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The brown algae *Fucus vesiculosus* and *Ascophyllum nodosum* reduce metabolic syndrome risk factors: a clinical study

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Fucus vesiculosus and *Ascophyllum nodosum* have been traditionally used for the treatment of obesity and several gastrointestinal diseases. We have recently demonstrated that the phytocomplex obtained from these algae (Gdue™) controls postprandial glucose levels in a mouse model of steatohepatitis, a condition often associated with obesity and type 2 diabetes mellitus. We analyzed the effect of Gdue™ on HOMA index, waist circumference, fasting blood glucose and insulin levels in overweight or obese subjects.
Waist circumference decreased significantly after 6 months of treatment (112 ± 17 at t_0 vs 105 ± 13 cm after 6 months of treatment; $p<0.0001$). Both blood glucose and insulin levels were significantly reduced after 6 months of treatment with Gdue™ (110 ± 15 at t_0 vs 98 ± 15 mg/dL after 6 months for glucose; $p<0.0001$; 22.6 ± 9.5 at t0 vs 17.8 ± 8.6 μU/mL after 6 months for insulin; $p<0.05$). Accordingly, HOMA index decreased significantly (6.103 ± 2.548 at t_0 vs 4.419 ± 2.382 after 6 months; $p<0.01$), suggesting an improvement of insulin sensitivity status. This phytocomplex represents a useful dietary supplement for controlling relevant metabolic syndrome risk factors, such as waist circumference, fasting insulin and glucose levels.

Keywords: *Fucus vesiculosus*, *Ascophyllum nodosum*, metabolic syndrome, overweight subjects, fasting blood glucose.

Edible seaweeds, an easily available food source, have been consumed by coastal communities since the dawn of times, especially in Asia [1]. Several studies reported that the consumption of seaweed fibers leads to a significant reduction of metabolic diseases, such as diabetes and obesity [2,3]. We recently demonstrated that a phytocomplex containing polysaccharides, polyphenolics, and fatty acids, obtained from the brown algae *Fucus vesiculosus* and *Ascophyllum nodosum* by water extraction controls postprandial plasma glucose levels in a mouse model of non-alcoholic steatohepatitis (NASH) [4]. This liver disease, which represents the most serious form of non-alcoholic fatty liver disease (NAFLD), is present in 25–30% of the patients affected by obesity or type 2 diabetes mellitus (T2DM) and more than 35% of severely obese patients who have T2DM [5–7]. NAFLD has been associated to metabolic syndrome (MetS) by a number of clinical and epidemiologic studies (reviewed by Asrih et al. [8]), and also to insulin resistance, which are in turn strongly associated with T2DM and abdominal obesity, representing pivotal pathogenic factors.

The presence of multiple metabolic abnormalities is significantly associated with the severity of liver disease. Physicians share now the opinion that only a behavioral approach to lifestyle correction will address the complex interplay of alterations characterizing the MetS, including metabolic liver disease. Indeed, the pivotal role of nutrition has been accepted for the treatment of MetS, based on the hypothesis that an appropriate intake of energy and nutrients can improve its control. It has been reported that the inhibition of the two intestinal enzymes α-amylase and α-glucosidase, which are both involved in carbohydrate digestion, can significantly lower the increase of blood glucose levels, after a mixed carbohydrate meal, by delaying glucose absorption. Therefore, α-glucosidase inhibitors, such as acarbose and voglibose, are currently used for the treatment of T2DM. It has been demonstrated by our group and others that *Fucus vesiculosus* and *Ascophyllum nodosum* act by inhibiting these two digestive enzymes, and that the inhibitory potencies of these algal mixtures on both enzymes are even higher than that of acarbose [4,9]. Furthermore, by means of an *in vivo* animal study, we demonstrated that this extract is able not only to delay but also to reduce glucose absorption, and this effect is particularly evident in mice fed with a high-fat diet [4].

On the basis of these considerations, in this study we analyzed the effect of a six-month administration of a commercial extract (Gdue™) of *Fucus vesiculosus* and *Ascophyllum nodosum* on HOMA index, waist circumference, fasting blood glucose and insulin levels in 50 overweight or obese subjects. The objective of this study was to ascertain whether these seaweed extracts may be useful for the control of MetS risk factors.

Fifty patients (18 men and 32 women) were enrolled in this study. The age range was broad (54 ± 12 years, min=28 y, max=79 y). Notably, 18 patients suffered from hypertension, 8 from thyroid disorders, and 6 were obese. No adverse effects due to the administration of the algal extract were noticed during this study.

Fasting blood glucose levels were significantly reduced after 3 months ($p<0.05$), and this drop reached an even higher statistical significance ($p<0.001$) after 6 months of treatment with Gdue™ (110 ± 15 at time 0 vs 103 ± 12 and 98 ± 15 mg/dL after 3 and 6 months, respectively). As shown in Fig. 1A, the mean of the fasting blood glucose levels of the patients was higher than the normal upper limit (99 mg/dL) before the treatment and after 3 months, whereas it reached the normal range (97.43 mg/dL) at the end of the study. Figure 1B reported the scatter plot of fasting blood glucose at the 3 time points analyzed in this study. One outlier could be identified, whose fasting glucose levels have been much higher than that of the other subjects for the entire study.
Fasting blood insulin levels were significantly reduced after 3 and 6 months of treatment with Gdue™ (22.6 ± 9.5 at time 0 vs 18.8 ± 9.3 and 17.8 ± 8.6 μU/mL after 3 and 6 months, respectively, for insulin levels; $p<0.05$). At variance with glucose levels, mean blood insulin was in the normal range since t_0 and decreased significantly with the administration of Gdue™ (Fig. 1C). The scatter plot revealed the presence of two outliers with particularly high insulin levels at the three considered time points (Fig. 1D).

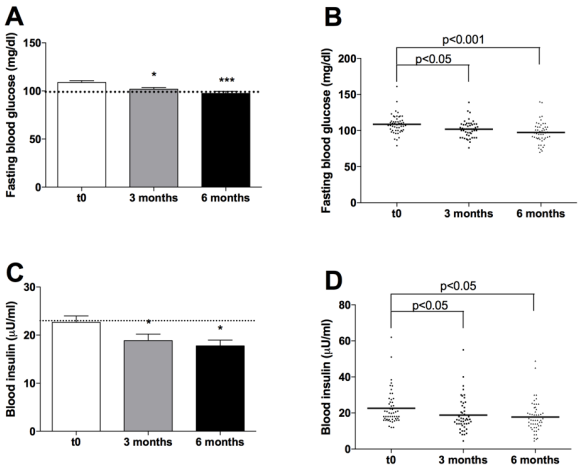

Figure 1: Fasting blood glucose (A) and insulin (C) levels before and 3 and 6 months after the treatment with *Fucus vesicolosus* and *Ascophyllum nodosum*. Data are reported as mean ± SEM. Scatter plot of glucose (B) and insulin (D) levels of the 50 subjects enrolled in this study. The horizontal bars represent the mean. * $p<0.05$ and *** $p<0.001$ vs t_0.

In order to evaluate the insulin resistance of the subjects enrolled in this study, and the effect of Gdue™ on this MetS risk factor, we calculated HOMA index, which represents a validated diagnostic index of insulin resistance (Wallace et al., 2004). Fig. 2A shows that all the subjects enrolled in this study had insulin resistance before the treatment with Gdue™, since the mean value of their HOMA index was 6.103. HOMA index decreased significantly after 3 and 6 months of algae administration (4.857 ± 2.655 and 4.419 ± 2.382 after 3 and 6 months, respectively vs 6.103 ± 2.548 at the beginning of the study), suggesting an improvement of insulin sensitivity status. In particular, when considering normal a HOMA index of 3 or lower, as suggested by some studies ([10] and refs. therein), none of the subjects enrolled in this study displayed this characteristic before the treatment, while 18% and 22% of them reached the normal range after 3 and 6 months of treatment, respectively (Fig. 2B).

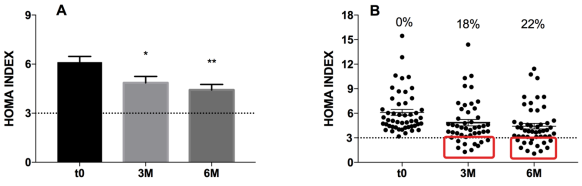

Figure 2: (A) HOMA index before and 3 and 6 months after the treatment with Fucus vesicolosus and Ascophyllum nodosum. Data are reported as mean ± SEM. (B) Scatter plot of the 50 subjects enrolled in this study. The horizontal bars represent the mean.
* $p<0.05$ and ** $p<0.01$ vs t_0.

In order to evaluate whether the effect of Gdue™ treatment on fasting blood glucose, insulin and HOMA index depends on the patients' characteristics, we calculated the difference between the values of these three parameters at the end and the beginning of the treatment and ascertain whether these differences correlate with their initial values. As clearly shown in Fig. 3, there is a significant negative correlation between the drop of all these three parameters and their initial values, meaning that the higher they were at the beginning the most they dropped during the treatment. In particular, the Pearson coefficients are -0,4795 (p=0.0003, Fig. 3A) for blood glucose, -0,4962 (p=0.0002, Fig. 3B) for blood insulin and -0.3781 (p=0.0043, Fig. 3C) for HOMA index. As shown in Fig. 3, the 75%, 86% and 94% of the subjects had a drop in their fasting blood glucose blood insulin and HOMA index, respectively, at the end of the treatment with Gdue™ with respect to the initial values.

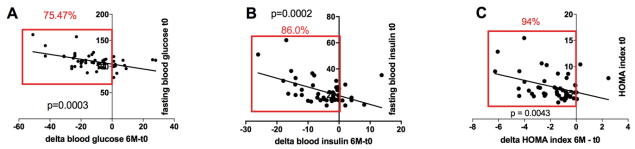

Figure 3: Pearson correlation analysis of the difference between the values of fasting blood glucose (A), fasting blood insulin (B) and HOMA index (C) at the end and the beginning of the treatment and their initial values.

The analysis of waist circumference was performed separately for male and female subjects, since the upper limits indicated by the World Health Organization [11] are different, i.e. 102 cm for men and 88 cm for women (Fig.4). As far as regards male subjects, Fig. 4A shows that mean waist circumference significantly decreased after 6 months of treatment (115 ± 10 vs 104 ± 13 cm; p=0.0021), getting quite close to the recommended upper limit (102 cm). The waist circumference of female subjects significantly dropped after 6 months of treatment (110 with Gdue™ as shown in Fig. 4C), although the mean value at the end of the study was still much higher than the recommended upper limit (105 cm vs 88 cm). No correlation between the drop of male and female waist circumference and their initial values could be observed (data not shown).

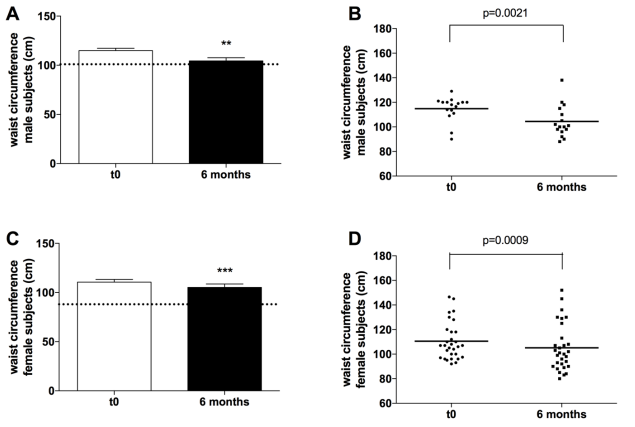

Figure 4: Waist circumference of male (A) and female (C) subjects before and 6 months after the treatment with Fucus vesicolosus and Ascophyllum nodosum. Data are reported as mean ± SEM. Scatter plot of the 18 men (B) and 32 women (D) enrolled in this study. The horizontal bars represent the mean. ** $p<0.01$ and *** $p<0.001$ vs t_0.

Preclinical and clinical studies have already demonstrated that the algal extracts obtained from *F. vesiculosus* and *A. nodosum* can reduce and delay glucose absorption by inhibiting digestive enzymes such as α-glucosidase and α-amylase [12–14]. We already reported that the inhibitory effect on the two digestive enzymes is due to the high content of two different bioactive compounds in the algal extract, such as phlorotannins (PHTs) and fatty acids. Their presence was confirmed by the fingerprint analysis of Gdue™ which was described in our previous study [4]. These two classes of compounds probably display a synergistic inhibition on α-glucosidase and α-amylase, thus increasing their pharmacological effect, as suggested by previous findings based on HR-bioassay and HPLC-HRMS-SPE-

NMR analyses [4 and refs. therein]. In particular, we recently demonstrated that this effect is particularly evident in mice fed with a diet particularly rich in fat, in which the administration of this phytocomplex is associated not only with a delay in carbohydrate digestion, but also with a significant drop in their assimilation. These results lead us to the hypothesis that these algal extracts can be used as dietary supplements, which can be administered before a meal with the aim to slow down the rate of carbohydrate digestion and assimilation, thereby changing the glycaemic response to high glycaemic index (GI) foods into one typical of foods with lower GI. This takes on importance since there is a theory stating that MetS is a consequence of an elevated intake of high GI foods [15]. A high GI diet has been associated with high blood glucose and insulin resistance, and other clinical abnormalities typical of MetS [16].

On the basis of these considerations, we designed this observational study in order to ascertain whether the phytocomplex obtained from these two algae and added with chromium picolinate might help in reducing MetS risk factors and is therefore useful in the clinical management of this condition. A previous clinical study analyzing the effect of the same algal extract without the addition of chromium picolinate [13] demonstrated that the acute administration of this brown seaweed extract was able to modulate insulin homeostasis after ingestion of a carbohydrate-rich meal. In particular, Paradis and collaborators observed an improvement of the Cederholm index, a surrogate marker of insulin resistance, calculated on the basis of both post-load glucose and insulin blood concentrations after an oral glucose tolerance test and anthropometric variables [17]. These authors concluded that a single dose of this extract could not modulate systemic insulin sensitivity to glucose and suggested to investigate the effect of the chronic administration of algal extracts on insulin concentration and resistance. Thanks to the results obtained in the present study, we have the possibility to add new information about the chronic effect of the extract obtained from these two algae, since we observed the consequences of a prolonged administration (6 months) on 4 risk factors of MetS, such as fasting blood insulin and glucose, insulin resistance (evaluated by HOMA index), and waist circumference.

To our knowledge, the effect on blood glucose and insulin of brown seaweed inhibiting α-glucosidase has so far been investigated in both pre-clinical and clinical studies only after a meal tolerance test [4,13,18–22]. In the present study, we demonstrated that the chronic administration of *F. vesicolosus* and *A. nodosum* has an impact on glucose–insulin homeostasis in overweight and obese subjects, thereby reducing the risk of the development of MetS. Interestingly, the chronic treatment with Gdue™ also lead to a drop of waist circumference in the study group, indicating that most of the subjects (i.e. 88% of men and 77% of women, data not shown) lost weight at the end of the 6-month treatment. Among the different nutritional approaches which have been recommended for the prevention and/or the management of MetS, it has been observed that low carbohydrate diets might be more effective than low fat diet in resolving MetS [23,24]. In this view, reducing carbohydrate digestion and absorption represents a valid and safe strategy, also considering the absence of adverse reactions observed in this study.

Although the number of subjects enrolled in this study is limited, our data clearly demonstrate the significative effect of the treatment. A wider study, including patients suffering from different diseases linked to the development of MetS would allow a multivariate analysis, in which the differential effect of the algal extract in different pathological conditions could be exploited. Furthermore, in this study a control group of healthy not overweight subjects was not included because, as already mentioned, several clinical studies have already analyzed the effect on algal extracts inhibiting α-glucosidase on glucose absorption in healthy subjects, and a chronic administration, as the one used in this study, should be recommended to subjects with high body weight and risk factors for MetS.

In conclusion, our data indicate that the algal extract obtained from *F. vesiculosus* and *A. nodosum* is a dietary supplement which can be useful for the management of metabolic syndrome risk factors in overweight or obese subjects.

Experimental

Study population: Fifty subjects participated in the study. The participants were recruited from general population by 6 general practitioners (listed in the acknowledgements). Inclusion criteria were women and men aged between 18 and 60 years, not-smokers, overweighed or obese (44 and 6 subjects, respectively). Woman of fertile age were considered eligible if using contraceptive methods for the entire duration of the study. Breastfeeding or pregnant women as well as those using unrecognized contraceptive methods were not eligible. This study has been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki and its later amendments.

Algal extract: The brown seaweed extract used in this study was supplied by Aesculapius Farmaceutici srl (Brescia, Italy) and is commercially available under the trade name Gdue™. Gdue™ is a food supplement containing InSea2® (a phytocomplex extracted from *A. nodosum* and *F. vesiculosus,* titrated in polyphenols to 20%), a registered trademark of InnoVactiv Inc. (Rimouski, Que., Canada) added with Chromium picolinate. The extract was prepared from the two brown algae, using a proprietary hot-water extraction, followed by filtration and ultrafiltration processes, and completed by spray-drying; its chemical composition has been characterized and described in detail [4].

Study design: The study was conducted between April and December 2016. Subjects came to the clinical investigation unit after a >12-h overnight fast (day 1). After the first measurement of waist circumference, fasting blood samples were collected to measure plasma glucose and insulin levels. At day 1, the subjects started to assume a hard capsule of Gdue™ three times a day before breakfast, lunch and dinner, and the administration of this food supplement was carried on for 6 months. Each hard capsule contains 237.5 mg of *Ascophyllum nodosum* extract, 12.5 mg of *Fucus vesiculosus* and 7.5 µg of chromium. After 3 months, fasting plasma glucose and insulin were measured in all subjects, waist circumference and fasting glucose and insulin levels were measured at the end of the study (6 months). HOMA-IR was computed with the formula fasting plasma glucose (mg/dL) times fasting serum insulin (mU/L) divided by 405.

Statistical analysis: Statistical analyses were performed by means of GraphPad Prism 7.0 (GraphPad Software Inc., San Diego, CA, USA) and SPSS (release 18.0.0; SPSS inc, Chicago, Illinois) software. Unless otherwise stated, the data are presented as means ± S.E.M. A comparison of the experimental data obtained at the different time point of the study was made by one-way analysis of variance (ANOVA), followed by the Newman-Keuls post-hoc test, or by the 122 Student's t-test, when appropriate. Since normal distribution of data could not be rejected, Pearson r was calculated for correlation studies. $p<0.05$ was considered statistically significant.

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