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1 Nutraceutical characteristics of the brown seaweed carotenoid fucoxanthin

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14  
15 **ABSTRACT**

16 Fucoxanthin (Fx), a major carotenoid found in brown seaweed, is known to show a  
17 unique and wide variety of biological activities. Upon absorption, Fx is metabolized to  
18 fucoxanthinol and amarouciaxanthin, and these metabolites mainly accumulate in  
19 visceral white adipose tissue (WAT). As seen in other carotenoids, Fx can quench  
20 singlet oxygen and scavenge a wide range of free radicals. The antioxidant activity is  
21 related to the neuroprotective, photoprotective, and hepatoprotective effects of Fx. Fx is  
22 also reported to show anti-cancer activity through the regulation of several biomolecules  
23 and signaling pathways that are involved in either cell cycle arrest, apoptosis, or  
24 metastasis suppression. Among the biological activities of Fx, anti-obesity is the most  
25 well-studied and most promising effect. This effect is primarily based on the  
26 upregulation of thermogenesis by uncoupling protein 1 expression and the increase in  
27 the metabolic rate induced by mitochondrial activation. In addition, Fx shows  
28 anti-diabetic effects by improving insulin resistance and promoting glucose utilization  
29 in skeletal muscle.

30  
31 *Keywords:* Brown seaweeds, Fucoxanthin, Anti-obesity, Anti-diabetes, Anti-cancer

32  
33 **1. Introduction**

34  
35 Seaweeds (marine macroalgae) are photosynthesizing plants that form the basic  
36 biomass in the intertidal zone. They are a varied group, with sizes ranging from a few

37 centimeters to 100 m in length. According to their color, they are divided into three  
38 main classes: green (chlorophytes), red (rhodophytes), and brown (phaeophytes). Brown  
39 seaweeds are the most consumed species, followed by red and green seaweeds [1]. As  
40 seaweeds lack many of the distinct organs (roots, stems, leaves) found in terrestrial  
41 plants, whole parts can be used as a source of food, cosmetics, and other products. They  
42 have high nutritional value, in both fresh and dried forms, and act as ingredients in a  
43 wide variety of prepared foods.

44 The unique and phenomenal biodiversity of the marine environment provides a large  
45 pool of novel and bioactive nutrients for marine organisms. Seaweed is one of the  
46 potential sources of these marine bioactive compounds. Seaweed has been consumed in  
47 East Asian countries for centuries, and lately, knowledge of the health benefits of  
48 dietary seaweed has gained attention in Western cultures [2,3]. According to the  
49 Seafood Source report, new products containing seaweed in the European market have  
50 increased yearly [2]. Seaweed is rich in non-starch polysaccharides, mainly dietary  
51 fibers, and essential minerals such as potassium and calcium [4,5]. The quality of  
52 seaweed protein is comparable to other vegetables, mainly due to its high content of  
53 essential amino acids. The lipid content of seaweed is generally low, but seaweeds  
54 contain high levels of functional omega-3 eicosapentaenoic acid and omega-6  
55 arachidonic acid [5]. Additionally, the value of edible seaweed in human nutrition is  
56 strongly recognized for its richness in several phycochemicals. Consequently, seaweed  
57 is currently considered a “superfood”, which is a market term recognizing health  
58 benefits including superior nutritional profile and richness in bioactive phytochemicals  
59 [6].

60 Primary metabolites of seaweed, such as polysaccharides, proteins, and lipids, are  
61 directly involved in their physiological functions under normal growth conditions, while  
62 the exposure to different stress conditions, (e.g., ultraviolet radiation, changes in  
63 temperature and salinity, or environmental pollutants) stimulates them to produce a  
64 wide range of secondary metabolites [6,7]. Among these secondary metabolites, much  
65 interest has been paid to seaweed antioxidants [2,6,8-13]. Compared to its terrestrial  
66 counterparts, seaweed is potentially a good source of antioxidants and has an advantage  
67 over many other organisms in that it can appropriately produce a large amount of  
68 desirable and specific bioactive compounds.

69 Alcoholic extracts from seaweed have been reported to show antioxidant activity  
70 due to the presence of polyphenols in the extracts. Red and green seaweeds contain  
71 bromophenols, phenolic acids, and flavonoids as the major polyphenols, while  
72 phlorotannins are only dominant in brown seaweed [8,14]. Phlorotannins are a group of

73 complex polymers of phloroglucinol (1,3,5-trihydroxybenzene). Based on the type of  
74 structural linkage between the phloroglucinol sub-units, they can be systematically  
75 classified into eckols, fucols, fuhalols, ishofuhalols, phloroethols, or fucophloroethols  
76 [2]. Many studies have shown that phenolic extracts from seaweeds have several kinds  
77 of biological activities [2,6,7,9,15-17]. Although these effects may be due to their  
78 ability to modulate oxidative stress and inflammatory cascades, the detailed mechanism  
79 remains unclear, mainly due to their complex chemical structure and the difficulty of  
80 identifying metabolites and active compounds after absorption.

81 Carotenoids are also major seaweed antioxidants. They are generally localized in  
82 photosynthetic organisms and play an important role in photochemical events [18].  
83  $\beta$ -Carotene,  $\alpha$ -carotene, zeaxanthin, lutein, violaxanthin, neoxanthin, and fucoxanthin  
84 (Fx) has been reported in several kinds of seaweed [19]. Among these carotenoids, Fx is  
85 a specific carotenoid only found in algae but not terrestrial plants. Fx is photosynthetic  
86 pigment mainly found in brown seaweed and Bacillariophyta (diatoms). These algae are  
87 widely distributed in cold and temperate ecosystems throughout the world; therefore, Fx  
88 is regarded as one of the most abundant carotenoids in nature [20]. In addition to the  
89 important role of Fx in the photosynthesis and photoprotection of algae, Fx also has  
90 health benefits [21].

91 This article focuses on the current scientific literature regarding the bioactive  
92 significance of Fx, including metabolic, anti-oxidant, anti-obese, anti-diabetic, and  
93 anti-cancer activities,.

94

## 95 **2. Structure and safety**

96

97 Fx has a distinctive structure with an allenic bond, a 5,6-monoepoxide, and nine  
98 conjugated double bonds (Fig. 1). The conjugated double bond system in the Fx  
99 molecule can quench singlet oxygen ( $^1O_2$ ) and the electron-rich status of Fx makes it  
100 more suitable to react with free radicals [21]. Therefore, Fx can protect cells, tissues and  
101 other structures against oxidative damage; however, it would be difficult to explain all  
102 of its beneficial health effects only by its antioxidant activity [22]. Much attention has  
103 been paid to the interaction between Fx or its metabolite and biological key molecules  
104 such as receptors and co-activators, where the specific chemical structure of a  
105 carotenoid may be essential for binding. To better understand the physiological effect of  
106 Fx, more efforts are needed to clarify the ability of Fx or its metabolites in modulating  
107 the expression of specific genes and proteins involved in biological systems (Fig. 2).

108 Fx-rich brown seaweed has been used in Southeast Asian countries as a traditional  
109 food. In addition to its use as a food source, the safety of Fx has been demonstrated  
110 through animal experiments. A single dose study indicated no mortality and no  
111 abnormalities in male and female ICR mice fed 1000 and 2000 mg/kg purified Fx. In a  
112 repeated dose study, no adverse effect of purified Fx was observed in mice given 500 and  
113 1000 mg/kg Fx for 30 days [23] and in rats given 200 mg/kg Fx as Fx-containing oil for  
114 13 weeks [24]. Additional animal studies [25-27] and a human study [28] confirmed that  
115 Fx caused no toxicities. On the basis of remarkable biological properties and safety, Fx  
116 can be considered a nutraceutical ingredient and can be utilized in the food industry and  
117 other fields to design new and improved nutraceuticals.

### 118 119 **3. Absorption and metabolism**

120  
121 An *In vitro* study showed the hydrolysis of Fx to fucoxanthinol (FxOH) during  
122 absorption by Caco-2 cells [29] and the transformation of FxOH into amarouciaxanthin  
123 A in human hepatoma HepG2 cells [30] (Fig. 2). An animal study also revealed that  
124 dietary Fx is rapidly hydrolyzed to FxOH in the gastrointestinal tract by digestive  
125 enzymes, such as lipase and cholesterol esterase within 2 h of administration [30,31].  
126 FxOH was further converted into amarouciaxanthin A through  
127 dehydrogenation/isomerization in mice liver microsomes and in HepG2 cells [30,32].  
128 Although FxOH and amarouciaxanthin A have been detected in plasma and all tissues of  
129 mice given Fx [30,32-34], most Fx metabolites preferentially accumulate as  
130 amarouciaxanthin A in the visceral WAT [33,35]. Yonekura et al. [36] have also reported  
131 the preferential accumulation of Fx metabolites in visceral WAT, while these researchers  
132 observed that lutein and its metabolites mostly accumulated in the liver. On the other  
133 hand, some researchers have demonstrated the absorption of Fx without conversion to  
134 any metabolites [33,37].

135 Several studies have discussed the bioavailability of Fx in cellular, animal, and human  
136 models. The absorption rate of Fx in Caco-2 cells was reported to be the lowest out of  
137 11 carotenoids tested [38]; however, the study only analyzed intact Fx but not its  
138 metabolites. Based on animal studies showed the ratio of absorbed Fx to the dose  
139 calculated using metabolite analysis, FxOH and amarouciaxanthin A exhibited higher  
140 levels than astaxanthin [33]. Other animal studies showed that Fx is absorbed in a  
141 similar fashion to  $\beta$ -carotene or lutein [30,39,40]. Furthermore, those authors concluded  
142 that Fx is more efficiently absorbed than lutein esters. In contrast, Hashimoto et al. [41]  
143 demonstrated from a human pharmacokinetics study that the bioaccessibility of Fx

144 seems to be lower than that of other carotenoids such as  $\beta$ -carotene, lutein or  
145 astaxanthin, while they demonstrated that the bioavailability of Fx was higher in human  
146 subjects than in mice. To increase bioaccessibility and stability of Fx, many challenges  
147 have been done [2]. They include encapsulation of Fx into nanoemulsions, nanoparticles,  
148 and other spray-dried powders.

149 Fx supplementation in a mouse model of obesity effectively decreased excess fat  
150 accumulation in abdominal white adipose tissue (WAT). This activity has been reported  
151 with at least 60 mg Fx intake/kg mouse/day [35]. On the other hand, a human study  
152 demonstrated a significant reduction in the abdominal WAT of obese female volunteers  
153 with an intake of Fx less than 0.024 mg/kg/day (2.4 mg intake/day for volunteers with  
154 100 kg average weight) [42]. This difference in the effectiveness between rodents and  
155 humans may be due to different absorption rates and/or different sensitivities to Fx.  
156 Overall, Fx may be effective even at low levels.

157

## 158 **4. Biological activities**

159

### 160 *4.1. Antioxidant activity*

161

162 As seen in other carotenoids, Fx can quench singlet oxygen through a physical  
163 interaction, where the excess energy of singlet oxygen is transferred to the conjugated  
164 polyene structure of Fx [43-45] (Fig. 1). Fx with added energy is excited to a triplet  
165 state upon losing energy as heat relaxes to a singlet state without structural changes. The  
166 singlet oxygen quenching activity of a carotenoid is generally influenced by the number  
167 of conjugated double bonds [45,46]. In addition, the activity is also affected by other  
168 factors such as the chain structure and functional groups of carotenoids, solvent  
169 viscosity, and substrate dispersion system [47-50]. Sachindra et al. [51] reported that Fx  
170 has 9 conjugated double bonds and has a lower quenching ability of singlet oxygen than  
171  $\beta$ -carotene (11 conjugated double bonds), while Hirayama et al. [48] reported little  
172 difference in quenching ability between Fx and  $\beta$ -carotene. Further, more effective  
173 prevention of lipid hydroperoxide formation by Fx was found in singlet  
174 oxygen-mediated plasma lipid oxidation than by  $\beta$ -carotene and  $\alpha$ -tocopherol [52].

175 Another role of Fx as an antioxidant is attributed to the scavenging of a wide range  
176 of free radicals (Fig. 1). Fx has a unique chemical structure including an allenic bond,  
177 epoxide group, and hydroxyl group. The electron-rich status of Fx makes it more  
178 suitable for reactions with the free radicals [21]. Several studies have reported an  
179 effective radical scavenging ability of Fx [53]. Although a detailed mechanism has not

180 yet been determined, Fx can quench different kinds of free radicals such as  
181 1,1-diphenyl-2-picrylhydrazyl (DPPH) [51,54-57], 2,2'-azinobis-3-ethylbenzo  
182 thizoline-6-sulphonate (ABTS) [51,57], hydroxyl [51,57], superoxide [51,57], and  
183 peroxy [58] radicals.

184 On the basis of the potential antioxidant properties of Fx, researchers examined the  
185 preventive effect of Fx on oxidative damage in biological systems. Murakami et al. [59]  
186 screened 19 natural carotenoids for their structure-function relationship with respect to  
187 radical scavenging activity. They found that the presence of an allenic bond, as seen in  
188 Fx and halocynthiaxanthin, increases the ability to inhibit the formation of superoxide in  
189 human promyelocytic HL-60 cells and of nitric oxide in mouse macrophage RAW  
190 264.7 cells. Fx significantly reduced ROS production and the viability of oxidatively  
191 damaged monkey kidney fibroblast cells [60], human HaCaT keratinocytes [61], human  
192 hematoma HepG2 cells [62], and normal human hepatic L02 cells [63]. The antioxidant  
193 activity of Fx has also been reported *in vivo*. When oxidative stress was induced by  
194 retinol deficiency in rats, Fx significantly reduced lipid hydroperoxide levels of plasma,  
195 liver, and liver microsomes [64]. In another animal experiment [65], Fx  
196 supplementation significantly increased the total antioxidant capacity in plasma. The  
197 antioxidant activity of Fx is not only based on its singlet oxygen and free radical  
198 scavenging activities but also strongly related to its upregulation of antioxidant enzymes  
199 such as catalase [64] and glutathione peroxidase [65]. In the upregulation pathway,  
200 several studies have demonstrated the involvement of the activation of Akt/nuclear  
201 factor-erythroid 2-related (Nrf2) by Fx [63,65,66].

202

#### 203 4.2. *Anti-obesity effect*

204

205 Many reviews have been published on the protective effects of Fx against various  
206 diseases [7,21,53,67-76] (Fig. 2). Of all characteristics of Fx, anti-obesity is certainly  
207 the most well-studied and promising [21,67,68,70,72,76].

208 Anti-obesity properties of Fx were first discovered in rats and mice given brown  
209 seaweed lipids containing Fx [77]. This effect has been confirmed using various animal  
210 models [78-84]. In addition, a comparative study indicated that Fx attenuates excess fat  
211 accumulation in the abdominal WAT of the obese KK-*A<sup>y</sup>* mice [79] and of C57BL/6J  
212 mice fed a high-fat diet [83], while no effect was found in the C57BL/6J mice fed a  
213 normal-fat diet [79]. These results suggest the suppressive effect of Fx on WAT weight  
214 gain is specific for adiposity in the development of obesity in mice. This specificity will  
215 be important for the safe application of Fx in human therapies for obesity. In a human

216 clinical trial, 2.4 mg Fx daily for volunteers (average weight 100 kg) resulted in a  
217 significant decrease in body fat, body weight, liver fat content, and serum triglyceride  
218 levels, which was accompanied by improvement in liver function tests [42]. The clinical  
219 trial also demonstrated an increase in the resting energy expenditure after >2.4 mg Fx  
220 intake. Hitoe and Shimoda [28] also examined the effect of Fx on mildly obese Japanese  
221 volunteers and reported a significant reduction of relative body weight, body mass index,  
222 and visceral fat area after 3 mg daily Fx intake for 4 weeks. Relative values of total fat  
223 mass, subcutaneous fat area, waist circumference, and right thigh circumference were  
224 also significantly lower after 1 mg Fx intake compared to the placebo group. Another  
225 study reported the induction of BAT expression by Fx intake in obese human subjects  
226 assessed by <sup>18</sup>F-fluorodeoxyglucose-positron emission tomography [85].

227 Obesity is defined as a condition of excess body fat induced by increased energy  
228 intake and/or reduced energy expenditure. Obesity is associated with a large number of  
229 metabolic disorders that induce cardiovascular and various other non-communicable  
230 diseases. Lifestyle interventions, such as a change in dietary habits and increased  
231 physical activity, are fundamentally important to obesity therapy [86]. In addition to  
232 these essential interventions, much attention has been paid to nutritional and dietary  
233 factors, especially metabolically active food compounds. Major molecular mechanisms  
234 for controlling obesity with nutrition include reducing food intake through the control of  
235 signals from the gut and adipose tissue; inhibiting nutrient absorption; increasing  
236 thermogenesis to dissipate food energy as heat; and modulating fat synthesis/lipolysis or  
237 adipose differentiation/apoptosis [87,88]. Many functional food components have been  
238 shown to alter energy metabolism by influencing fat absorption, substrate utilization  
239 rate, and thermogenesis.

240 Upregulation of sympathetically mediated thermogenesis is the most targeted  
241 component when developing functional foods for obesity therapy. Uncoupling protein 1  
242 (UCP1) expression is a major factor in this thermogenic process. UCP1 can be induced  
243 in brown adipose tissue (BAT) [89-91] and in beige adipocytes of WAT [92-94].  
244 Although several mechanisms have been proposed for the anti-obesity effects of Fx,  
245 adaptive thermogenesis via UCP1 induction in adipose tissue is the major target of Fx  
246 [21,22,53,68,72,73,95] (Fig. 3). Activated UCP1, short circuits the electrochemical  
247 gradient normally used to drive adenosine triphosphate (ATP) synthesis. This can occur  
248 with the re-entry of protons into the mitochondrial matrix, bypassing ATP synthase. The  
249 uncoupling of oxidative phosphorylation releases excess energy intake as heat. Feeding  
250 Fx to mice increased BAT weight and induced UCP1 mRNA and protein expressions in  
251 abdominal WAT [77,81,82], suggesting that the anti-obesity effect of Fx is derived from



252 an increase in the adaptive thermogenesis through UCP1 expression. In addition, Fx  
253 supplementation in animal models increased the mRNA and protein expression of  
254 several biomolecules, such as  $\beta$ 3-adrenarine receptor ( $\beta$ 3Ad) and peroxisome  
255 proliferator-activated receptor gamma co-activator 1 (PGC-1), in WAT [83,96]. The  
256 upregulation of  $\beta$ 3Ad and PGC-1 are known to positively regulate UCP-1 expression  
257 [21]. Furthermore, PGC-1 upregulation can also induce mitochondrial biogenesis.  
258 Therefore, the anti-obesity effect of Fx would also be related to the increase in  
259 metabolic rate induced by mitochondrial activation [97] (Fig. 3).

260 Several studies have also shown that Fx ameliorates obesity through its effects on  
261 lipid metabolism. Woo et al. [98] reported an increase in the content of non-digested  
262 fecal lipids and a decrease in hepatic lipid and plasma triacylglycerol levels by Fx  
263 supplementation to C57BL/6N mice fed a high-fat diet. The effect of Fx could be  
264 explained by reduced activity of hepatic lipogenesis and enhanced activity of fatty acid  
265  $\beta$ -oxidation [98,99]. In another study using obese mice fed a high fat diet [84], the  
266 mRNA expression and activity of lipogenic enzymes were significantly downregulated  
267 in a dose-dependent manner in epididymal adipose tissue, with simultaneous  
268 upregulation of fatty acid  $\beta$ -oxidation. In addition, Fx increased the activities of key  
269 enzymes in lipid metabolism, such as AMP-activated protein kinase and its downstream  
270 target acetyl-CoA carboxylase in epididymal adipose tissue of diet-induced obese mice  
271 [80]. Moreover, Fx and FxOH have been demonstrated to inhibit pancreatic lipase  
272 activity and suppress triacylglycerol absorption after oral infusion with oil [31].  
273 Improvement of hepatic lipid metabolism by Fx intake would be related to risk  
274 reduction in non-alcoholic fatty liver disease (NAFLD) [100]. Potential protective  
275 functions of Fx against the development of NAFLD have also been recognized in a  
276 human study [42]. Consumption of an Fx supplement containing 2.4 mg of pure Fx for  
277 16 weeks decreased liver fat content and serum concentrations of TAG and C-reactive  
278 protein in obese premenopausal women with NAFLD.

279 The regulatory effect of Fx and Fx metabolites on adipocyte differentiation may be  
280 involved in its anti-obesity effects [21,73]. These compounds suppressed murine  
281 pre-adipocyte differentiation to mature adipocytes by inhibiting intracellular lipid  
282 accumulation and decreasing glycerol-3-phosphate dehydrogenase activity [101,102].  
283 When the suppressive effect of Fx and Fx metabolites on the differentiation of 3T3-L1  
284 preadipocytes to adipocytes was compared [101,102], amarouciaxanthin A showed the  
285 strongest effect, followed by FxOH and Fx. Fx and FxOH downregulated adipogenic  
286 genes, such as peroxisome proliferator-activated receptor  $\gamma$  (PPAR $\gamma$ ) and  
287 CCAAT/enhancer binding protein  $\alpha$  (C/EBP $\alpha$ ), in a dose-dependent manner. On the

288 other hand, Kang et al. [103] has demonstrated that the effect of Fx on adipocytes  
289 depends on its differentiation stage, early (days 0-2), intermediate (days 2-4), and late  
290 stage (days 4-7). Fx paradoxically promoted adipocyte differentiation during the first  
291 two days by increasing the expression of PPAR $\gamma$ , C/EBP $\alpha$ , sterol regulatory  
292 element-binding protein 1c, and adipocyte fatty acid-binding protein, while it inhibited  
293 differentiation at later stages by reducing these protein expression levels.

294 Okada et al. [104] compared the suppressive effect of 13 naturally occurring  
295 carotenoids on the differentiation of 3T3-L1 adipose cells. Among these carotenoids,  
296 neoxanthin did show suppressive effects on lipid accumulation, glycerol-3-phosphate  
297 dehydrogenase (GPDH) activity and adipocyte protein 2 expression in the 3T3-L1  
298 differentiation. However, treatment with (rac)- $\alpha$ -carotene, carotenoids with keto group  
299 (citraxanthin, rhodoxanthin, canthaxanthin) and an epoxy group ( $\beta$ -carotene  
300 5,6-epoxide) did not result in apparent changes in the level of GPDH activity. The same  
301 was true for hydroxyl carotenoid ( $\beta$ -cryptoxanthin, lutein), epoxy-hydroxy carotenoids  
302 (violaxanthin, antheraxanthin, lutein epoxide), and keto-hydroxy cerotenoids  
303 (capsorubin). Interestingly, neoxanthin contain allenic bond, a 5,6-monoepoxide, and  
304 three hydroxyl groups and this structure is very similar in structure to FxOH and  
305 amarousiaxanthin A, major metabolites of Fx. These findings provide further evidence  
306 for the theory that suppressive effect on adipocyte differentiation in carotenoids are  
307 related to structural properties, where allenic bond is essential for the expression of the  
308 activity.

309

#### 310 *4.3. Anti-diabetic effect*

311

312 Obesity has been recognized as a driver of type 2 diabetes [105]. Indeed, the  
313 majority of patients with type 2 diabetes are obese [106], and the increased incidence of  
314 type 2 diabetes has paralleled that of obesity. Excess fat accumulation in abdominal  
315 WAT in obese individuals increases the secretion of biologically active mediators,  
316 termed adipokines/chemokines, from adipocytes, as part of the endocrine system [21].  
317 Development of obesity leads to an increase in pro-inflammatory adipokines such as  
318 tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ), interleukin-6 (IL-6) and monocyte chemoattractant  
319 protein-1. These pro-inflammatory adipokines induce macrophage infiltration into the  
320 abdominal WAT, leading to chronic low-grade inflammation. Furthermore, saturated  
321 fatty acid and TNF- $\alpha$  derived from adipocytes and macrophages, respectively, initiate a  
322 paracrine loop that leads to inflammation in the adipose tissue and upregulation of  
323 pro-inflammatory adipokine secretions. These adipokines are reported to increase

324 insulin sensitivity [107]. On the other hand, Fx supplementation significantly inhibited  
325 macrophage infiltration and downregulated pro-inflammatory adipokine expression and  
326 secretion in the abdominal WAT of obese/diabetes KK-*A*<sup>y</sup> mice, resulting in the  
327 improvement of insulin resistance and subsequent blood glucose levels [79,82].  
328 Normalization of blood glucose levels by Fx intake has also been observed in C57BL/6J  
329 mice fed a high fat diet [83,98,99], while Fx did not affect the blood glucose levels in  
330 C57BL/6J mice fed a normal diet [79], suggesting the specificity of the lowering effect  
331 of Fx on diabetes.

332 Another possible mechanism for the anti-diabetic effect of Fx is the regulation of  
333 glucose transporter 4 (GLUT4) [83,96]. GLUT4 protein is the predominant isoform of  
334 the glucose transporters expressed abundantly in skeletal muscle and adipose tissue.  
335 With insulin and other stimuli, GLUT4 expression is upregulated, quickly moves to the  
336 plasma membrane from an intracellular location, and promotes glucose uptake [108].  
337 However, in type 2 diabetes mellitus, insulin signaling is impaired and GLUT4  
338 expression and its translocation is attenuated [109]. When mice were fed high fat (HF)  
339 or normal fat (NF) diets, the HF group experienced hyperglycemia, hyperinsulinemia  
340 and hyperleptinemia with a significant decrease in GLUT4 mRNA levels in skeletal  
341 muscle compared to the NF group [83]. These disorders were completely normalized by  
342 the addition of Fx to the HF diet, and GLUT4 mRNA levels in the HF group with Fx  
343 were restored to levels observed in the NF group. These anti-diabetic effects of Fx were  
344 recapitulated in obese/diabetes KK-*A*<sup>y</sup> mice [96]. A significant increase in GLUT4  
345 levels was found in the extensor digitorum longus muscle of the obese/diabetes mouse  
346 model together with an upregulation of PGC-1 $\alpha$  expression [96]. PGC-1 $\alpha$  is an  
347 important co-activator that has been implicated in the regulation of mitochondrial  
348 biogenesis and the activation of GLUT4 [110,111]. In addition, Fx supplementation  
349 significantly increased GLUT4 translocation to plasma membranes from the cytosol in  
350 the soleus muscle of KK-*A*<sup>y</sup> mice.

351

#### 352 4.4. Anticancer activity

353

354 Compared with other carotinoids, Fx is known to exhibit stronger anti-proliferative  
355 effects on several cancer cell types [69,112]. Fx strongly decreased the viability of  
356 many cancer cell types such as human neuroblastoma GOTT [113], leukemia (HD-60)  
357 [114], epithelial colorectal adenocarcinoma (Caco-2, DLD-1 and HT-29) [115], human  
358 prostate cancer (PC-3) [116], mouse melanoma (B16) and human colorectal carcinoma  
359 (HCT116) [117], while it did not affect the normal cell viability [117]. Kotake-Nara et

360 al. [118] compared the effect of 15 kinds of carotenoids (phytoene, phytofluene,  
361  $\xi$ -carotene, lycopene,  $\alpha$ -carotene,  $\beta$ -carotene,  $\beta$ -cryptoxanthin, canthaxanthin,  
362 astaxanthin, capsanthin, lutein, zeaxanthin, vioxanthin, neoxanthin, and fucoxanthin)  
363 present in foodstuffs on the growth of human prostate cancer cell lines (PC-3, DU 145  
364 and LNCap). Among the carotenoids evaluated, allenic carotenoids, neoxanthin and Fx,  
365 showed the higher activity in the growth reduction of these prostate cancer cells as  
366 compared with other kinds of carotenoids without allenic bond, suggesting the  
367 importance of allenic bond in the anti-proliferative ability of carotenoids.

368 Fx intake also suppressed the number and growth of tumors in animal models  
369 [119-122]. In addition, brown seaweed extract (containing Fx showed chemopreventive  
370 activity against the formation of aberrant crypt foci, a preneoplastic marker for colon  
371 cancer, in rats [123,124]. To investigate the underlying mechanisms of the anti-cancer  
372 potential of Fx, many studies have focused on several biomolecules and signaling  
373 pathways involved in either cell cycle arrest, apoptosis, or metastasis suppression  
374 [37,67,69,71,112]. These studies suggest that Fx could arrest the cell cycle of tumor  
375 cells in the G0/G1 and/or G2/M phase by altering the expression of various genes  
376 including upregulating GADD45, p21 and p27 and downregulating cyclin D1, cyclin  
377 D2, CDK4, and survivin [37,67,69,71,75,112,125]. The apoptotic effect of Fx is  
378 well-studied because apoptosis of cancer cells is a promising method to control and treat  
379 cancer. Fx induces apoptosis by targeting different molecular pathways including Bcl-2,  
380 caspase, mitogen-activated protein kinase, nuclear factor kappa B families, and others  
381 [69,71,75].

382 Recently, efforts have focused on the chemopreventive effect of Fx in colorectal  
383 cancer. Takahashi et al. [126] found that FxOH showed higher anti-proliferative activity  
384 than Fx on different kinds of colorectal cancer cell lines including DLD-1, HCT116,  
385 SW620, Caco-2, Colo205, and WiDr. The anti-proliferative capacity of FxOH was also  
386 effective in colorectal cancer stem cells (CD44<sup>high</sup> and EpCAM<sup>high</sup> cells) [127]. These  
387 cells initiate colorectal carcinogenesis and play a central role in tumor development,  
388 exhibiting several biological properties including self-renewal, multipotential,  
389 drug-resistance, sphere formation, and tumor formation in xenograft models. FxOH  
390 significantly inhibited the growth of the colorectal cancer stem cells [127]. Fx also  
391 suppressed sphere-forming activity, migration and invasion of colorectal cancer stem  
392 cells in a dose-dependent manner [128] and downregulated several biomolecules related  
393 to cell proliferation, cell cycle, metastasis, and extracellular adhesion [127]. In addition,  
394 Fx induced anchorage-dependent apoptosis in human colorectal cancer (CRC) cells  
395 through the suppression of integrin signals [129,130]. In animal models, Fx

396 administration significantly suppressed tumor development in a xenograft model of  
397 colorectal cancer [127,131], decreased the number of colorectal polyps, and decreased  
398 colonic lesions compared to untreated control mice [132]. In addition, Fx administration  
399 resulted in significantly lower numbers of colorectal cancer stem cell-like cells,  
400 cancer-associated fibroblast-like, tumor-associated macrophage-like and dendritic  
401 cell-like cells in colonic mucosa compared to untreated control mice [132].

402

#### 403 *4.5. Other activities*

404

405 Given that metabolic syndrome and obesity are regarded as major risk factors for the  
406 induction of cardiovascular disease (CVD), much attention has been paid to the  
407 anti-obesity and anti-diabetic effects Fx. In addition, Fx is known to show *in vivo*  
408 anti-oxidant, anti-inflammatory, and anti-hypertensive activities. These abilities are also  
409 important in the context of CVD. The antioxidant activity of Fx has been well-described,  
410 and several studies have reported anti-inflammatory [7] and anti-hypertensive [53,133]  
411 activities of Fx. Effective downregulation of lipopolysaccharide-induced inflammatory  
412 signaling pathways has been found in cellular models supplemented with Fx [134-136].  
413 In these models, Fx significantly suppressed the expression and secretion of  
414 inflammatory mediators such as nitric oxide, prostaglandin E<sub>2</sub>, TNF- $\alpha$ , IL-6, and IL-1 $\beta$   
415 and inflammatory cytokines such as cyclooxygenase (COX) and inducible nitric oxide  
416 synthase (iNOS) [137]. The levels of inflammatory markers such as IL-1 $\beta$ , TNF- $\alpha$ ,  
417 iNOS, and COX-2 were downregulated in an obese mouse model [138]. The protective  
418 effect of fucoxanthin was further described in UV-induced inflammation in cellular  
419 [139] and animal [140] models. Fx has also been reported exhibit anti-hypertensive  
420 properties. Supplementation of brown seaweed containing Fx delayed the incidence of  
421 stroke symptoms and increased the life span of stroke-prone spontaneously hypertensive  
422 rats [141], although there was no significant difference in the blood pressure with Fx  
423 intake. Fx isolated from brown seaweed may also have a preventive effect on ischemic  
424 cultured neuronal cell death. An interesting, extra metabolic effect of Fx is the  
425 promotion of the synthesis of docosahexaenoic acid (DHA) in the liver, resulting in  
426 improvements in the lipid profile [34,142]. DHA is known to positively influence  
427 human nutrition and health including cardioprotection. DHA could inhibit the  
428 development of inflammation in endothelial cells, alter the function and regulation of  
429 vascular biomarkers, and reduce cardiovascular risk [143]. It can also improve  
430 hypertriglyceridemia, which is known to increase cardiovascular risk [144]. Therefore,  
431 the protective effect of Fx on CVD maybe explained by an increase in DHA levels.

432 Fucoxanthin protected neuronal cells against oxidative damage induced by H<sub>2</sub>O<sub>2</sub>  
433 [145,146] and oligomers of  $\beta$ -amyloid (A $\beta$ ) [147,148]. This effect involved the  
434 activation of PI3-K/Akt cascade and inhibition of ERK pathway by Fx [146,148]. A $\beta$   
435 oligomers are known as major neurotoxins in Alzheimer's disease (AD). Fx potently  
436 reduced the formation of A $\beta$  oligomers *in vitro* and *in vivo* [149]. In the brains of AD  
437 patients, neuronal degeneration is accompanied by markers of microglial activation and  
438 inflammation, as well as oxidant damage. Fx can ameliorate oxidative stress and  
439 inflammation in A $\beta$ 42-induced BV2 microglia cells [150]. In addition, Fx plays a  
440 protective role in animal models of traumatic brain injury [151] and middle cerebral  
441 artery occlusion [152]. Although the underlying mechanism of the neuroprotective  
442 effect of Fx has not been fully elucidated, molecular docking studies suggest the  
443 importance of interactions between Fx and key proteins related to brain function [153].  
444 Paudel et al. [154] have shown that Fx could serve as a potent dopamine D3/D4 agonist  
445 that might be useful in the management of neurodegenerative diseases, especially  
446 Parkinson's disease. On the other hand,  $\beta$ -site amyloid precursor protein cleaving  
447 enzyme 1 (BACE1) levels are known to be elevated in sporadic AD brains at disease  
448 onset. The BACE1 levels are upregulated under stress conditions such as cerebral  
449 ischemia, hypoxia, and oxidative stress. Fx inhibited the BACE1 activity through the  
450 interaction between two hydroxyl groups on the Fx molecule and two additional  
451 BACE1 residues (Gly11 and Ala127) [155].

452

## 453 **5. Fx source and its extraction**

454

455 Although Fx can be synthesized chemically, extraction from brown seaweed is a  
456 more accessible, safe, and economic method [75]. The Fx content in brown seaweed  
457 varies greatly by species, geographical location, season, temperature, salinity, and light  
458 intensity, as well as interactions among these factors [5]. Comparative studies on 15  
459 brown seaweed samples collected near the northern coast of Japan have revealed that  
460 higher levels of Fx were found in *Sargassum horneri* (Turner) (370 mg/100 g dry  
461 weight) and *Cystoseira hakodatensis* (Yendo) (240 mg/100 g dry weight) [156]. The  
462 same research group also examined seasonal changes in the lipid components of the two  
463 brown seaweeds, *S. horneri* and *C. hakodatensis* [157]. They collected young thalli of  
464 both brown seaweeds cultivated in the northern coast of Japan. The Fx content of both  
465 brown seaweeds increased from October/November, reached a maximum in January,  
466 and decreased thereafter. In January, the Fx content of *S. horneri* and *C. hakodatensis*  
467 was 449 mg and 363 mg/100 g dry weight, respectively. Furthermore, by controlling the

468 cultivation conditions, such as temperature, light, and depth, a high content of Fx (1080  
469 mg/100 g dry weight) was found in *S. horneri* cultivated in cold water [158].

470 Fx is generally extracted from brown seaweed with organic solvents [159-166].  
471 However, recovery remains low due to the presence of various physical and chemical  
472 barriers in the complex matrix. In addition, conventional extraction methods are  
473 time-consuming and require a large amount of organic solvent. To overcome the  
474 disadvantages of conventional solvent extractions, several studies have reported  
475 advanced techniques, such as pressurized liquid extraction [167], enzyme-assisted  
476 extraction [168], and extraction with supercritical CO<sub>2</sub> [169-173]. Much interest has  
477 been generated in the development of new, eco-friendly alternatives to petrochemical  
478 organic solvents. These ideal alternative “green” solvents, should be nontoxic,  
479 bio-based, and environmentally friendly. Supercritical CO<sub>2</sub> is regarded as a green  
480 solvent. However, this method requires expensive investment and a complex operating  
481 system with high operating costs [174-176]. In addition, the solubility of Fx in  
482 supercritical CO<sub>2</sub> is low, requiring the use of cosolvents [172].

483 Considering the increase in consumer concern regarding organic solvent  
484 contamination in the final food product, the development of new eco-friendly solvents is  
485 still required. The use of edible oils for the extraction of Fx from brown seaweed is  
486 promising. Edible oils are regarded as green alternative solvents with no volatile organic  
487 compounds, low toxicity for humans, and a limited impact on the environment. Due to  
488 their oil solubility, carotenoids can be extracted with edible oils from natural resources.  
489 Products from carotenoid extraction with edible oils can be directly used as food  
490 materials without purification, and the oil protects carotenoids from degrading [177].  
491 This advantage is not found using other green extraction methods. When the edible oil  
492 used for carotenoid extraction shows any type of nutritional functionality, as seen in  
493 omega-3 oils [178,179], medium-chain triacylglycerol (TAG) (MCT) [180-182], olive  
494 oil [183,184], and others, the extracts are expected to show the combined biological  
495 activities originating from phytochemicals such as carotenoids and from the functional  
496 oils.

497 Although several studies have highlighted many advantages for extracting  
498 carotenoids with edible oils from shrimp waste [185-188], crawfish waste [189-191],  
499 microalgae, *Haematococcus pluvialis* [192,193], and fresh carrots [194], little is known  
500 about their application to Fx extraction. Recently, Teramukai et al. [195] reported  
501 effective extraction of Fx from brown seaweed, *Sargassum horneri*. When the  
502 extraction rate was compared with 12 types of edible oils, more Fx could be extracted  
503 with short-chain (tributylin, C4 and tricaprionin, C6) TAG, medium-chain (tricaprylin,

504 C8) TAG (MCT), and fish oil compared with other edible oils; e.g., rice bran, rice germ,  
505 rapeseed, sesame, corn, soybean, and linseed [195]. MCT reportedly increases the  
506 anti-obesity effect of Fx [81]. WAT weight gain was markedly lower in diabetic/obese  
507 KK-*A*<sup>y</sup> mice fed a mixture of Fx (0.1%) and MCT (0.9%) than in mice fed Fx (0.1%)  
508 alone. In addition, the expression of UCP1 was also markedly increased by MCT  
509 co-supplementation with Fx. Furthermore, an increase in anti-obesity and anti-diabetic  
510 effects of Fx has been reported with the combination of fish oil rich in EPA and DHA  
511 [82]. These results suggest that Fx extracts from brown seaweed with MCT and fish oil  
512 may show higher biological activities than those with organic solvent.

513

## 514 **6. Conclusion**

515

516 Since the discovery of the anti-obesity effect of Fx with UCP1 induction in  
517 abdominal WAT, many studies have been conducted on the nutritional impact of Fx.  
518 Increasing data clearly shows that Fx is effective at reducing the risk of a surprisingly  
519 wide variety of dysfunctions and diseases, including metabolic syndrome, obesity, heart  
520 disease, diabetes, cancer, hypertension, and reactive oxygen species- and  
521 inflammation-associated disorders. To explain the underlying mechanism, studies have  
522 demonstrated the modulation effect of Fx or its metabolites on signaling pathways  
523 related to these dysfunctions and diseases. The biological activities of Fx may be due to  
524 its unique chemical structure and its interaction with important biomolecules such as  
525 receptor proteins. Thus, more research is required to clarify the interactions by  
526 analyzing binding sites of the Fx molecule, the affinity for specific biomolecules, the  
527 molecular docking, etc.

528

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Figure legends

- 1224 Fig. 1. Antioxidant activity of Fx.
- 1225 Fig. 2. Major biological impact of Fx.
- 1226 Fig. 3. Major possible mechanism for the anti-obesity effect of Fx.

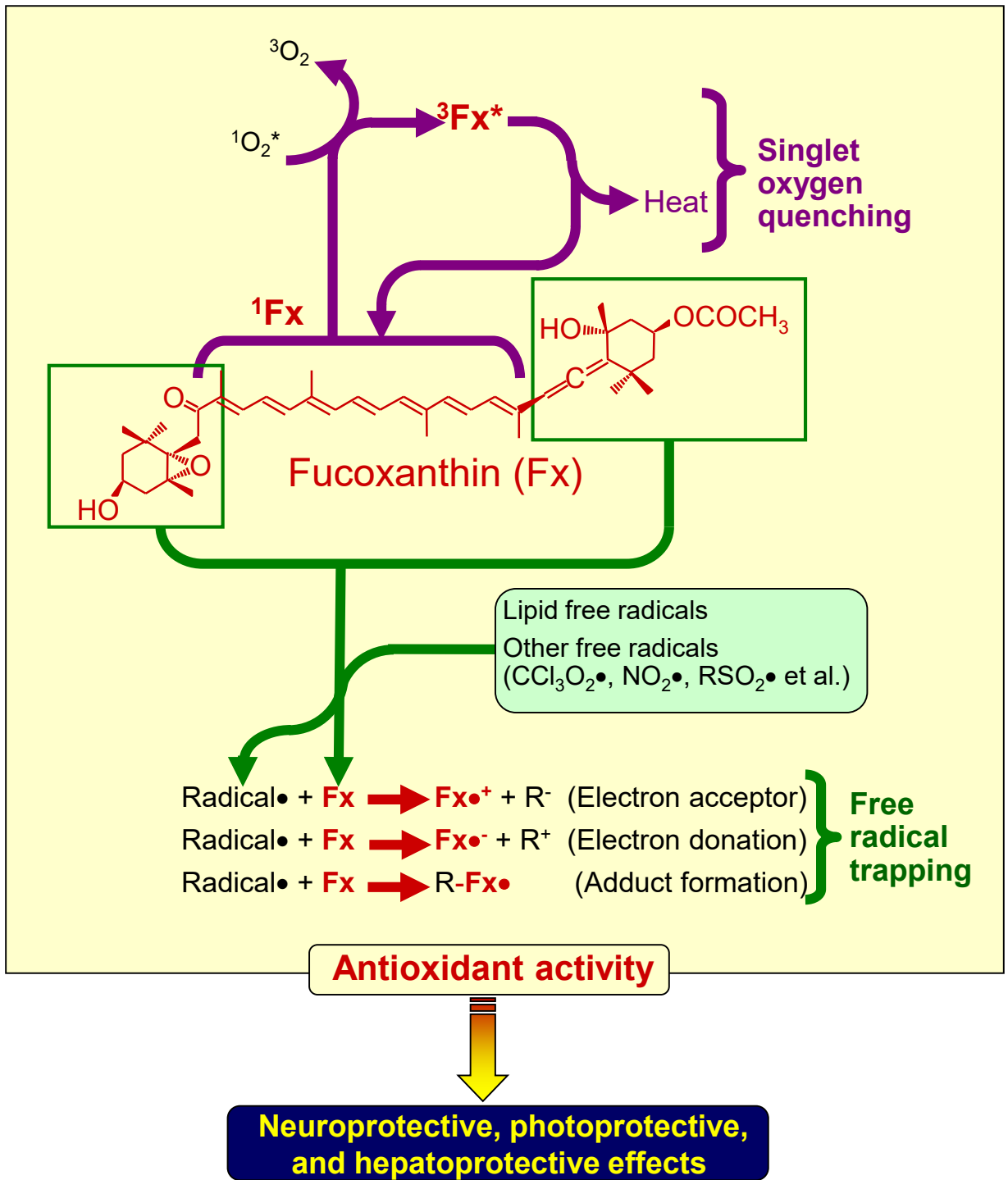


Fig. 1

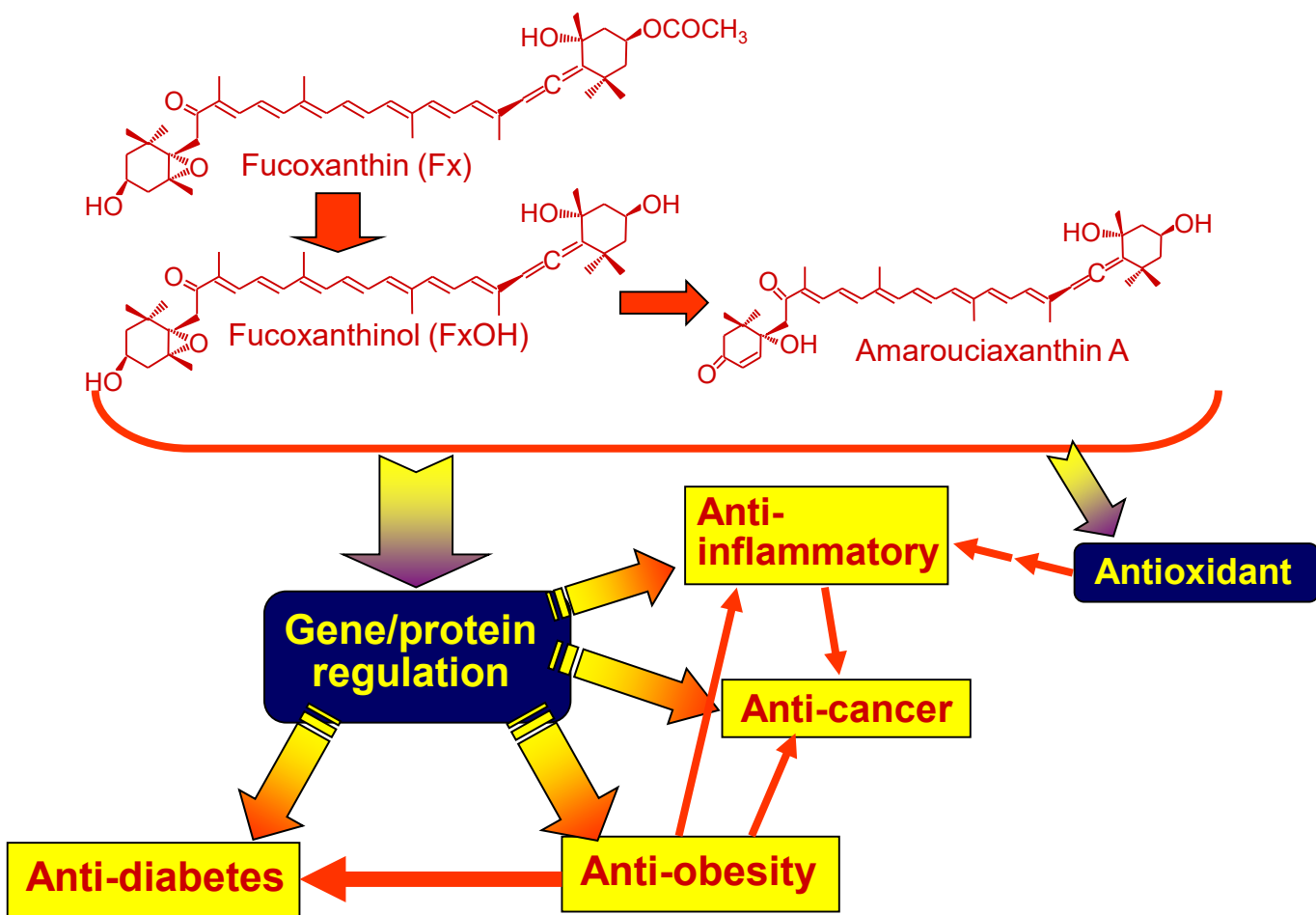


Fig. 2

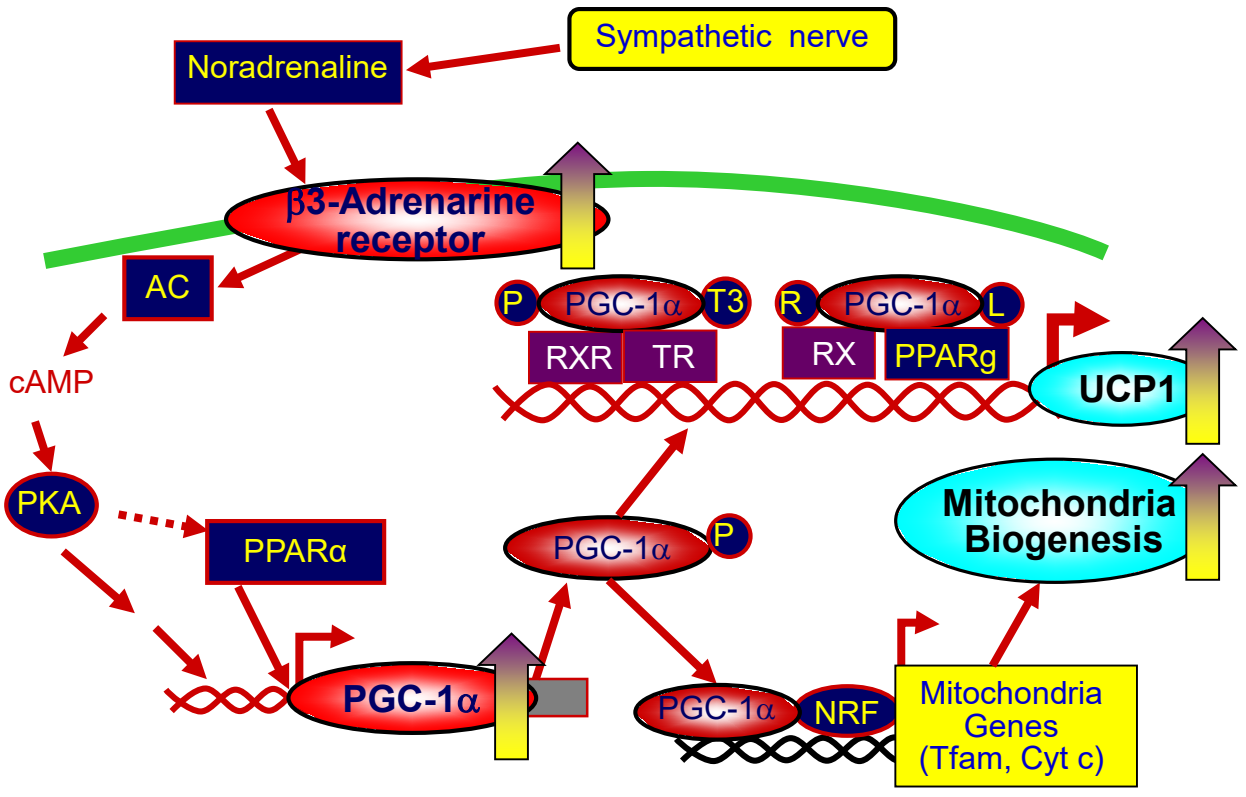


Fig. 3