Kuroshio Science **7**-1, 17-29, 2013

Anti-diabetic Effects of Algae with a Sustainable Society in View: In Focus on Regulatory T Cells and the Flora of Intestinal Bacteria

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Abstract

Due to the lifestyle changes caused by mechanization and urbanization, high nutrient availability and an energy dense diet along with decreasing physical activity levels have predisposed people to obesity and type 2 diabetes. This drastic change of life style in Asia also increased the onset of allergic diseases, and changed the type of tumors. For example, the world prevalence of diabetes has been reported on and it is estimated that more than 400 million adults will suffer from diabetes by 2030. Health organizations also reported that the prevalence of type 2 diabetes has reached epidemic levels in Asia and that this epidemic threatens to overwhelm health-care systems in the region. It has been suggested that certain changes in lifestyle can be effective in the prevention of type 2 diabetes, especially for those with impaired glucose tolerance. This is also true for allergies and tumors.

In Asian countries, various species of algae have traditionally been considered as foods good for health. In Japan, for example, the *Ecklonia* species have been thought to improve the properties of blood. The precise function of these algae, however, has not been well analyzed. Here, we have focused on the following two topics.

1) The regulatory effect of algae on the development of type 2 diabetes. At first, we examined the effects of various algae on the blood glucose level in leptin receptor deficient *db/db* mice and found that gametophytes of *Ecklonia kurome* (*E. kurome*) down-regulate blood levels of glucose and triacylglycerol. Sporophytes of *E. kurome* did not have any effects on these levels. Since we administered the algae homogenates orally, we examined the bacterial flora in the intestine by using 16S rRNA nucleotides as a probe. We found the increase of *Lactobacillus* species and *Bacteroides* species in the mice administered with gametophytes of *E. kurome*. Interestingly, we found that regulatory T lymphocytes (Treg) had decreased in the mice administered with gametophytes of *E. kurome*.

2) We also examined the effect of gametophytes of *E. kurome* in wild type mice. We established prediabetic status in C57BL/6J by administrating a high fat diet (HFD) containing 32% fatty acids. We found that gametophytes of *E. kurome* down-regulated the blood glucose in prediabetic C57BL/6J wild type mice. We found that a cytokine, interferon-γ (IFN-γ) involved in the thermogenesis is also engaged in the regulation of blood levels of glucose and triacylglycerol. We also noted that Treg cells had increased in the absence of interferon-γ. Although we found that both gametophytes and sporophytes of *E. kurome* down-regulated the Treg cells in prediabetic mice of wild type strain, only gametophytes of *E. kurome* down-regulated the blood glucose in pre-diabetic wild type mice. This result suggests that Treg cells are down-regulated by substances contained in both sporophytes and gametophytes of *E. kurome* and that the level of blood glucose is not simply controlled by Treg cells. We summarize the recent advances in the area of regulation of immune responses by edible algae in view of a sustainable society.

Key words: edible algae, sustainable society, diabetes, Treg cells, intestinal flora, db/db mice, IFN-γ KO mice

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1. Introduction

Modern humans, *Homo sapiens* drove *Homo Neanderthalensis* to extinction, but retained a trace amount of their DNA in our genome. It has been hypothesized that about 100,000 years ago, human beings subsisted on as many as 300 species of plants, similar to modern primates today, such as chimpanzees and gorillas (Engel, 2002). Gradually, humans narrowed down the species of plants for their diet. Regarding crops, we now largely rely on several species such as corn, wheat, rice, and millet. Humans' life style changed drastically after the Industrial Revolution. It is estimated that ordinary people used to walk 30,000 steps per day during the Edo period (1603~1867) while people in Japan only walk 5000 to 10,000 steps nowadays. We are consuming larger amount of animal fats and higher calorie diets compared with human in the Edo period. This high calorie consumption and decreased level of physical activity have caused life-style related diseases such as cancer, cerebrovascular disease, cardiovascular disease, obesity, diabetes, and arteriosclerosis. A hundred years is too short to introduce changes in the genome of *Homo sapiens*.

An increase of patients with allergies has also been caused by the drastic changes in the environment. Indeed, allergic patients are more frequently found in northern Europe than in southern Europe. Lipopolysaccharides (LPS) from *Escherichia coli* are more abundant in the atmosphere in southern Europe. LPS is known to stimulate macrophages to induce T lymphocytes to produce IFN-γ that suppresses the production of IgE which triggers allergic reactions.

In modern society, huge amounts of efforts are taken to develop medicines, resulting in the high medical expenses. Not all people can afford to purchase the medicines developed by pharmaceutical companies. In Asian countries, algae have traditionally been considered as a food for health. In Japan, algae is believed to reduce blood pressure or to ameliorate atherosclerosis. In particular, it is believed that *Ecklonia kurome* (*E. kurome*) has been to improve the circulation of blood. In this paper, we have focused on the anti-diabetic effect of algae found in the island where we live, Shikoku Island.

In this review, we focused on the usage of edible algae to ameliorate diseases such as allergies, cancers, and diabetes. We focus on treating diabetes, in particular using leptin receptor-deficient *db/db* mice as a model for type 2 diabetes. We think that it is beneficial for fishermen's villages to introduce the cultivation of algae, because it results in financial support for the marine protected areas. For this purposes, we have tried to find the usefulness of algae for human health.

2. Gametophytes of *Ecklonia kurome* are effective to decrease the blood glucose level of type 2 diabetes mice.

Among the life-style related diseases, it is estimated that more than four hundred million adults will suffer from diabetes by 2030 (Table 1., Shaw *et al.,* 2010). Particularly in Asia, it has been reported that the prevalence of type 2 diabetes has reached epidemic levels (Yoon *et al.,* 2006). Due to the lifestyle changes caused by motorization, urbanization, and the subsequent decrease in physical activity combined high nutrient

Table 1. Prevalence of diabetes and estimated of diabetes numbers among adults.

	Adults with diabetes 2010 (x1000)	Adults with diabetes 2030 (x1000)	Annual increment Mean (x1000)
China	43,157	62,553	970
India	50,768	87,036	1,813
Indonesia	6,964	11,980	251
Japan	7,089	6,879	-11
Malaysia	1,846	3,245	70
Philippines	3,398	6,164	138
Taiwan	816	1,232	21
Vietnam	1,647	3,415	88
Australia	1,086	1,503	21
France	4,164	5,201	52
Germany	7,494	8,014	26
Russian Federation	9,625	10,330	35
Spain	2,939	3,866	46
USA	26,814	35,958	457

Shaw, J.E., Sicree, R.A., Zimmet, P.Z. 2010. Diabetes Research and Clinical Practice, 87, 4-14.
Adults mean aged 20-79.

availability as part of an energy dense diet, a larger percentage of the population has become predisposed to both obesity and type 2 diabetes (Yoon *et al.,* 2006 and Peng *et al.,* 2011). Table 1 shows a prediction of the increase in diabetes by 2030 in Asian countries compared with Europeans (Shaw *et al.,* 2010). There are some differences at the genomic level between Asians and Europeans. One of the major differences between Asians and Europeans is the production of insulin. It is known that Europeans produce 3 to 5 times more insulin than Japanese. Many Asians become diabetic when they become obese. One exception is Mongolians, because they are not predisposed to diabetes even though they have a high body mass index (data not shown). Mongolians have become more adapted to a meat and high fat diet they over a 20,000 years or longer time period, and this is reflected in their genome. In contrast, the genome of many Asian people has adapted to their high carbohydrate diet. Pima Indians who immigrated to North America have a high frequency occurrence of diabetes after changing their food from carbohydrates to high fat diet (Boyce *et al.,* 1993).

Fig. 1 shows the interrelation between fats, carbohydrates, and proteins (Greenstein and Wood, 2011). *Homo sapiens* were out of Africa about 100, 000 years ago and lived in an age when it was a constant struggle to obtain enough food. Although there are several hormones that increase the blood glucose level such as glucagon, adrenalin, glucocorticoid and growth hormone, there is only one hormone, insulin that decreases the blood glucose. This fact probably suggests that humans had a constantly low level of blood glucose at the time when their genome was established. We focused on two molecules in this review. One is leptin, which is secreted from fat tissues and stimulates the hypothalamic arcuate nucleus to suppress appetite and increase the consumption of energy. Leptin production is induced by eating, glucocorticoid, insulin, TNFα (a pro-inflammatory cytokine secreted from macrophages), IL-1 (interleukin 1, an pro-inflammatory cytokine secreted from macrophages and dendritic cells), and neuropeptide Y. Leptin production is suppressed by fasting, coldness, thiazolin derivative, catecholamine, and cAMP.

Leptin is encoded by *ob* (obese) gene and mutations of this gene cause obesity. A mutant of leptin receptor gene (*db/db*: BKS.Cg-+*Lepr*^{db}/+*Lepr*^{db}/Jcl) provides a very good model of type 2 diabetes (Chen *et al.,* 1996, Hummel *et al.,* 1996, Lee *et al.,* 1996). Since this mouse eats twice as much as wild type mice, its body weight is two-fold heavier than wild type mouse. The body weight of *db/db* mice are from 50 to 60 g and they do not move as wild type mice do. Their condition is similar to that of

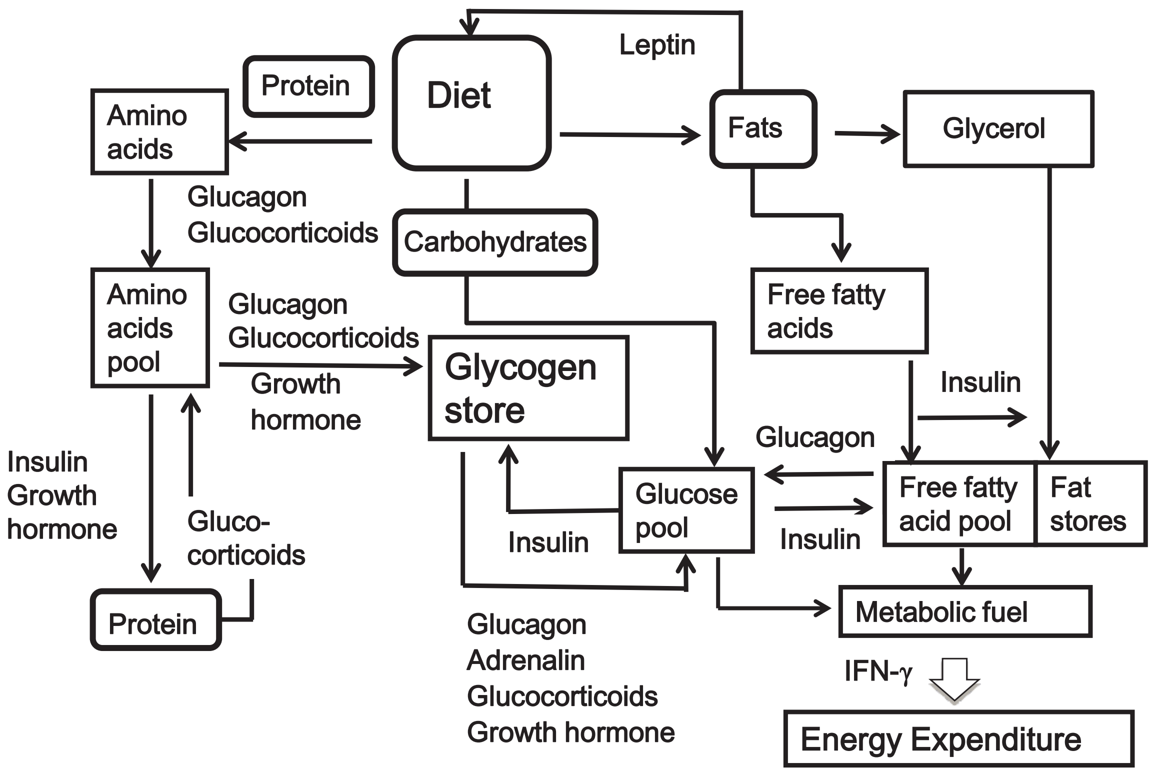

Fig. 1. Metabolic relations among fatty acids, carbohydrates, proteins.
Modified from reference by Greenstein B. and Wood, D., 2011.

humans afflicted with the life-style related disease, type 2 diabetes. Leptin stimulates the energy consumption by inducing IFN-γ-secreting helper T lymphocytes (type 1 helper T), Th1 (De Rosa *et al.,* 2007). IFN-γ is a cytokine that has anti-cancerous and anti-infection effects, and is involved in thermogenesis.

Here, we started administration of algae homogenates after the blood glucose level of *db/db* mice reached the 500 mg/dL (blood glucose level of wild type mice are 90-140 mg/dL). We administered 10 mg of algae homogenate in 0.2 mL of distilled water orally every other day using a capillary. The algae we administered were *Spirulina pacifica*, *Ulva meridionalis* (Horimoto *et al.,* 2011), gametophytes and sporophytes of *Ecklonia kurome*, and an unidentified filamentous blue green alga. Fig. 2 shows the levels of blood glucose of *db/db* mice ten weeks after starting administration of these algae homogenates (Fig. 2A, B). Administration of gametophytes but not sporophytes of *E. kurome* decreased the blood glucose level of *db/db* mice.

3. Gametophytes of *Ecklonia kurome* increased the *Lactobacillus* and *Bacteroides* species in the large intestine of type 2 diabetes mice.

Since algae homogenates were administered orally, we examined the bacterial flora of intestines of *db/db* mice. We examined the group of bacteria using 16S rRNA as a probe. At first, we measured the total number of bacteria. Although it varies from140 to 180 x 10^8/g, there is no significant difference in the contents of the large intestines between any combination of groups administered with algae homogenates or extracts (Fig. 3A, D). We compared numbers of *Lactobacillus* species among groups administered with different algae homogenate (Fig.3B, E). Mice administered with gametophytes of *E. kurome* had a higher level of *Lactobacillus* species than other groups. We also found an increased level of *Bacteroides* species in the gametophytes of *E. kurome*-administered group compared with the control group (administered with DW, Fig. 3C, F).

4. Both gametophytes and sporophytes of *E. kurome* decreased Treg cells in type 2 diabetes mice.

Next, we examined the level of regulatory T (Treg) cells, which are reportedly induced to proliferate by leptin (De Rosa *et al.,* 2007). We found that *E. kurome* decreased the Treg cells. By analyzing 50,000 peripheral blood lymphocytes pooled from each group, we found an interesting feature of lymphocyte subpopulation in the above experiment using *db/db* mice. The percentage of Treg cells ($CD4^+CD25^+$ T cells) in peripheral blood lymphocytes of the group administered with gametophytes of *E. kurome* was lower than that of the DW group (Table 2, Exp. 1). Next, we asked if gametophytes of *E. kurome* were effective in down-regulating blood glucose in pre-

Table 2. Effects of algae on the Treg cells.

Exp. 1. Percentages of $CD4^+CD25^+$ T cells among total lymphocytes in *db/db* mice.

	No treat	DW	*E. kurome* G	*Ulva* sp.2	Fil. Alga	*S. pacifica*
$CD4^+CD25^+$ T cells (%)	3.01	2.73	1.94	2.41	2.52	3.21

No treat: without any oral administration. In experiments described in Fig. 2A, two weeks before the sampling of sera, lymphocytes were collected from peripheral blood of each mouse. Lymphocytes pooled from each group were stained with PerCP-anti-CD4 Ab and FITC-anti-CD25 Ab, and fifty thousand cells were analyzed. *Ulva* sp.2: *Ulva* meridionalis, Fil. Alga: filamentous blue-green alga, *E. kurome* G: *Ecklonia kurome* gametophytes.

Exp. 2. Percentages of $CD4^+CD25^+$ T cells among total lymphocytes in C57BL/6J mice

	Group 1 ND (CE-2)	Group 2 HFD + DW	Group 3 HFD + gametophytes *E. kurome*	Group 4 HFD + sporphytes *E. kurome*	Group 6 HFD + *Porphyra* sp
$CD4^+CD25^+$ T cells (%)	2.93	2.76	2.50	2.54	3.14

Exp. 3. Percentages of $CD4^+CD25^+$ T cells among total lymphocytes in IFN-γ KO mice.

	Group 7 ND (CE-2)	Group 8 HFD + DW	Group 9 HFD + gametophytes *E. kurome*
$CD4^+CD25^+$ T cells (%)	4.02	3.09	2.13

Exp. 2 and Exp. 3: At the Stage of Fig.5, one day before being sacrificed, lymphocytes were collected from peripheral blood of each mouse. Lymphocytes pooled from each group were stained with PerCP-anti-CD4 antibodies and PE-anti-CD25 Ab, and fifty thousand cells were analyzed. All experiments are from Dwiranti *et al*., 2012.

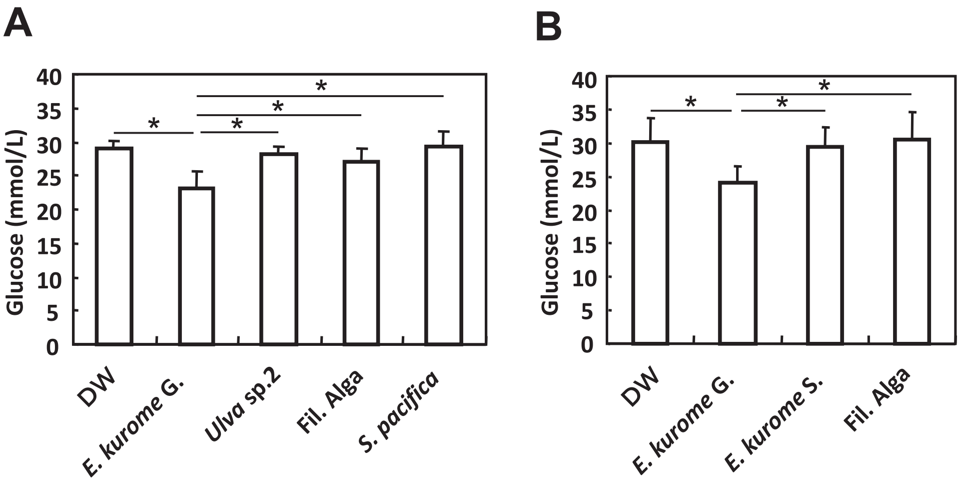

Fig. 2. Effects of algae on blood glucose of *db/db* mice.
E. kurome G: gametophytes of *Ecklonia kurome* including both male and female gametophytes.
Ulva sp.2: *Ulva meridionalis*, Fil. Alga: filamentous blue green algae not identified, *S. pacifica*: *Spirulina pacifica*. Algae were homogenated by polytoron in distilled water and treated at 80°C for one hour. When the level of blood glucose reaches 500 mg/dL (28 mmol/L), algae homogenates were administered every other day for ten weeks (10 mg/0.2 ml/mose). *Db/db*: BKS.Cg-+*Lepr*^{db}/+*lepr*^{db}/Jcl. Results are expressed as average ± S.D. (A: n=5 female mice, B: n=8 female mice). *: One way ANOVA, *p*<0.05. From Dwiranti *et al*. 2012.

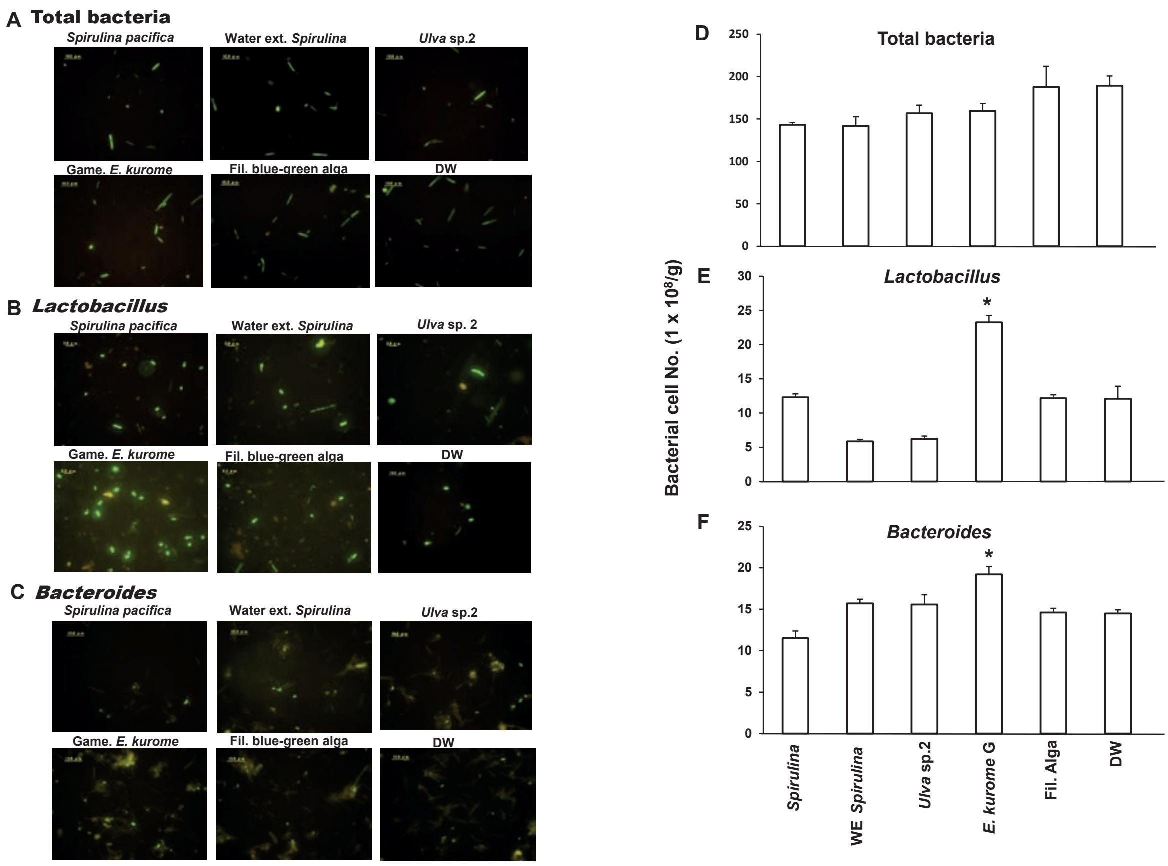

Fig. 3. Bacteria in the large intestine were analyzed.
Bacteria of large intestine of Fig. 2A mice were hybridized with the following 16S rRNA probes coupled with fluorescein according to the protocol (Ribo Technologies, Groningen, Holland). A: total bacterial probe, B: *Lactobacillus* species probe, C: *Bacteroides* species probe. Hybridized bacteria with each probe were counted and expressed as 1 x 10^8/g. D: Bacterial cell numbers hybridized with 16S rRNA probe that can detect all bacteria. E: Bacterial cell numbers hybridized with *Lactobacillus* 16S rRNA probe. F: Bacterial cell numbers hybridized with *Bacteroides*16S rRNA probe. At least 14 fields were measured and the bacterial cell No./g of the contents of large intestine were expressed as average ± S.D.. *: ANOVA, Tukey-Krammer's post hoc test. *p*<0.01. (*E. kurome* G-administered group versus DW-administered group)

diabetic wild type mice whose condition was caused by a high fat diet. A high fat diet containing 32% fatty acids (HFD) was administered to C57BL/6J and C57BL/6J-IFN-γ KO mice, C57BL/6J-*Ifng^tm1Ts^* (Dalton *et al.,* 1993). Eight weeks after feeding normal diet (ND), we found a higher level of blood glucose one hour after glucose administration in the oral glucose tolerance test (OGTT) of IFN-γ KO mice compared with that of parental C57BL/6J mice (Fig. 4 A. B). We confirmed that HFD caused not only higher body weight but also prediabetic status in both strains of mice (Surwit *et al.,* 1988). (Impaired glucose tolerance: Fasting plasma glucose: < 7.0 mmol/L (126 mg/dL) and 2 hours plasma glucose: ≥ 7.8 and < 11.1 mmol/L (140 mg/dL and 200 mg/dL), WHO, 2006.). The effect of HFD was much greater in C57BL/6J mice. We found that one hour after administrating glucose, the level of blood glucose was more than two times higher in C57BL/6J mice as compared with ND (normal diet containing 4.8% crude fat) (Fig. 4A). In contrast, the effects of HFD were not seen one hour after glucose administration in IFN-γ KO mice (Fig. 4B). A higher level of glucose was observed three hours after glucose administration in the HFD-treated group (8.96 ± 0.7 mmol/L) compared with the ND-treated group (6.32 ± 0.41 mmol/L) in IFN-γ KO mice (Fig. 4B).

Administration of gametophytes of *E. kurome* for four weeks to HFD-fed mice did not have any effects on the OGTT in either C57BL/6J or IFN-γ KO mice. Next, we evaluated the effect of gametophytes of *E. kurome* three weeks after switching the food from HFD to ND. At this stage, both strains of mice were still prediabetic. Blood glucose levels two hours after glucose administration to C57BL/6J and C57BL/6J-IFN-γ KO mice were 7.91 ± 0.46 mmol/L and 8.59 ± 0.68 mmol/L, respectively (Fig. 4C, D). At this stage, gametophytes but not sporophytes of *E. kurome* were effective in the OGTT compared with the control group in C57BL/6J mice (Fig. 4 C, D, and data not shown). *Porphyra* species and *Cladosiphon okamuranus* did not have any effects on OGTT in C57BL/6J mice. Gametophytes of *E. kurome*

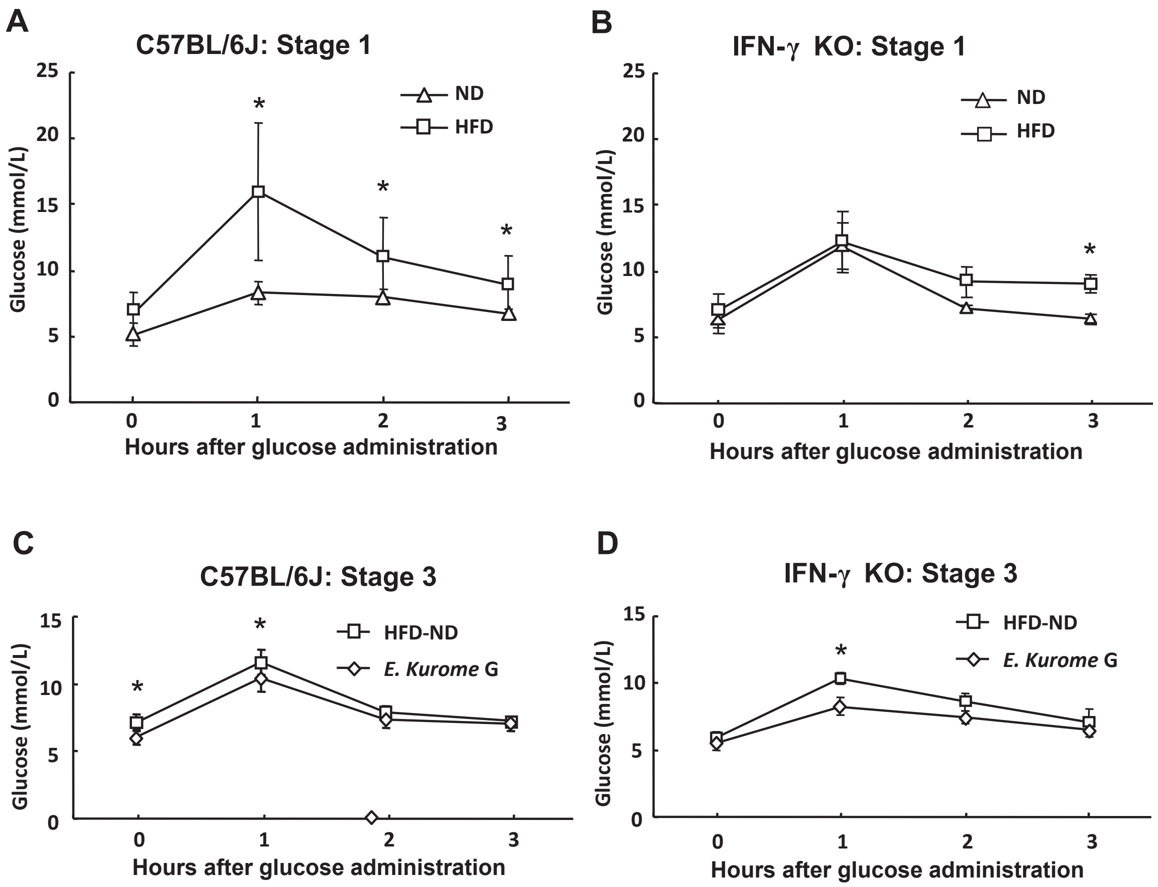

Fig. 4. Effects of algae homogenates on oral glucose tolerance test of HFD-treated wild type and IFN-γ KO mice.
Wild type mice: C57BL/6J, IFN-γ KO: C57BL/6J-*Ifng^tm1Ts^*. *E. kurome* G: male and female gametophytes of *Ecklonia kurome* were administered together with HFD or ND according to the following protocol: eight weeks HFD/four weeks HFD + algae/three weeks ND + algae. After 18 hours starvation, glucose (2 g/kg body weight) was administered and the blood glucose level was measured hourly. Results were expressed as average ± S.D. (wild type mice: n=8 female mice, IFN-γ KO mice: n=5 female mice). *: ANOVA, Tukey-Krammer's post hoc test. $p<0.05$. From Dwiranti *et al*. 2012.

were also effective in ameliorating the impaired glucose tolerance compared with the control group (HFD-HD) in IFN-γ KO mice. To maintain the prediabetic status, we changed the food from ND to HFD again and continued to administer algae homogenate to mice. Ten days after changing the food, we measured serum glucose after fasting the mice for 18 hours (Fig. 5). Among the algae homogenate examined, only gametophytes were effective in down-regulating the blood glucose in C57BL/6J mice. In contrast, gametophytes of *E. kurome* did not have any effect on the serum level of glucose in IFN-γ KO mice. It is speculated that IFN-γ was involved in this down-regulation of blood glucose.

We measured the weight of adipose fat tissue around the uterus at the end stage. Adipose fat tissue accumulated in the groups of HFD-fed mice in both C57BL/6J and IFN-γ KO mice (Fig. 6). Sporophytes but not gametophytes of *E. kurome* augmented adipose tissue slightly compared with the HFD group in C57BL/6J mice. The adipose tissue weight of the group administered with gametophytes was significantly reduced compared with the weight of the group administered with HFD alone in IFN-γ KO mice. There was no significant difference in body weight among HFD-fed groups in either C57BL/6J or IFN-γ KO mice.

By measuring the percentage of Treg cells in C57BL/6J or IFN-γ KO mice, we found a slight decrease of $CD4^+CD25^+$ T cells in peripheral blood from group treated with either gametophytes or sporophytes of *E. kurome* in C57BL/6J mice (Table 2, Exp 2). IFN-γ KO mice had a higher level of $CD4^+CD25^+$ T cells in peripheral blood of ND-treated group compared with that of C57BL/6J mice, suggesting that IFN-γ has a suppressive effect on the development of Treg cells (Table 2, Exp. 3). Interestingly, we found a lower percentage of $CD4^+CD25^+$ T cells in peripheral blood from gametophytes of *E. kurome*-treated group than that of the DW-treated group in IFN-γ KO mice. We confirmed these results by analyzing $CD4^+FOXP3^+$ T cells and $CD25^+FOXP3^+$ T cells (Fig. 7 A, B). FOXP3 is a forkhead-winged-helix transcription factor which is specifically expressed in Treg cells. A similar tendency in the level of $CD4^+CD25^+$ T cells was observed in both $CD4^+FOXP3^+$ T cells and $CD25^+FOXP3^+$ T cells.

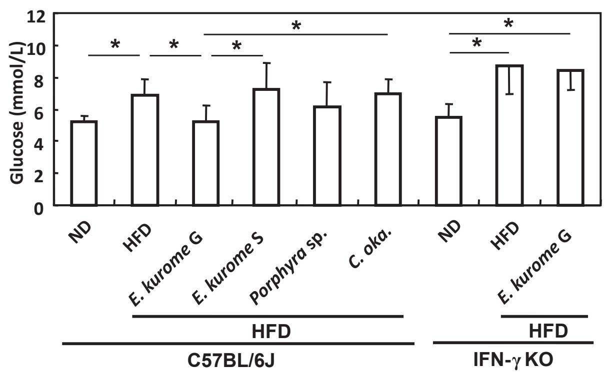

Fig. 5. Effects of algae homogenates on the blood glucose level of HFD-treated prediabetic wild type and IFN-γ KO mice.
ND: Normal diet, HFD: High fat diet, *E. kurome* G: gametophytes of *Ecklonia kurome*, *E. kurome* S: *E. kurome* sporophytes, *Porphyra* sp.: Porphyra species, *C. oka*.: *Cladosiphon okamuranus*. At the end of the serial treatment (eight weeks HFD/four weeks HFD + algae/three weeks ND + algae/three weeks HFD + algae), blood glucose levels in serum were measured. Results were expressed as average ± S.D. (wild type mice: n=8 female mice, IFN-γ KO mice: n=5 female mice). *: One way ANOVA, $p<0.05$. From Dwiranti *et al*. 2012.

5. Effects of algae on obesity and diabetes in relation to the role of leptin and IFN-γ.

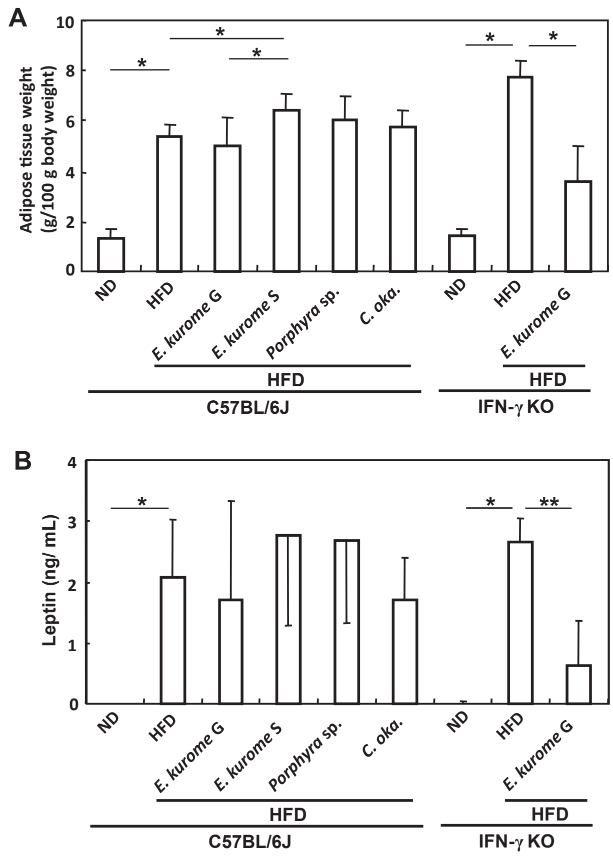

Fig. 6. Effects of edible algae on the development of adipose tissue and the serum level of leptin.
At the end of the serial treatment (eight weeks HFD/four weeks HFD + algae/three weeks ND + algae/three weeks HFD + algae), adipose tissue around the uterus was measured. Results were expressed as average adipose tissue weight (g/100g body weight) ± S.D. (wild type mice: n=8 female mice, IFN-γ KO mice: n=5 female mice). Leptin was measured by ELISA (Shibayagi co., Ltd, Gunma, Japan). *: ANOVA, Tukey-Krammer's post hoc test. $p<0.05$. **: One way ANOVA, $p<0.05$. From Dwiranti *et al*. 2012.

Since it is reported that overnight fasting nearly depletes glycogen stores in mice, we examined the effect of IFN-γ by using OGTT after overnight fasting. We found IFN-γ is certainly involved in the regulation of glucose usage. Although both gametophytes and sporophytes of *E. kurome* reduced Treg cells, neither of them was effective in reducing adipose tissue in C57BL/6J mice. In IFN-γ KO mice, however, gametophytes of *E. kurome* reduced adipose fat tissue compared with other groups. This may suggest that IFN-γ inhibits the role of gametophytes of *E. kurome* in reducing adipose fat tissue.

Interestingly, we found that gametophytes but not sporophytes of *E. kurome* were effective in down-regulating blood glucose in both *db/db* mice and prediabetic C57BL/6J mice, suggesting that active compounds are enriched in the gametophytes. Since we used a mixture of female and male gametophytes, active compounds may exist in either gametophyte. These compounds are not likely to reduce the absorption of foods, because body weights of mice did not change significantly among HFD-fed groups, though different algae were administered.

One of the well-studied active compounds extracted from edible brown algae to down-regulate blood glucose is fucoxanthin. It down-regulated not only the level of blood glucose and plasma insulin but also the expression level of leptin and TNF-α mRNAs in white adipose tissue of the diabetes/obesity mice (Maeda *et al.,* 2007, Jeon *et al.,* 2010). Although fucoxanthin is extracted from sporophytes of various brown algae such as *Undaria pinnatifida*, *Sargassum fulvellum*, *Saccharina*

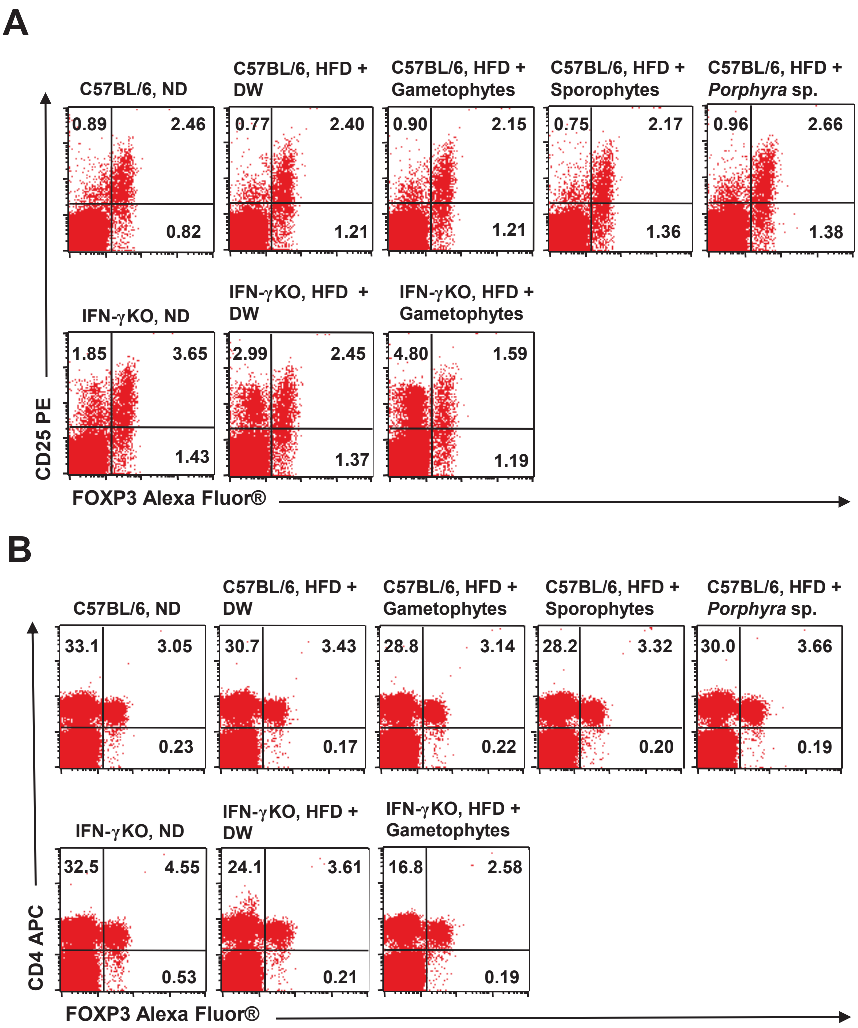

Fig. 7. Effects of algae on the Treg cells in prediabetic wild type mice and IFN-γ KO mice.
One day before measuring adipose tissue weight in Fig. 6, we stained Treg cells in peripheral blood by two different methods. A: Phycoerythrin (PE) coupled anti-CD25 antibodies and Alexa Fluor® coupled anti-FOXP3 antibodies. B: Allophycocyanin (APC) coupled anti-CD4 antibodies and Alexa Fluor® coupled anti-FOXP3 antibodies according to the protocol described (BioLegend, San Diego, CA). Fifty thousands cells were analyzed by a flow cytometer (FACS Calibur, Becton Dickinson and Co., Mountain View, CA). Numbers shown in each quadrant are percentages of the population of the area among total lymphocytes.

japonica, *Sargassum fusiformis*, there is no study which has compared the quantity of fucoxanthin in the life cycle stage of brown algae. Since fucoxanthin is rich in brown algae and brown alga, *C. okamuranus* did not decrease the blood glucose, it is unlikely that fucoxanthin is one of the major compounds in gametophytes of *E. kurome* that down-regulates the blood glucose. The question of whether or not there are active compounds selectively produced or accumulated in gametophytes of *E. kurome* remains to be asked. We think it is possible that gametophytes accumulate triacylglycerol or carbohydrates as energy sources by converting glucose before fertilization, because it is common for plants to accumulate energy sources at a certain stage of the life cycle such as when producing seeds. This hypothesis may be supported by the fact that gametophytes but not sporophytes of *E. kurome* recovered the reduced level of triacylglycerol by HFD to the level of ND group in C57BL/6J mice (Dwiranti *et al.,* 2012). This effect of gametophytes of *E. kurome* was not observed in IFN-γ KO mice (Dwiranti *et al.,* 2012). In *db/db* mice, the serum level of triacylglycerol was decreased by administrating gametophytes but not sporophytes of *E. kurome*. These results suggest that both leptin and IFN-γ are involved in the regulation of the metabolism of glucose and triacylglycerol.

It is reported that T cells from obese adipose tissue produce more IFN-γ than those from controls (Rocha *et al.,* 2008). This may explain why the weight of adipose tissue was reduced in IFN-γ KO mice treated with gametophytes of *E. kurome* (Fig. 6).

Leptin is not a simple satiety signal to prevent obesity in times of energy excess. Falling leptin concentration is a critical signal that initiates the neuroendocrine response to starvation, including limiting procreation, decreasing thyroid thermogenesis, and increasing secretion of stress steroids, which together are likely to have survival value during prolonged nutritional deprivation (Ahima *et al.,* 1996). It is also suggested that leptin may be the key link between nutritional status and an optimal immune response (Lord *et al.,* 1998). De Rosa *et al.* reported that leptin down-regulates the Treg cells and stimulates the proliferation of IFN-γ secreting Th1 cells (De Rosa *et al.,* 2007). In *db/db* mice, Treg cells were reduced in the group administered with gametophytes of *E. kurome* (Table 2). This may suggest that gametophytes of *E. kurome* may replace the function of leptin, in part, in the regulation of Treg cells. However, the serum level of IFN-γ was not increased in the group administered with gametophytes of *E. kurome* (data not shown).

In order to clarify the role of Treg cells in the down-regulation of blood glucose, we examined the effects of *E. kurome* on the population of Treg cells in C57BL/6J and IFN-γ KO mice. Although there was a slight down-regulation of Treg cells in the gametophytes *E. kurome*-administered group compared with the control DW- or *Porphyra* sp.-administered group, there was no significant difference between the gametophytes-administered group and the sporophytes-administered group in C57BL/6J mice (Table 2, Fig. 7A, B). Although gametophytes of *E. kurome* decreased Treg cells, they had no effect on the serum level of glucose in IFN-γ KO mice (Fig. 5), suggesting that Treg cells are not directly involved in the regulation of glucose in these mice. However, Treg cells may be engaged in the regulation of adipose fat tissue and leptin production (Table 2 and Fig. 7A, B). At any rate, IFN-γ is certainly involved in glucose metabolism (Wong *et al.,* 2011).

6. Effects of algae on obesity and diabetes in relation to the gut bacteria.

Another important point is that germ-free C57BL/6J mice are resistant to HFD-induced insulin resistance and have altered cholesterol. Rabot *et al.* have demonstrated that lower calorie consumption and increased lipid excretion contributed to the obesity-resistant phenotype of germ-free and HFD-fed mice (Rabot *et al.,* 2010). They also revealed that insulin sensitivity and cholesterol metabolism are metabolic targets influenced by the gut microbiota. Mazmanian *et al.* reported that colonization with wild-type *Bacteroides fragilis* alone is sufficient to correct the defect in IFN-γ expression in germ-free mice, with levels nearly as high as those in conventional mice (Mazmanian *et al.,* 2005). This effect of IFN-γ expression depends on the bacterial polysaccharides of *Bacteroides fragilis*. In accordance with this fact, gametophytes of *E. kurome* increased the *Bacteroides* group in the gut (Fig. 3C, F).

These results suggest that gametophytes of *E. kurome* are effective in regulating the metabolism through the manipulation of cytokines such as IFN-γ or leptin, resulting in the down-regulation of blood glucose. In conclusion, gametophytes of *E. kurome* are useful in down-regulating blood glucose and are good candidates to be used as an alternative medicine, because they are one stage of an edible alga. Anti-diabetic effects of gametophytes of edible algae other than *E. kurome* remain to be studied.

7. Allergic reaction was caused by the imbalance between human genome and the environment.

Nowadays, we find *Aspergillus fumigatus* in the filter of air conditioners. This fungus often causes an allergic reaction. Since we have changed our living environment to an artificial one with ferroconcrete, glasses, and metals, some species live in a niche different from their original one. *Dermatofagoides farinae* proliferates in futons, blankets, and pillows. In humid conditions, 100,000 mites are found in one futon. In case of mites, allergic reactions are caused by a serine protease. Live mites are not necessary to cause allergic reactions, because this protease is contained in mite feces. Several typical allergens in cedar pollen are pectate lyase and polymethylgalacturonase. Lysosomal enzymes called cathepsins digest protein allergens into peptides consisting of 8 to 30 amino acids in antigen presenting cells such as macrophages and dendritic cells. Digested peptides are presented in a groove of major histocompatibility complex (MHC) protein on the membrane of antigen presenting cells. In case of humans, this MHC molecule is named HLA, or human leukocyte antigen. T lymphocytes recognize both HLA and antigen peptides and proliferate. In other words, T lymphocytes proliferate in response to HLA plus antigen peptides. If T lymphocytes produce interleukin (IL)-4, this cytokine induces B lymphocytes to differentiate to produce immunoglobulin E (IgE). When T lymphocytes secrete IFN-γ, this cytokine induces B lymphocytes to differentiate to produce most abundant IgG antibodies. IFN-γ production does not cause allergic reactions. IFN-γ suppresses the function of IL-4, and vise versa. Throughout the human history, IgE has been useful in attacking parasites. When lipopolysaccharides (LPS) are present in the atmosphere, IFN-γ is produced by T lymphocytes, resulting in the suppression of allergic reaction. Less LPS in the atmosphere in the countries of Northern Europe probably caused a frequent occurrence of allergic diseases. Interestingly, a receptor for fungi molecules was found as the receptor for a dorsalizing factor Spätzle named toll in *Drosophila*. This toll-like receptor found in drosophila transfer signals against the pathogens not only in invertebrates but also in vertebrates. Because this immune system works without immunization, this is called natural immunity and is mediated mainly by macrophages, neutrophils, and natural killer cells. On the other hand, acquired immunity including vaccination or allergic reactions is mediated by lymphocytes and antibodies. More than 10 toll-like receptors (TLR) are found to react with pathogen molecules such as LPS, peptidoglycan, GC-rich DNA, and double strand RNA.

8. Anti-allergic effects of algae.

We have examined TLR binding molecules from algae. Blue green alga *Spirulina* is also classified as a Gram-negative cyanobacterium. We found that *Spirulina* complex polysaccharides (CPS) induce NFκB by signaling through either TLR2 or TLR4. In contrast, LPS of *E. coli* induces NFκB though TLR4. Since both of them lose NFκB inducing ability after pre-incubation with polymixin B, they probably share the same chemical nature. Algae polysaccharides, alginic acid, fucoidan, pectin, and carrageenan bind to NFκB to induce TLR4. *Petalonia binghamiae* polysaccharides binds to TLR4 and TLR2 to induce NFκB just like *Spirulina* CPS. Here, we summarize the effects these polysaccharides have on delayed-type hypersensitivity against picryl chlodide (PCl: 2,4,6-trinitrochlorobenzene). Among *Spirulina* CPS, alginic acid, fucoidan, and *Petalonia* polysaccharides, only *Petalonia* polysaccharides effectively suppressed this allergic reaction. This suppression was observed in wild type C3H/HeN mice but not in TLR4 deficient C3H/HeJ mice, suggesting that the suppressive signal is transferred through TLR4. Since other TLR4 binding molecules did not exert suppressive effects, a signal through TLR2 may be involved in this suppression. When administered intraperitoneally, *Petalonia* polysaccharides suppressed the DTH response against PCl by decreasing the blood level of IL-17. The level of IL-17 was not decreased, even though the DTH reaction was suppressed when *Petalonia* polysaccharides were administered orally. Therefore, there must be other common factors which are suppressed by both way of administration of *Petalonia* polysaccharides. We found that migration of eosinophils to the site of inflammation were inhibited in either case (Tominaga *et al.,* 2010, 2011).

9. Effects of algae on tumor growth.

We have also examined the anti-tumor effects of algae polysaccharides, alginic acid, fucoidan, carrageenan, and *Petalonia binghamiae* polysaccharides. Interestingly, fucoidan, *Petalonia binghamiae* polysaccharides and *E. coli* LPS suppressed the growth of hepatoma (MH134) in C3H/HeN but not in TLR4 mutant C3H/HeJ (Tominaga *et al.,* 2010, data not shown). In contrast, alginic acid and *Eucheuma* λ carrageenan suppressed the hepatoma growth in both C3H/NeN and C3H/HeJ. *Spirulina* CPS suppressed the growth of glioma in C3H/HeN but not in TLR4 mutant C3H/HeJ (Kawanishi *et al.,* 2013). Among these polysaccharides, *Petalonia binghamiae* polysaccharides and *Spirulina* CPS induce NFκB equally in response to TLR2 and TLR4 at a low level. Other polysaccharides induce NFκB in response to TLR4 but not TLR2. At this moment, we do not know why alginic acid and *Eucheuma* λ carrageenan suppressed the hepatoma growth not only in C3H/NeN but also in C3H/HeJ mice.

Another interesting point is that *E. coli* LPS suppressed the glioma growth by natural killer cells without reducing the angiogenesis by tumor cells. In this case, no memory of vaccination was found, because T lymphocytes were not involved in the anti-tumor effects. On the other hand, *Spirulina* CPS suppressed the growth of glioma by T lymphocytes and macrophages but not by natural killer cells. *Spirulina* CPS suppressed the angiogenesis significantly (Kawanishi *et al.,* 2013). So, there are at least two strategies for polysaccharides from microorganisms to suppress tumor growth. The strategy to suppress tumor growth using polysaccharides may depend on their reactivity to TLR2 or TLR4.

10. Algae and a sustainable society.

As summarized in Table 3, edible algae showed us anti-diabetic, anti-allergic, and anti-tumor activities with different mechanisms. After starting agriculture, humans have decreased the number of plants they consume compared with those consumed by primates. It is estimated that humans only eat one tenth of the plant species consumed by chimpanzees and gorillas (Engel, 2002). These cultural changes of food and decrease of exercises have predisposed people towards obesity, diabetes, cerebrovascular diseases, cardiovascular diseases, and arteriosclerosis. Allergies are also related to changes in the living environment. Although we can use algae in our daily diet, we cannot decrease the blood glucose level while we are continuing to subsist on high calorie diets. We suggest for obese people to have algae as part of a lower calorie diet and also to get enough physical exercise.

It is reported that 30% of obese people are genetically predisposed to obesity (Greenstein *et al.,* 2011). As for the remaining 70%, ten percent of obese people are influenced specifically by cultural factors, and the remaining 60% of obese people are mainly influenced by lifestyle factors. Therefore, 70 percent of the people with obesity can be helped through changing their diet, because their obesity depends primarily on their life style. Here, we would like to think of culture and personality. Personality is developed by the interaction among human genome, environment, and culture (Fig. 8).

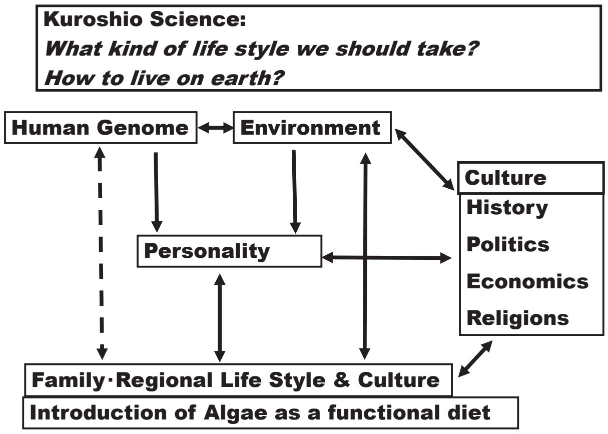

Fig. 8. How personality and regional life style and culture are developed.

Although there are many marine protected areas in Kuroshio region, not all of them are functioning well. One of the major reasons is that fishermen's income has decreased because of restrictions on catching fish. Cultivation of algae will be beneficial for fishermen to protect the marine environment. For this purpose, we need to show the beneficial effects of algae for human health. We believe this would be helpful to achieve a sustainable society (Fig. 9).

Acknowlegements

This review is based on my plenary lecture in

Table 3. Summary of function of algae or algae polysaccharides.

	Anti-allergic	Anti-tumor	Anti-diabetic
Alginic acid	-	+	
Fucoidan	-	+	
Carrageenan	+	+	
***Petalonia* PS**	+	+	
***Spirulina* CPS**	±	+	±
Game. *E. kurome*			+
Spo. *E. kurome*			-

Alginic acid is prepared from *Macrocystis pyrifera* (kelp)(sigma-Aldrich), Molecular Mass (MS): 420100 ~ 420700. Fucoidan is prepared from *Fucus vesiculosus* (sigma-Aldrich), MS: 841388 ~ 1113156. Carrageenan is prepared from *Eucheuma*. *Petalonia* PS is prepared from *Petalonia binghamiae* polysaccharides. MS:27355 ~353476. *Spirulina* CPS is prepared from *Spirulina pacifica*. MS: 1284 ~ 28315. Game.: gametophytes, Spo.: sporophytes.

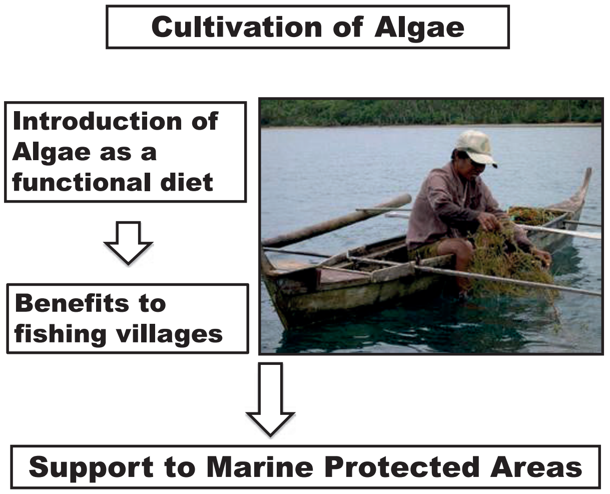

Fig. 9. Cultivation of algae to support marine protected areas.

the 6th International Symposium on Kuroshio Science in Tabaco Campus, Bicol University. We appreciate the Organizing Committee, especially, Dr. Plutomeo Nieves (Dean of Tabaco Campus), Dr. Ninfa R. Pelea (Director of Consortiam), and Dr. Fay Lea Patria M. Lauraya (President of Bicol University). We are grateful to Dr. Gerald Cysewski, Cyanotech Cooperation and Mr. Nobuyuki Miyaji, Toyo Koso Kagaku Co., LTD, for their generous supply of *Spirulina pacifica*. We are also grateful to Mr. Ippei Yanagida for his generous gift of Okinawa Mozuku. We are also grateful for support from Fuji Sangyo Co., LTD. We also appreciate Ms. Sayo Kataoka for her skillful help in the analysis by flow cytometry. We are grateful to Mr. Daniel Brownson Ribble for his English editing.

I deeply appreciate emeritus professor Yoshinori Morooka who introduced me to the cultivation field of *Eucheuma*, Rapu-Rapu Island in Albay province in Philippines for the first time and suggested me to think of usage of cultivated algae for the marine protected areas.

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Letter from GSKS Alumni 1

Life in Japan and Canada

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From Dhaka to Kochi

I moved to Nankoku-shi, Japan from Dhaka, Bangladesh in July 2001 with my husband when he was a foreign student at the Faculty of Agriculture, Kochi University. It was my first time in a foreign country and I was so excited to be in a wonderful place like Kochi-ken. From the very beginning, I was interested to know about Japanese culture which is very unique. I was delighted to see that Kochi people have a wonderful quality of being very friendly and helpful to foreigners, particularly to foreign students and their families. I was easy for me to adapt to life in Kochi mainly due to the cordial help and support from my Japanese host families, volunteers and friends. I also worked as a volunteer and guest speaker in schools and community events organized by the Nankoku International Association and the Kochi International Association. It was the most effective way for me to mix with local people, make new friends and habituate with foods, norms and values of Japanese people. Soon Kochi became my most favorite place and second home.

After living in Kochi for a few years, I decided to pursue my higher studies at Kochi University. I was looking for a suitable course relevant to my previous education that would fulfil my academic and research interests. My desire would not be successful without the then new Graduate School of Kuroshio Science. This faculty has a unique combination of many disciplines including agriculture, economics, social science and medical science. This faculty is giving invaluable opportunities to students from various disciplines to conduct study and research under one umbrella. We had to present research updates periodically in seminars. Professors from various departments were present there to give thoughtful comments which I believe was an excellent opportunity to exchange knowledge and enrich student's academic excellence and be steered in the right direction to continue their research.

I was really lucky to be a Ph.D. student of the first batch of the Graduate School of Kuroshio Science at Kochi University and had the privilege to be taught, guided and inspired by many renowned professors, particularly, Dr. Yoshiaki Iiguni (my main supervisor), Dr. Yoshinori Morooka (my co-supervisor) and Dr. Masayuki Takahashi (the then Dean).

From Kochi to Mississauga

I moved to Mississauga City in Canada from Japan in November 2008 with my family. It was a long way across the Pacific Ocean. It was a very meaningful change in our life because we decided to settle down in this country of immigrants. We came here at the beginning of the winter in Canada and it was little shocking to see freezing temperatures and lots of snow even in mid-November and December.

But it was more shocking when we found that the job market was very tight because of the world economic recession. Some people we had known previously were giving us much frustrating information about how new immigrants have been struggling in Canada. We quickly realized that integrating into Canadian life is not so easy. We found that there are some non-profit organizations (NPOs) that provide different programs for the newcomers and help them learning about Canadian society, culture, norms, values and the job market. We attended a program with one of the NPOs close to our home. Gradually, we came to know more about this country. Both my husband and I obtained regular full time jobs within a few months after arrival in Canada and our life became smooth here.

The most wonderful thing in Canadian society is the multiculturalism. In 1971, Canada became the first country in the world to declare multiculturalism as the state policy. This bold step charted the path to a vibrant and evolving cultural mosaic premised on mutual respect for Canadians of all backgrounds and ancestry. We can find people from everywhere in the world (including Japan) living in harmony in the big cities of Canada. Food from every country is available here, especially in the Greater Toronto Area (Mississauga City is located in the Greater Toronto Area). We sometimes go to Japanese restaurants as we like Japanese foods very much. We are now settled down here, but always remember our wonderful time in Japan.

I have been working in a private company for last four years which is providing fire protection and safety services to provincial/municipal government housing. I always had deemed of working in the public sector to contribute directly to the advancement of the quality of life in society. To achieve this career goal, I need to know more about Canadian municipal, provincial, and federal public administrations systems and policies to fulfil the needs of a rapid growing diversified community.

I have enrolled in the Master of Public Service (MPS) 2013 Fall Program at the University of Waterloo, Canada, and will equip me with adequate knowledge and skills to become an effective public service employee in Canada. The MPS is a source of knowledge and skills, ethical sensitivity, modern communication skills, sophisticated financial management, and excellent leadership to become an effective public servant in any branch of the Canadian public service.

Revisit to Kochi in my dreams

I strongly believe that my study in Kuroshio Science has had a significant impact on my life. It broadened my understanding of the world via learning about diverse socio-economic issues of development, social capital and women's empowerment. It has also significantly increased my research skills, analytical ability, adaptability and moral values as a human being. In this way, Kuroshio Science will be very important t me throughout my life. Again, living in Kochi was the most beautiful period of my life.

I always consider Kochi as my second home. My son Shinjan (Shin-chan) was born in the Nankoku Nokyo Hospital and he lived in Kochi until he was four years old. He wants to go to Kochi to meet his "hoikuen (nursery school)" friends and teachers. I will definitely go to Kochi some day with my son to show him his birth place and the place where we spent the best times of our life.