids and F2 isoprostanes

<table>
<thead>
<tr>
<th>Blood marker</th>
<th>Average baseline value</th>
<th>Placebo treatment effect</th>
<th>SPE treatment effect</th>
<th>P value</th>
<th>Average baseline value</th>
<th>Placebo treatment effect</th>
<th>SPE treatment effect</th>
<th>P value</th>
<th>Average baseline value</th>
<th>Placebo treatment effect</th>
<th>SPE treatment effect</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total (n=78)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DNA damage - basal (% Tail)</td>
<td>6.72 ± 2.48 0.74 ± 2.86 -0.41 ± 3.13 0.350</td>
<td>6.59 ± 2.80 0.32 ± 2.14</td>
<td>0.57 ± 3.24 0.129</td>
<td></td>
<td>6.91 ± 2.00 1.81 ± 4.50</td>
<td>0.15 ± 2.93</td>
<td>0.044</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DNA damage - H2O2 (%Tail)</td>
<td>34.2 ± 7.00 -1.56 ± 6.60 -2.03 ± 6.40 0.390</td>
<td>35.2 ± 7.11 0.13 ± 2.19</td>
<td>0.76 ± 1.66 0.062</td>
<td></td>
<td>32.8 ± 6.69 1.26 ± 2.09</td>
<td>0.54 ± 1.54</td>
<td>0.111</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CRP (mg/ml)</td>
<td>2.67 ± 3.9 0.01 ± 3.3 -0.83 ± 4.9 0.429</td>
<td>2.59 ± 4.69 0.00 ± 3.40</td>
<td>-1.32 ± 6.22 0.348</td>
<td></td>
<td>2.80 ± 2.56 -1.81 ± 11.82</td>
<td>0.72 ± 5.15</td>
<td>0.258</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cholesterol (mmol/l)</td>
<td>5.20 ± 0.77 -0.06 ± 0.57 -0.10 ± 0.57 0.256</td>
<td>5.18 ± 0.82 0.01 ± 0.55</td>
<td>-0.53 ± 0.54 0.201</td>
<td></td>
<td>5.24 ± 0.73 -0.13 ± 0.58</td>
<td>-0.15 ± 0.60</td>
<td>0.419</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Triglycerides (mmol/l)</td>
<td>1.51 ± 0.94 0.01 ± 0.82 0.04 ± 0.96 0.385</td>
<td>1.34 ± 0.72 0.07 ± 0.79</td>
<td>-0.04 ± 0.48 0.278</td>
<td></td>
<td>1.75 ± 1.15 -0.06 ± 0.85</td>
<td>-0.02 ± 1.34</td>
<td>0.497</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HDL (mmol/l)</td>
<td>1.37 ± 0.32 -0.01 ± 0.15 0.03 ± 0.15 0.187</td>
<td>1.46 ± 0.31 0.01 ± 0.16</td>
<td>-0.03 ± 0.15 0.150</td>
<td></td>
<td>1.25 ± 0.32 -0.04 ± 0.11</td>
<td>-0.04 ± 0.14</td>
<td>0.446</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LDL (mmol/l)</td>
<td>3.16 ± 0.1 -0.08 ± 0.5 -0.06 ± 0.50 0.478</td>
<td>3.13 ± 0.69 -0.07 ± 0.57</td>
<td>0.01 ± 0.45 0.383</td>
<td></td>
<td>3.20 ± 0.67 -0.09 ± 0.56</td>
<td>-0.13 ± 0.56</td>
<td>0.412</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>F2-Isoprostanes (pg/ml)</td>
<td>393 ± 220 -10 ± 182 -6 ± 138 0.374</td>
<td>342 ± 128 28 ± 152</td>
<td>-29 ± 108 0.130</td>
<td></td>
<td>439 ± 272 -44 ± 204</td>
<td>14 ± 158</td>
<td>0.283</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Data is presented as treatment affects, calculated based on individual differences between pre- and post- values for both control and treatment phases for each subject. Paired T-tests were then carried out on the difference scores (post-treatment value minus pre-treatment values) between treatment (Seaweed phenolic extract capsule) and placebo control phase (maltodextrin). Significance level was set at P<0.05 (one-tailed T test).
**Table 5.** Phlorotannins metabolites tentatively identified in human urine samples from Group 1 (70 subjects) and Group 2 (8 subjects) after seaweed capsule consumption.

<table>
<thead>
<tr>
<th>ID</th>
<th>VIP value</th>
<th>Rt (min)</th>
<th>Experimental Molecular Weight</th>
<th>Predicted Formula</th>
<th>Metabolite putative identification</th>
<th>Ratio¹</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Group 1 (72 subjects)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>652</td>
<td>2.06</td>
<td>25.6</td>
<td>205.9881</td>
<td>C6H6O6S</td>
<td>Pyrogallol/phloroglucinol sulfate</td>
<td>1.5</td>
</tr>
<tr>
<td>800</td>
<td>2.28</td>
<td>25.8</td>
<td>205.9881</td>
<td>C6H6O6S</td>
<td>Pyrogallol/phloroglucinol sulfate</td>
<td>1.2</td>
</tr>
<tr>
<td>1472</td>
<td>3.86</td>
<td>24.9</td>
<td>205.9881</td>
<td>C6H6O6S</td>
<td>Pyrogallol/phloroglucinol sulfate</td>
<td>1.1</td>
</tr>
<tr>
<td>1352</td>
<td>4.26</td>
<td>25.6</td>
<td>486.1727</td>
<td>C26H30O7S</td>
<td>Hydroxytrifluoroal A-glucuronide</td>
<td>2.0</td>
</tr>
<tr>
<td>1453</td>
<td>3.83</td>
<td>25.4</td>
<td>486.1727</td>
<td>C26H30O7S</td>
<td>Hydroxytrifluoroal A-glucuronide</td>
<td>1.9</td>
</tr>
<tr>
<td>1458</td>
<td>4.23</td>
<td>25.3</td>
<td>486.1727</td>
<td>C26H30O7S</td>
<td>Hydroxytrifluoroal A-glucuronide</td>
<td>2.2</td>
</tr>
<tr>
<td>1483</td>
<td>4.39</td>
<td>25.3</td>
<td>486.1727</td>
<td>C22H30O12</td>
<td>Hydroxytrifluoroal A-glucuronide</td>
<td>2.2</td>
</tr>
<tr>
<td>1917</td>
<td>2.11</td>
<td>31.4</td>
<td>544.2881</td>
<td>C27H44O11</td>
<td>Dioxinodehydroeckol glucuronide</td>
<td>1.1</td>
</tr>
<tr>
<td><strong>Group 2 (8 subjects)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>293</td>
<td>2.08</td>
<td>9.5</td>
<td>327.0951</td>
<td>C12H8O9S</td>
<td>C-O-C dimer of phloroglucinol-sulfate</td>
<td>1.2</td>
</tr>
<tr>
<td>702</td>
<td>2.20</td>
<td>24.1</td>
<td>248.0315</td>
<td>C12H8O6</td>
<td>C-O-C dimer of phloroglucinol</td>
<td>1.2</td>
</tr>
<tr>
<td>853</td>
<td>2.08</td>
<td>27.8</td>
<td>797.3186</td>
<td>C45H49O13</td>
<td>Fucophloroethol glucuronide</td>
<td>1.1</td>
</tr>
<tr>
<td>1293</td>
<td>2.28</td>
<td>34.2</td>
<td>330.1675</td>
<td>C12H1009S</td>
<td>Diphlorethol sulfate</td>
<td>1.9</td>
</tr>
<tr>
<td>1356</td>
<td>2.31</td>
<td>34.1</td>
<td>330.1675</td>
<td>C12H1009S</td>
<td>Diphlorethol sulfate</td>
<td>1.7</td>
</tr>
<tr>
<td>1633</td>
<td>2.07</td>
<td>35.1</td>
<td>544.2152</td>
<td>C27H44O11</td>
<td>Dioxinodehydroeckol glucuronide</td>
<td>1.2</td>
</tr>
</tbody>
</table>

ID, identification number. VIP (variable influence in projection) is a variable that summarizes the importance of X variables to the OPLS-DA model. Variables with values > 2 were the most influential in the model. All predicted formula derived with < 5 ppm mass accuracy data.

¹Ratio: seaweed capsule consumption/placebo consumption.
FIGURE 1. CONSORT DIAGRAM. Progress of participants through the intervention study.

FIGURE 2. Comparison of the effects of SPE supplementation phase (■) with that of a placebo phase (□) on total oxidative capacity (TOC) in the total study population (n=78), in overweight (n=42), and in obese (n=36). All values were measured in duplicate expressed as mean ± SD. TOC is represented as the mean of the individual difference values (after – before supplementation) in the SPE and placebo treatment phases.

FIGURE 3. Comparison of the effects of SPE supplementation phase (■) with that of a placebo phase (□) on cytokine levels in the total study population (n=78), in overweight (n=42), and in obese (n=36). All values were measured in duplicate expressed as mean ± SD. Cytokines were measured as the mean of the individual difference value (after – before supplementation) in the SPE and placebo treatment phases. Change in mean treatment group values were not significantly different between treatment phases, P>0.05 (Paired T Test; one-tailed test). M.F.I.; Mean Fluorescence Intensity.

FIGURE 4. Principal Component Analysis (PCA) (A) and Hierarchical Cluster Analysis Cluster (HCA) (B) of urinary profiles before (♦) and after (■) seaweed consumption by 80 participants. The HCA was calculated based on Euclidean distances and the Ward hierarchical agglomerative method. The PC explained 23.14% of the total variance (PC-1 14.8% and PC-2 8.34%).

FIGURE 5 A) OPLS-DA scores and B) loadings of the urine samples belong to group of participants 1 (70 participants). C) OPLS-DA scores and D) loading of urine samples belong to group of participants 2 (8 participants) before (♦) and after (■) seaweed ingestion. (Circles shown in the graph represent a confidence of 95%). LV1: latent variable 1; LV 2: latent variable 2. The cut off VIP value selected to be 2. For VIP scores identification see Table 5 and Supplemental Table 1.
FIGURE 1

Enrolment
Assessed for eligibility (n=244)

Excluded (n=157)
- BMI <25 kg/m² (n=71)
- Taking supplements (n=7)
- Taking medication/diagnosed medical condition (n=53)
- Age <30 years (n=13)
- Pregnant (n=4)
- Opted out due to personal reasons (n=7)
- Smoker (n=2)

Suitable (n=87)

Randomised (n=80)

Intervention phase (n=40)
Seaweed capsule
1 x 400mg/day for 8 weeks

Control phase (n=40)
Placebo capsule
1 x 400mg/day for 8 weeks

Allocation

8-week washout phase
Discontinued intervention
Due to personal reasons (n=1)

Follow-up

8-week washout phase
Discontinued intervention
Due to personal reasons (n=1)

Control phase (n=39)
Placebo capsule
1 x 400mg/day for 8 weeks

Completed study

Intervention phase (n=39)
Seaweed capsule
1 x 400mg/day for 8 weeks

Analysed (n=39)
Excluded from analysis (n=0)

Analysis

Analysed (n=39)
Excluded from analysis (n=0)
FIGURE 2.
FIGURE 4.
FIGURE 5