A randomised crossover placebo-controlled trial investigating the effect of brown seaweed (*Ascophyllum nodosum* and *Fucus vesiculosus*) on postchallenge plasma glucose and insulin levels in men and women

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Abstract: This study examined the impact of brown seaweed on post-load plasma glucose and insulin concentrations in men and women. Twenty-three participants (11 men, 12 women) aged 19–59 years were recruited in this double-blind, randomized, placebo-controlled crossover study. The test product consisted of a commercially available blend of brown seaweed (*Ascophyllum nodosum* and *Fucus vesiculosus*) with known inhibitory action on α-amylase and α-glucosidase activities (InSea^2). Two 250 mg seaweed capsules and 2 placebo capsules were consumed on each occasion 30 min prior to the consumption of 50 g of carbohydrates from bread. Plasma glucose and insulin concentrations were measured over a period of 3 h postcarbohydrate ingestion at predetermined time points. Both treatments were separated by a 1-week washout period. Data were analysed using mixed models for repeated measures. Compared with placebo, consumption of seaweed was associated with a 12.1% reduction in the insulin incremental area under the curve ($p = 0.04$, adjusted for baseline) and a 7.9% increase in the Cederholm index of insulin sensitivity ($p < 0.05$). The single ingestion of 500 mg of brown seaweed had no significant effect on the glucose response ($p = 0.24$, adjusted for baseline). Glucose and insulin responses were similar between men and women. Consumption of the seaweed capsules was not associated with any adverse event. These data suggest that brown seaweed may alter the insulin homeostasis in response to carbohydrate ingestion.

Key words: α-glucosidase inhibitor, α-amylase inhibitor, oral glucose tolerance test, postprandial glycemia, postprandial insulinemia, *Ascophyllum nodosum*, *Fucus vesiculosus*, blood glucose response.

Résumé : Cette étude avait pour but d'examiner l'impact d'algues brunes sur la concentration plasmatique de glucose et d'insuline suite à la consommation d'un aliment-test chez les hommes et les femmes. Vingt-trois participants (11 hommes, 12 femmes) âgés entre 19–59 ans ont été recrutés pour cette étude randomisée à double-insu, contrôlée avec un placébo suivant un devis en chassé-croisé. Le produit à l'étude consistait en une poudre d'algues brunes (*Ascophyllum nodosum* et *Fucus vesiculosus*) commercialisée sous le nom de InSea^2 et ayant une action inhibitrice sur les enzymes α-amylase et α-glucosidase. Deux capsules de 250 mg de poudre d'algues brunes et 2 capsules de placebo ont été prises à chacune des visites 30 minutes avant la consommation de 50 g de glucides provenant de pain blanc. Les concentrations plasmatiques de glucose et d'insuline ont été mesurées à des temps prédéterminés sur une période de 3 heures suivant l'ingestion de l'aliment-test. Les 2 traitements étaient espacés par une période d'une semaine. Les données ont été analysées à l'aide de modèles mixtes pour les mesures répétées. Comparée au placébo, la consommation de la poudre d'algues brunes a été associée à une baisse de 12,1 % dans l'aire sous la courbe incrémentale de l'insuline ($p = 0,04$, ajusté pour les valeurs de départ) et à une augmentation de 7,9 % de l'indice Cederholm, un indicateur de la sensibilité à l'insuline ($p < 0,05$). La prise unique de 500 mg de poudre d'algues brunes n'a pas eu d'effet sur la réponse glycémique ($p = 0,24$, ajusté pour les valeurs de départ). Les réponses glycémiques et insulinémiques étaient similaires entre les hommes et les femmes. La consommation des capsules d'algues brunes n'était pas associée à des événements indésirables. Ces données suggèrent que la poudre d'algues brunes peut moduler l'homéostasie de l'insuline en réponse à l'ingestion de glucides.

Mots-clés : inhibiteur d'α-glucosidase, inhibiteur d'α-amylase, test oral de tolérance au glucose, glycémie postprandiale, insulinémie postprandiale, *Ascophyllum nodosum*, *Fucus vesiculosus*, réponse glycémique.

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Introduction

Optimal control of blood glucose levels has been shown to reduce the incidence of diabetes-related complications (Diabetes Control and Complications Trial Research Group 1993; UK Prospective Diabetes Study (UKPDS) Group 1998). Adequate management of blood glucose involves measures such as diet and exercise but also oral glucose-lowering medication in patients with more advanced disease states. Food ingredients that attenuate postprandial glucose response, such as α-glucosidase and α-amylase inhibitors, also have particular relevance for the prevention of diabetes.

α-glucosidase and α-amylase are enzymes that are implicated in digestion of dietary starch and carbohydrates into absorbable monosaccharides. Inhibition of these enzymes reduces carbohydrate absorption, thereby contributing to an improved glycemic control and postprandial insulinemia (van de Laar et al. 2005). Antidiabetic drugs, such as acarbose, miglitol, and voglibose, work through inhibiting α-glucosidase enzymatic activity.

Some herbal supplements have been shown to have α-glucosidase–inhibiting activities. For instance, several plants from the *Salacia* genus (eg, *S. prinoides*, *S. reticulata*, and *S. oblonga*) have been used for many years as part of traditional Indian medicine to treat diabetic conditions (Vaidyaratnam 1996). A variety of foods, such as fermented soybean (Fujita et al. 2001), purple sweet potato (Matsui et al. 2001), propolis (Matsui et al. 2004), leaf tea (Youn et al. 2004), fish protein hydrolysates (Matsui et al. 1999), and seaweeds (Kurihara et al. 1999; Nwosu et al. 2010; Zhang et al. 2007), also contain compounds that inhibit α-glucosidase activity.

The primary objective of this study was to investigate the impact of a single dose of 500 mg of InSea^2, a commercially available extract from brown seaweeds (*Ascophyllum nodosum* and *Fucus vesiculosus*) on the response to a standard test-food as measured by postprandial glycemia and insulinemia in men and women. This extract has been shown previously to inhibit in vitro activities of both α-amylase and α-glucosidase (Roy et al. 2011). We were also interested to document any side effects related to the single and acute use of this brown seaweed extract.

Material and methods

Subjects

Participants were recruited from the general population. Inclusion criteria were women and men aged between 18 and 60 years, nonsmokers, with a body mass index (BMI) between 20 and 30 kg·m^{-2}. Female volunteers of childbearing age were eligible if they were using contraceptive methods (oral contraceptives, condoms, etc.) for the duration of the study. Potential volunteers were excluded from the study if they were allergic to iodine, suffering from gastrointestinal problems, diabetes, thyroid dysfunction, liver dysfunction, or other diseases that could compromise their safety. Volunteers with untreated hypertension (systolic blood pressure (SBP) > 140 mm Hg and (or) diastolic blood pressure (DBP) > 90 mm Hg) as well as those with a SBP > 130 mm Hg and (or) DBP > 85 mm Hg along with ≥3 other cardiovascular risk factors according to the Canadian Hypertension Education Program (Canadian Hypertension Education Program 2009) were excluded from the study. Volunteers who had undergone major surgery (including surgery of the digestive tract) were also excluded. Breastfeeding or pregnant women as well as those using unrecognized contraceptive methods were not eligible. Volunteers consuming more than 2 alcoholic drinks per day or more than 9 alcoholic drinks per week for the duration of the project as well as those using natural health products were also excluded.

Seaweed product and placebo composition

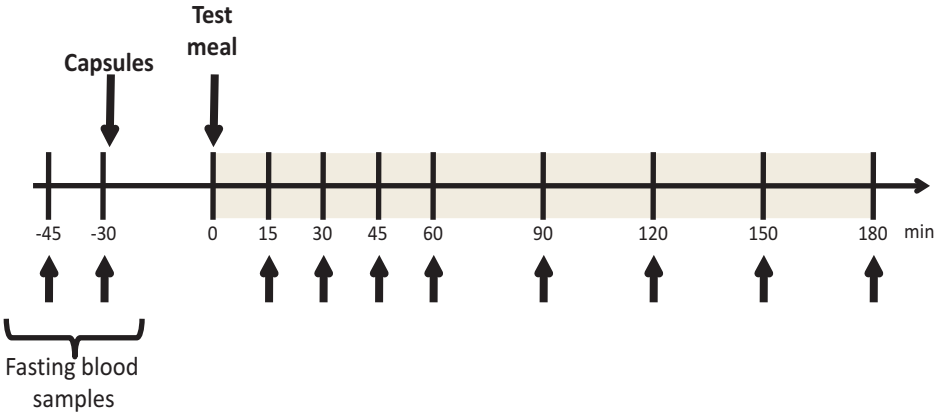
The brown seaweed extract under study was supplied by innoVactiv Inc. (Rimouski, Que., Canada) and is available commercially under the tradename InSea2. Typically, it is prepared from *A. nodosum* and *F. vesiculosus* following hot water extraction, a series of filtration and ultrafiltration, and completed by spray drying. The extract has been cleared of alginates and partially demineralized. The extract is characterized by a minimum of 10% polyphenols (chlorogenic acid equivalent) with the remaining constituents being mostly composed of fibers (algal polysaccharides) and minerals.

The test product was delivered as a 508-mg capsule comprising 250 mg of the brown seaweed extract, 100 mg of microcrystalline cellulose, 150 mg of calcium phosphate dibasic, 5 mg of magnesium stearate, and 3 mg of croscarmellose. The product was supplied by innoVactiv Inc. The placebo was provided in an opaque capsule containing 191 mg of microcrystalline cellulose, 287 mg of calcium phosphate dibasic, 25 mg of caramel (colour), and 5 mg of magnesium stearate.

Study design

The study was performed according to the International Conference on Harmonization guidelines for Good Clinical Practice; the Helsinki Declaration of 1975, as revised in 2000; and the Canadian Natural Health Product Regulations. The study protocol had been approved by the Research Ethical Board of Université Laval. The study was conducted between June and October 2009. This trial was registered at clinicaltrials.gov as NCT00936754. The study was performed using a randomized double-blind, placebo-controlled crossover design in which subjects participated in two 3-h meal tolerance tests that were taken 1 week apart. Assignment of treatment sequence was conducted via the use of random sequence of numbers. Subjects were instructed to avoid intense physical exercise 48 h before the experiment. Subjects came to the clinical investigation unit after a >12-h overnight fast. Anthropometric characteristics (weight, height), blood pressure, and heart rate were measured prior to randomization. Fasting blood samples were collected at –45 min and –30 min of the testing schedule (refer to Fig. 1 for timeline of samples collection). Immediately after these 2 blood draws, the capsules (test or placebo) were consumed and then, at 0 min, the test meal was consumed. Test meals were provided 30 min after the capsules were administered to give enough time for the capsules to dissolve. The test meal consisted of 50 g of available carbohydrates that was provided as 4 slices of white bread (110 g) served with water (maximum 500 mL). This test food had to be consumed within a period of 7 min. Blood samples were subsequently collected through a venous catheter from an antecubital vein at 15,

Fig. 1. Timeline for samples collection. The shaded area corresponds to the meal postchallenge period.



30, 45, 60, 90, 120, 150, and 180 min for the determination of plasma glucose and insulin concentrations. The averages of plasma glucose levels (and insulin levels) at time –45 and –30 min were used as the fasting (time "zero") value.

Volunteers were asked to refrain from using natural health products or drugs known to have inhibitory activities on α-amylase and α-glucosidase (such as acarbose, white bean extract, extracts of brown seaweed, etc.) during the entire period of the study, including a 2-week run-in period. One week after the first test, subjects came back to the Clinical Investigation Unit after overnight fasting and repeated the test protocol for the second treatment according to the randomization sequence.

Blood glucose and insulin analysis

Blood samples obtained before (–45, –30 min) and after ingestion of the test food (15, 30, 45, 60, 90, 120, 150, and 180 min) were used to determine plasma glucose and insulin concentrations. Glucose concentrations were determined enzymatically (Richterich and Dauwalder 1971), whereas plasma insulin was measured by radioimmunoassay with polyethylene glycol separation (Desbuquois and Aurbach 1971) as previously described (Piché et al. 2005).

Intolerance symptoms

Subjective tolerance scaled ratings of the frequency and intensity of side effects were obtained on site 3 h after the ingestion of the capsules. Side effects of interest were headache, anxiety, fatigue–exhaustion, lack of energy, tendency to become exhausted quickly, decreased appetite, increased appetite, hiccup, nausea, vomiting, indigestion, stomach or abdominal pain, constipation, diarrhea, flatulence, abdominal bloating, palpitations, balance disorders, decreased ability to concentrate, internal pressure, flushing, feeling cold, joints or members pain, numbness, burning or itching (feet–hands), and dark or depressing thoughts. Participants had to indicate whether each side effect was absent (0), of mild intensity (1), of moderate intensity (2), or of severe intensity (3). Subjects were also asked to report the frequency and intensity of these side effects during the 7-day period following each treatment, using the same scale.

Statistical analyses

The impact of the brown seaweed extract on postprandial glycemia and insulinemia compared with placebo was investigated using mixed models for repeated measures with the Statistical Analysis Software (version 9.2, 2008, SAS Institute Inc., Cary, N.C., USA). Treatment interactions were tested using appropriate terms in the mixed models. Glucose and insulin area under the curve (AUC) during the 3-h oral meal tolerance test was determined by the trapezoidal method. The incremental glucose and insulin AUC (IAUC) was calculated by subtracting the baseline values in the fasting state from the AUC. The Cederholm index provides an indication of insulin sensitivity based on an oral glucose tolerance test and anthropometric variables and has been show to correlate strongly with insulin sensitivity measured by the euglycemic–hyperinsulinemic clamp (Piché et al. 2007). In the present study, the meal tolerance test provided 50 g of available carbohydrates and this number was used for the calculation of the Cederholm index: [50000 + (fasting glucose – glucose at 2 h) × 1.15 × 180 × 0.19 × body weight]/[120 × log(mean insulin) × mean glucose] (Cederholm and Wibell 1990). Variables not distributed normally were log-transformed prior to analysis. The frequency and intensity of side effects between the 2 treatments was examined using Wilcoxon nonparametric test. Values of $p \leq 0.05$ were considered statistically significant. This study involving 23 subjects had 86% power to detect a 20% change in glucose response (IAUC) based on an estimated coefficient of variation of 30% at a level of $p < 0.05$.

Results

Baseline characteristics of subjects are presented in Table 1. Twenty-three volunteers (11 men and 12 nonpregnant, nonbreastfeeding women) were included in this study. There were no drop outs. Subjects were aged between 19 and 59 years (mean (SD): 39.9 (12.7) years), had a mean BMI of 24.9 (3.2) $kg \cdot m^{-2}$, and were not taking any medication. DBP and SBP were in a normal range. As a group, subjects also had a normal plasma lipid profile and glucose status.

Postprandial glucose response

No significant difference was observed between treatments for fasting plasma glucose concentrations prior to ingestion of the test foods (mean (SD): 5.55 (0.41) and 5.58 (0.46) $mmol \cdot L^{-1}$ for the brown seaweed extract and the placebo, respectively, $p = 0.45$). Plasma glucose concentrations did not differ between treatments at each postprandial time points

Table 1. Characteristics of subjects at baseline (N = 23).

Variable	Mean (SD)	Range
Subjects (male–female)	11–12	—
Age (y)	39.9 (12.7)	19.0–59.0
BMI (kg·m^{-2})	24.9 (3.2)	20.1–29.5
SBP (mm Hg)	108.5 (9.6)	91.5–130.0
DBP (mm Hg)	73.9 (7.3)	62.0–87.5
Total cholesterol (mmol·L^{-1})	4.80 (1.11)	2.90–7.00
LDL-cholesterol (mmol·L^{-1})	2.72 (1.08)	1.09–5.11
HDL-cholesterol (mmol·L^{-1})	1.60 (0.55)	0.96–3.13
Cholesterol/HDL-cholesterol	3.28 (1.25)	1.69–6.77
Total triglycerides (mmol·L^{-1})	1.04 (0.66)	0.37–3.50
Fasting blood glucose (mmol·L^{-1})	4.94 (0.37)	4.30–5.70

Note: BMI, Body mass index; DBP, diastolic blood pressure; LDL, low density lipoprotein; HDL, high density lipoprotein; SBP, systolic blood pressure.

(Fig. 2A) and no significant difference was seen in the glucose IAUC between brown seaweed and placebo (p = 0.24, adjusted for baseline, Fig. 2B). There was, however, a significant treatment × sequence interaction in the plasma glucose response to the test meal (p = 0.05). Consumption of the brown seaweed extract compared with placebo was associated with a lower plasma glucose AUC by 9.0% (mean (SD): brown seaweed: 1081.7 (148.5) mmol·L^{-1}·min^{-1}; placebo: 1188.4 (166.9) mmol·L^{-1}·min^{-1}) and IAUC by 48.3% (brown seaweed: 94.0 (136.5) mmol·L^{-1}·min^{-1}; placebo: 181.9 (142.2) mmol·L^{-1}·min^{-1}) in the group that received the placebo first. No such effects were seen in those who received the brown seaweed extract first. There was no difference in the glucose response to treatments between men and women (not shown).

Postprandial insulin response

Baseline-adjusted IAUC in plasma insulin was 12.1% lower with brown seaweed extract vs. placebo (mean (SD): brown seaweed: 279.1 (29.7) × 10^2 pmol·L^{-1}·min^{-1}; placebo: 317.5 (34.9) × 10^2 pmol·L^{-1}·min^{-1}, p = 0.04, Fig. 3B). However, there was no difference in plasma insulin values at each individual time points between the 2 treatments (Fig. 3A). Finally, brown seaweed extract treatment increased the Cederholm index by 7.9% (mean (SD): brown seaweed: 12.1 (3.1) %; placebo: 11.2 (2.4) %) when compared with placebo (p = 0.05). Again, responses were similar between men and women (not shown).

Side effects

The number of subjects who reported having experimented intolerance symptoms was the same between the 2 treatments (N = 13 in each treatment) and were of minimal intensity (1 on the arbitrary scale of 0 to 3) for most of the symptoms following ingestion. There was no significant difference between the brown seaweed extract and the placebo in the intensity or frequency of discomfort symptoms.

Discussion

We investigated in this randomized crossover, double-blind, placebo-controlled trial the impact of an extract from the brown seaweeds *A. nodosum* and *F. vesiculosus* with demonstrated α-glucosidase and α-amylase in vitro inhibiting activities (Roy et al. 2011) on post-load plasma glucose and insulin concentrations. Intake of the brown seaweed extract was associated with a small but significant reduction in post-load plasma insulin concentrations as well as with an increase in the Cederholm index compared with placebo, which suggests an apparent acute improvement in insulin sensitivity. The extract also tended to reduce plasma glucose concentrations after ingestion of the standardized carbohydrate load but this reduction did not reach statistical significance. Acute intake of the brown seaweed extract had no apparent side effect.

Fig. 2. Plasma glucose concentrations and incremental area under the curve (IAUC) during the 180 min following consumption of 50 g of carbohydrates 30 min after intake of brown seaweed extract or placebo. (A) Plasma glucose concentrations over time. The baseline value (0 min) is the mean of fasting samples obtained at –45 and –30 min prior to the test, before the ingestion of the test meal. (B) Mean IAUC of plasma glucose (brown seaweed: 135.0 ± 32.2 mmol·L^{-1}·min^{-1}; placebo: 170.7 ± 26.1 mmol·L^{-1}·min^{-1}). Data are means ± SE. Baseline values for each variable were included in the comparisons of the 2 treatments. Seaweed extract is represented by closed squares and placebo is represented by open triangles. P_{IAUC}, p value of the difference in IAUC between placebo and brown seaweed.

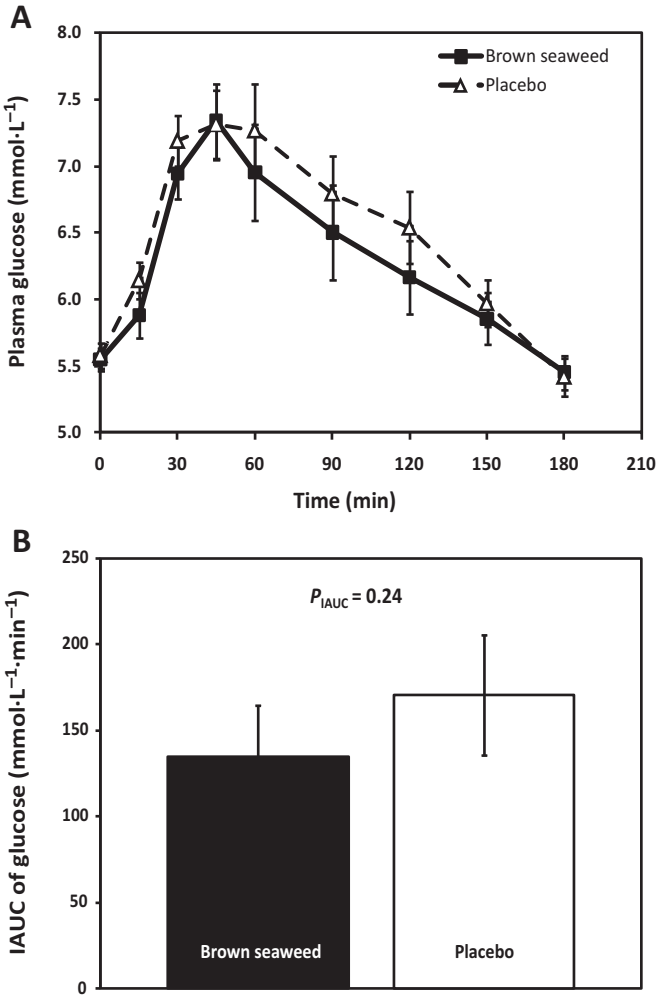
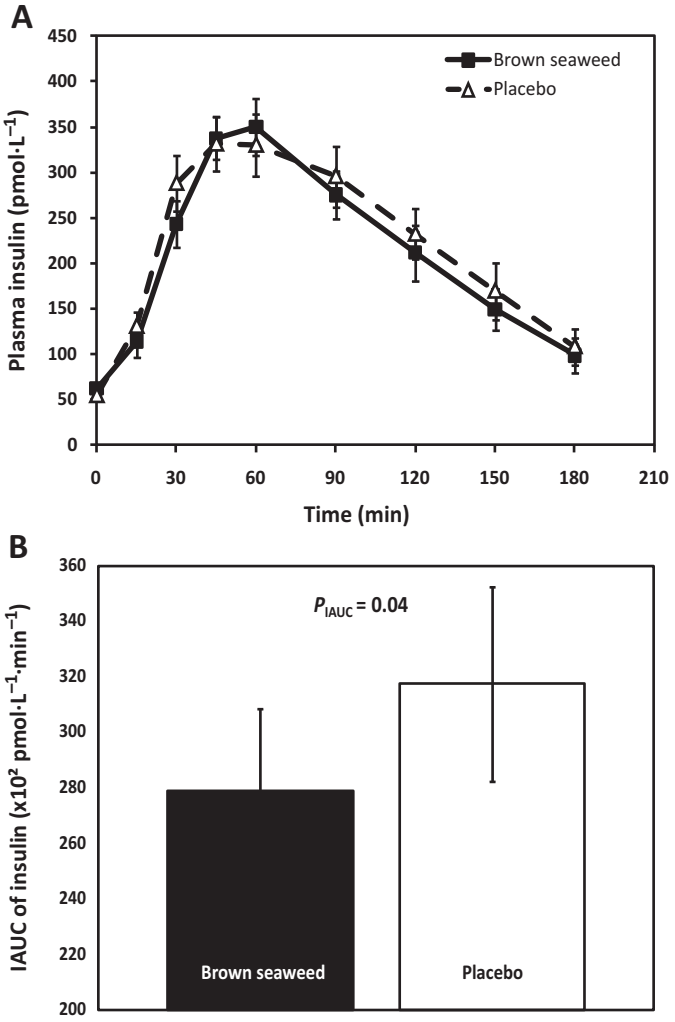


Fig. 3. Plasma insulin concentrations and incremental area under the curve (IAUC) during the 180 min following consumption of 50 g of carbohydrates 30 min after intake of brown seaweed extract or placebo. (A) Plasma insulin concentrations over time. The baseline value (0 min) is the mean of fasting samples obtained at –45 and –30 min, prior to the test and before the ingestion of the test meal. (B) Mean IAUC of plasma insulin (brown seaweed: 279.1 ± 29.7 × 10^2 pmol·L^{-1}·min^{-1}; placebo: 317.5 ± 34.9 × 10^2 pmol·L^{-1}·min^{-1}). Data are means ± SE. Baseline values for each variable were included in the comparisons of the 2 treatments. Seaweed extract is represented by closed squares and placebo is represented by open triangles. P_{IAUC}, p value of the difference in IAUC between placebo and brown seaweed.



Glycemia

Other studies have investigated the effect of food ingredients inhibiting α-glucosidase activity on glycemia in humans on reduction in post-load glycemia (Williams et al. 2007; Collene et al. 2005; Heacock et al. 2005). A reduction of 14%–24% of the plasma glucose IAUC has been reported in healthy individuals and in patients with type 2 diabetes for doses of *S. oblonga* ranging from 240 to 1000 mg (Williams et al. 2007; Collene et al. 2005; Heacock et al. 2005).

The results of the present study showed that the ingestion of 500 mg of brown seaweed prior to a carbohydrate load did not affect significantly plasma glucose levels in men and women. This absence of effect has been confounded by a significant treatment × sequence interaction. When each treatment sequence was analysed separately, brown seaweed extract reduced plasma glucose AUC and IAUC by –9.0% and –48.3%, respectively, compared with placebo in the group that received the placebo first. On the other hand, no effect was seen in participants who received the brown seaweed extract first. This sequence effect was unexpected as subjects were tested in the same conditions on the 2 occasions (testing at the same day of week and hour of day). Also, while subjects were instructed not to engage in strenuous physical activity 2 days prior to the carbohydrate load tests, this was not recorded. Our study is also limited by the fact that the participants' diet prior to the testing of brown seaweed extract and placebo was not controlled. This may have interfered with the test and may explain the significant treatment × sequence interaction on the blood glucose response to the brown seaweed extract. The possibility of a carry-over effect of the brown seaweed extract on plasma glucose in the group that received this treatment is unlikely given the design of the study and the nature of the product tested. Nevertheless, we conclude that the acute impact of the brown seaweed extract on post-load plasma glucose concentrations are mitigated and need further investigation.

Insulinemia

Interestingly, consumption of brown seaweed extract significantly reduced postmeal insulin concentrations and improved a surrogate marker of insulin sensitivity, the Cederholm index. The Cederholm index is a validated index of insulin sensitivity derived from postload glucose and insulin concentrations after an oral glucose tolerance test and using anthropometric variables in its definition (Piché et al. 2007). It is very unlikely that a single dose of this extract modulated systemic insulin sensitivity to glucose. Rather, we suggest that the absorption rate of dietary carbohydrates within the gut may have been altered enough by the consumption of the brown seaweed extract to require less insulin for its subsequent processing within the plasma compartment and that these changes are particularly apparent when plasma glucose and insulin changes are integrated into an index of insulin sensitivity. However, as AUC of glucose were not different between brown seaweed and placebo, this hypothesis has to be further investigated. Other groups have reported positive effects of α-glucosidase inhibitors on insulinemia. For instance, in healthy volunteers, *S. oblonga* (an α-glucosidase inhibitor) consumed after a meal tolerance test reduced insulin IAUC over 120 min at doses ranging from 240 to 1000 mg (Williams et al. 2007; Collene et al. 2005; Heacock et al. 2005). It will be of interest in the future to investigate the impact of brown seaweed extract when used chronically rather than on a single occasion on glucose–insulin homeostasis in healthy but also in prediabetic subjects as well.

Tolerability

α-glucosidase inhibitors such as *S. oblonga* (Williams et al. 2007) and acarbose (Wolever et al. 1998) have been associated with gastrointestinal side effects possibly because of fermentation of undigested carbohydrates in the bowel (Mertes 2001). It was of interest to observe that a relatively

minimal dose of α-glucosidase and α-amylase inhibitors from a brown seaweed extract was not accompanied by gastrointestinal intolerance or discomfort.

Conclusions

In summary, this study indicates that consumption of a brown seaweed extract with demonstrated in vitro α-glucosidase and α-amylase inhibitory activities (Roy et al. 2011) modulates insulin homeostasis after ingestion of a carbohydrate-rich meal in men and women. Future studies are warranted to investigate further different doses as well as the chronic impact of these compounds in healthy as well as in prediabetic and diabetic subjects.

Contribution

B.L. and P.C. designed this study. P.C. was responsible for the screening and medical supervision of the study participants. M.E.P. and B.L. participated in analyses and interpretation of data, and drafting and revision of the manuscript, which was reviewed critically by all authors.

Competing interests

The authors declare that they have no competing interests.

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