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MODERATION IN TAKING NUTRITION OF VITAMIN H (BIOTIN) KEEPS THE DOCTOR AWAY.

Kou Hayakawa[1]*
*[1]*Most recently retired) Graduate School of Health Sciences, Gunma University 3-39-15 Showa-achi, Maebashi, Gunma 371-8514, Japan. (Formerly retired) Department of Endocrinology and Metabolism, National Research Institute for Child Health and Development, 2-10-1 Okura, Setagaya-ku, Tokyo 157-8535, Japan.*
To whom correspondence should be addressed.
2-5-B-308 Fukiage-Fujimi, Kohnosu-city, Saitama 369-0121, Japan Tel; +81-48-548-1733 Fax; +81-48-548-1733

Corresponding Author:
E-mail; amokha123@true.ocn.ne.jp

Abstracts: -
*English maxim says that "Feed by measure and defy the physician". In this review, I would like to demonstrate the truth of this proverb for **biotin (vitamin H)** and **lipoic acid (thioctic acid)**. Recently, I have found that D-biotin regulates the membrane biosynthesis in the cells. Thus, both too extreme and too few amounts of biotin reduces the ratio of the membrane components leading to the disease. Further, the polysaccharide fucoidan derived from the edible brown alga of **Japanese Mozuku** gives the adequate ratio of the membrane components via giving the measure of biotin and lipoic acid. Thus, adequate lipoic acid-. fucoidan-, biotin-, and balanced-nutrition- intakes are expected to be the chief of all medicines combatting against such severe diseases as primary biliary cirrhosis, human liver cancer, depression, mania, cleft lip, Down syndrome, diabetes, atopic dermati- tis, amyotrophic lateral sclerosis, dementia, Alzheimer disease, and against Middle-East-respiratory- syndrome corona and human immunodeficiency viruses.*

Keywords:-
Biotin (vitamin H).Thiol-type biotinidase/lipoamidase. Membrane components. Fucoidan. lipoic acid. avidin (biotin/lipoate-binding glycoprotein).

Abbreviations:
PDMD protein-direct-micro sequencing-deciphering; thiol-BIN/LIP thiol-type bio- tinidase/lipoamidase; ester-LIP esterase-type lipoamidase; brain-LIP complex-type (thiol/esterase/ metallo-type) brain-lipoamidase; LA lipoic acid; LC liver cirrhosis; PBC primary biliary cirrhosis; HCV hepatitis C virus; HIV human immunodeficiency virus; MERS Middle East respiratory syndrome; ALS amyotrophic lateral sclerosis; BSE bovine spongiform encephalopathy.

I. INTRODUCTION

Homo sapiens has long been aware that moderation is the most important issue in human life from ancient times; i.e., the ancient Greek temple of Apollo at Delphi (BC 8 C) has borne the inscription "Meden Agan (μηδὲν ἄγαν; nothing in excess)", and such ancient philosophers as Aristotle (Greece; BC 4 C), Confucius (China; BC 6 C), and Gotama Siddhattha (India; BC 6 C) have already indicated about the importance of "Golden or Happy Mean". In 1712 (Edo period of Japan), Ekken Kaibara (clansman of Kuroda-Han, Fukuoka) has described that"Hara-hachibun-me ni Isha-irazu (Taking a moderate amount of foodstuffs is rejecting the physician)" in his handwritten book "Youjoh-Kun (Instruction to Preserve the Health)" [1]. In this review, it will be pointed out that this maxim is surely true for the biotin (vitamin H) and lipoic acid (LA).

Biotin (vitamin H) is an essential growth factor originally found by Fritz Kögl from duck's egg-yolk [2]. Biotin incorporates the carbon dioxide (CO_2) into organic acid to elongate the acyl chain as a co-enzyme of carboxylase [3, 4]. Biotin is de novo synthesized in plants, fungi (yeast), algae, and many bacteria, but is not synthesized in some infecting/symbiotic bacteria with mamma- lian animals and plants; i.e., such as milk-invading bacteria of Lactobacillus casei, Lactobacillus arabinosus, Propionibacterium freudenreichii (shermani), skin-infecting bacteria of Staphylococcus aureus, gut bacteria of Enterococcus (Streptococcus) faecalis, and nitrogen-fixing legume-symbiotic bacteria of Rhizobium leguminosarum (biovar trifolii). Thus, these bacteria have been employed in the bioassay of biotin; i.e., by Lactobacillus casei ε [5], Lactobacillus plantarum ATCC 8014 [6], and Rhizobium leguminosarum (biovar trifolii) [7]. These bacteria can use biotin-amide, and have biotinamide amidohydrolase (biotinidase; EC 3.5.1.12) [8]. Originally, yeast has also been used for the biotin measurement [2]. Yeast can biosynthesize biotin [9] and has biotinidase [8], but the biotin biosynthesis may be repressed if biotin is present in the medium. Microbiological- and avidin-binding- assays have long been employed in vain, since these assays use non-linear calibra- tion curve obtained from the spectrophotometer [5]. Therefore, the classical data for biotin determination employing the bioassay using biotin-deficient white-rat (the Japanese Shiro-Nezumi) without using photometer by Umetaro Suzuki [10] has now only be coincided with the data obtained from the newly developed HPLC-fluorometric method [11].

Both D-biotin and D-lipoic acid (D-LA) is not biosynthesized in the adult humans, so they are designated as vitamins. However, both biotin and also LA has recently been found to be biosynthesized in the human cultured cells (Hc; derived from normal fetal liver, HepG2; derived from HCC tissue of 15 y American Caucasoid) [12], and possibly fetal tissues, then the designation of vitamin should not be applied during the fetal growth-period of humans. It has recently firstly been described about the presence of both biotin synthetase and LA synthetase in the human im- mortal cultured liver-cell-lines, although they are not found in adult human liver-tissue specimens, sera and milk [12]. Therefore, supplementations of D-biotin and D, L-LA (α-LA) in the culture medium are effective to accelerate the growth rate of immortal human-cell-lines. But, it is note- worthy that LA in the culture medium at 0.242 mM is toxic due to unknown reason in case of the immortal rat-cell-lines such as TRL1215 (from the liver of *Fischer F344*) and NBT-T2 (from the bladder of Rattus norvegiscus) (unpublished observation).

Biotinidase (EC 3.5.1.12) is an important amidohydrolase for some bacteria, animals, and humans recycling vitamin H (D-biotin) [13 - 16]. Both high concentration of biotin [11] and high biotinidase activity [12] has been found in the chicken egg yolk. Therefore, it seems in animals that biotin concentration and biotinidase activity is strongly correlated. Human serum biotinidase (thiol-BIN/LIP) is a known thiol-type [17, 18] and glycoprotein [19, 20] enzyme. Mean specific acitivity (V) of thiol-BIN/LIP is highest (86.6 pmol/min/mg of serum protein) at the age of 18.7 years, and decreases to 0 at the age of 174 years old; i.e., destined length of the human life has been estimated to be 174 years old by using parametric quadratic-regression curve [16]. Since the distribution pattern of thiol-BIN/LIP activity at an age is not a normal or Gaussian, median values are used instead and recalculated to obtain the non-parametric life span; i.e., the median value of the human life-span recalculated by the Fig. 4 of [16] becomes to be c.a. 250 years.

Mercuric ions (Hg^{++}) at 5 μM completely inhibit the purified human serum thiol-BIN/LIP, but they interestingly activate the enzyme activity (V; 140% of the original) at the concentration of 5 nM [17]. Therefore, the ancient Chinese-Emperor of Shi Huangdi (Qin dynasty; BC 3 C), who believes the "Taoism" and finds "the elixir of immortality by mercury", may have possessed a good intuition. Although gold ion has not yet been tested on the thiol-BIN/LIP activity, an English philosopher of Roger Bacon (1214-1292) has already recommended the oral intake of gold as "the elixir of immortality" in his book of "Liber Sex Scientiarum" [21]. The addition of Fe^{+++} at 250 nM in the cell-culture medium is interestingly also effective to fast growth (unpublished obser- vation), and the additions of Hg and Au ions to the culture medium should then also be re-studied.

Glycochain of human thiol-BIN/LIP is important in its enzyme kinetics; i.e., the treatment with sialidase (neuraminidase) on purified human serum thiol-BIN/LIP has decreased the affinity (increased the K_m value) suggesting that N-acetylneuraminic acid (NANA) on the glycochain in- crease the affinity for biotinamide-substrate (unpublished result). Further, it is also recently found that the serum thiol-BIN/LIP with biotin deficiency is sensitive to glycosidases (sialidase and/or fucosidase), although the thiol-BIN/LIP of healthy serum is relatively resistant [22]. Therefore, heat-instable thiol-

BIN/LIP of the biotin-deficient patient's serum is cured by fucoidan or fucan and heparin (co-polymer of sulphated iduronic acid and glucosamine; M_r 5,500) [22]. Further, the elevated K_{ip} value (inhibition constant by the product biotin) of thiol-BIN/LIP in the hepato- cellular carcinoma (HCC) tissue is normalized by sulfated poly-fucose (Okinawa-fucan containing D-glucuronic acid derived from the edible brown-alga Futo-Mozuku *Cladosiphon okamuraus*; M_r 200,000) and by heparin is also observed [23]. But, L(-)-fucose, chondroitin sulfate C (co-poly- mer of sulphated D-glucuronic acid and N-acetyl-D-galactosamine; M_r 25,000), and hyaluronic acid (non-sulphated co-polymer of D-glucuronic acid and D-N-acetyl glucosamine; M_r 3,500,000) are in- effective at all [23]. Fucan has been taken up into the hepatoblastoma HuH-6 cells (derived from the liver of pediatric cancer of male 1-y Japanese), and has normalized K_{ip} value *in vivo* within two- days of fucan-treatment at 0.20 mg/mL [23]. Recently, it has been found that fucoidan derived from the Ishi-Mozuku (*Sphaerotrichia divaricate*; an alga grown at the seashores of Noto peninsula, Ishikawa, Japan) retards the growth rate of a hepatocarcinoma HepG2 cells *via* changing the mem- brane glycoprotein metabolism [12].

Biotinidase and lipoamidase activities in human sera are due to the same enzyme protein (thiol-BIN/LIP; M_r 76,000 with asparagine-linked (N-linked) two/three-anntenary complex-type glycochains; M_r 66,000 without glycochain) [24, 25], which suggests that both biotin and LA is handled with a closely-linked manner in humans and rats; i.e., mutual allosteric-effects (11-46% increases in A_{mo}) between biotin and LA are observed [16]. On the contrary, bacterial biotinidase (non-thiol-type BIN/LIP) of *Lactobacillus casei (Shirota)* handles both LA- and biotin- amides as competitive manner; i.e., K_{is} (competitive inhibition or occupation of the active center between biotin-amide substrate and LA) of LA for biotin-amide substrate is 55 μM, and K_{is} of biotin for LA-amide substrate is 543 μM, respectively [16]. Furthermore, it has recently been found that LA is the vitamin for the adult fish [26]. Although the biosynthesis of LA in chicken has not yet been

Studied, chicken egg-white avidin has recently been found to deal with both LA and biotin equally;
i.e., affinity for LA is a little stronger than D-biotin as assessed by the retention time in the HPLC- affinity (trypsin-treated avidin-bound column) chromatography [27]. Furthermore, bacterial lipo- amidase (LIP) of *Bacillus natto* has no biotinidase (BIN) activity, which suggests that *Bacillus natto* can biosynthesize biotin [16]. Thus, LIP activities are present on three distinctive glycoproteins in mammals and humans; i.e., serine-type ester-LIP (PMSF-inhibited ester-LIP; M_r 140,000 in human breast milk [28]; identical to pancreatic bile salt-dependent lipase [29]; recognizes only LA), complex-active-center-type brain-LIP (thiol/esterase/metallo-type; M_r 140,000 in pig brain [30]; recognizes only LA), and thiol-BIN/LIP (thiol-type BIN/LIP; M_r 66,000 in human organs; M_r 76,000 in human serum; recognizes both LA and biotin), although only thiol-BIN/LIP is present in the human serum [31]. Thus, biotin and LA have been recently found to be the equivalently im- portant vitamins for the adult humans.

Lipoic acid (LA) has firstly found by E.E. Snell et al. [32] in the potato as a growth factor of lactic acid bacteria. LA is important in obtaining the essential energy *via* pyruvate dehydrogenase (PDH), which produces important energy of NADH [33]. However, both biotin and LA has some other roles than the growth or survival of the nerve cells in the brain; i.e., thiol-BIN/LIP is rich in the cerebellum of pig and guinea pig [34] and in the left cerebrum of LEW rat [16], and brain-LIP is also rich in the cerebellum of pig and guinea pig [30]. Recently, direct detection of biotin has been possible in the selected regions of rat brain; i.e., high-expression in the cerebellar motor system and in the cerebellar *Purkinje cells* is observed [35]. The presence of vitamin in the selected-region in the organ has also been recognized; i.e. the subcellular distribution of thiol-BIN/LIP in the pig cerebrum [34] and in the LEW rat kidney [16] is in both of compartment membrane- and soluble- subfractions [31], on the other hand the thiol-BIN/LIP of guinea pig livers (and possibly of humans) is solely present mainly in the microsomal membrane-subfraction [36]. Affinity (inverse of K_m) for LIP substrate of membrane ester- and/or brain-LIP in LEW rat is strongest in the cerebellum in male, and is in the liver and cerebellum in female [16]. Thus, it has been understood that the intractable liver disease of primary biliary cirrhosis (PBC) is mainly occurred in human ladies of advanced ages due to ester-LIP of the livers [37], but LA-responsive cerebellar diseases are general- ly occurred in both human genders due to brain-LIP [38]. Then, this brain-LIP may have other role than recycling of LA, and may become the useful glycoprotein-drug for the brain diseases such as the senile dementia, Alzheimer disease, and prevention of the ageing.

Furthermore, the moderation of vitamins (biotin and LA) is crucially important for the animals to survive. It has been estimated that the ancestor animals of *Burgess Shale type fauna* (such as anomalocaris ("abnormal shrimp") and opabinia with lobopodia) seems to perished during the Cam- brian-Ordovician extinction event (c.a. 500 million years ago) possibly due to the excess of nutrition [39]. On the other hand, *Mammoth* (a relation of the elephant) has been perished during Pleisto- cene age (ice age), and disappeared at c.a. 4,000 years ago possibly due to the inflammation by in- vaded bacteria and viruses *via* deficiency of nutrition [40]. Therefore, the importance of biotin and LA together with the thiol-BIN/LIP, ester-LIP, and brain-LIP in the various tragic and severe human diseases is summarized and discussed in this review.

II. BIOTIN DEFICIENCY

II-I. EGG-WHITE INJURY

In order to guard the nutrition within the chicken egg-yolk against the invasion of microbes, avidin (a strong biotin-binding- [41] and LA-binding- glycoprotein [42]), conalbumin (ovo- transferrin; iron-binding glycoprotein), and lysozyme (N-acety lmuramide glycanhydrolase) are present in the chicken egg-white. Slow-growing bacteria (high GC-content bacteria) such as *Actinobacteria* in soil can combat against the fast-growing bacteria by producing various antibiotics [43], and *Streptomyces avidinii* also produces a strong biotin-binding protein of streptavidin [44]. Egg-white injury occurred by avidin of egg-white is a biotin deficiency in humans [45], and the healing effect by oral administration of free biotin in this injury is remarkable [10, 46]. It also has been studied on the biotin deficiency using dried chicken-egg-white feed with *Sprague-Dawley* (SD) rat, and found that the loss of hair (mainly abdominal) and the reddish eczema around eyes and labial skins are occurred [10]. This biochemical study on SD rat has also indicated as follows; i.e., specific activity (V) of thiol-BIN/LIP in rat serum is not changed at all, however the storage stability (at 4 ℃) of four-kinds of biotin-containing carboxylases (such as pyruvate carboxylase, acetyl- coenzyme A carboxylase, propionyl-coenzyme A carboxylase, and 3-methylcrotonyl-coenzyme A carboxylase [47]) of the liver homogenates is impaired (unpublished observation). Administered free-form biotin to the rat liver mitochondria might directly be bound to lysine residues of the apo-form carboxylases by the reverse-reaction of biotinidase (thiol-BIN/LIP) using ATP [48]. Recently, it is also found by PDMD method that the amount of human serum thiol-BIN/LIP proteins is not changed at all by the administration of free-form biotin [22]. Therefore, the shortage of free-biotin in the rat seems to induce the production of labile non-biotinylated carboxylases (apo-form carboxylases); i.e., although these four mitochondrial carboxylases are not glycosylated, apo-form of carboxylase may be unstable during the storage at 4 ℃ due to absence of bound-form Biotin.

Furthermore, it is noteworthy that the strong binding-ability of avidin (alkaline hydrophilic glycoprotein of pI = 10.5, M_r 17,000, and hydrophobicity = 0.45) to biotin is considerably resistant against the heat, alcohol, and trypsin treatment as assessed by the HPLC-affinity chromatography [11]. Therefore, taking the chicken egg-white as foodstuffs is very dangerous even though it is boiled, cooked, or griddled, and it is recommended to eat only the chicken egg-yolk separately.

II-II. CHILDREN'S AND BABIES' BIOTIN DEFICIENCY ASSOCIATED WITH ALOPECIA

Recently, a case report about a girl with alopecia (3-y female) with biotin-deficiency (together with GSD-1b (glycogen storage disease type 1b) has been reported [49]. This child patient takes glycogen storage disease-related formula (GSD formula Day and Night; Meiji Dairies Co., Tokyo, Japan), which contains no total- and free-biotin at all [49, 50], and the biotin-deficiency is accordingly occurred in this patient possessing the labile thiol-BIN/LIP [50]. This patient's total biotin (3.08 μg/mL) is normal, but free-form biotin is not detected in her serum (Table 1), and free/total ratio of biotin (%) is 0.00 (Table 2). Her serum enzyme is thermo-instable; i.e., K_m of her thiol-BIN/LIP increased from normal of 8.38 μM to 23.4 μM (2.8-fold) after the heat treatment at 37℃ for 4 h (Table. 3). Her heat-instable K_m of serum enzyme is not cured at all by the liver- transplantation operation at after 2 months. Therefore, the origin of human serum thiol-BIN/LIP is not the liver, but is the other organs. Thus, amino acid sequences of N-terminal region of the serum thiol-BIN/LIP are surely varying as reportedly [20]. Then, the serum biotinidase sequence reported by Cole et al. [51] using liver DNA-bank has turned out to be the fetal/inflammatory-type thiol-BIN/LIP [12].

With respect to this GSD-1b patient, her gene for glucose-6-phosphate transporter (G6PT; membrane glycoprotein) is mutated only at the two amino-acid residues among 429 amino acids (0.47%) [49], which suggests other chemical-structure than core-protein may be changed. Therefore, glycochain of this membrane glycoprotein G6PT may have become heat labile due to changed glycochain, or integration of G6PT into endoplasmic-reticulum membrane may be injured by biotin deficiency *via* the labile thiol-BIN/LIP. Thus, this girl patient may have been cured by the biotin and fucoidan administrations without dangerous operation of liver-transplantation. Similar consideration on the thiol-BIN/LIP deficiency is now possible; i.e., thiol-BIN/LIP deficiency due to the recessive gene has not yet been discovered in Japan contrary to the observations reported in USA. Thiol-BIN/LIP deficiency in Japan has now turned out to be due to the heat-labile thiol- BIN/LIP, which has an altered-glycochain [22, 50]. Therefore, tragic and intractable diseases, which has long been believed to be due to the recessive genes and considered to be necessary to perform the transplantation operation, are healed completely and safely by solely administrating the adequate foodstuffs. Thus, surgeons are not always necessary and the internists are more helpful to the patient who requires the incredibly high-fee operation; i.e., Hippocrates von Kos (BC 460 – 370; Greece) has already recognized the importance of healing by the foodstuffs.

Further, another three biotin-deficient babies with alopecia (3 and 4 mo boys, and a 1 y girl) have also been studied by using their sera (Table 1, 2). The 4-mo boy's total biotin (2.31 μg/mL) and the 3-mo boy's total biotin (1.87 μg/mL) are low (Table 1), and the free/total-ratio of biotin (%) of both patients are very high. Both of total- and free-biotin of the 1-y girl's serum is low (Table 1). The 1-y girl and 4-mo boy have the serum thiol-BIN/LIP with heat-labile K_m, and have been given the allergy milk (Milfy; Meiji Co., Tokyo, Japan) containing low free-form biotin (0.056 μg/mL) and low ratio (11.4%) of free biotin (ratio of the normal human breast milk; 55.6%) [50]. The 3-mo boy's enzyme is heat stable but fresh K_m is large (Table 3), and has been given the allergy milk (New MA-1; Morinaga Co., Tokyo, Japan) containing low free-biotin (0.164 μg/mL) and low ratio (30.8%) of free biotin. Therefore, biotin deficiency is occurred through patients'

heat-labile and/or large K_m thiol-BIN/LIP possessing changed glycochain to induce extreme values in the free/total-ratio of biotin (%) (Table 2). Then, these allergy patients with thermo-instable serum thiol-BIN/LIP should drink the soy-bean milk [50] instead of cow's milk, since commercial allergy milks are usually scanty of biotin [50].

Interestingly, a bronchitis child (without biotin deficiency) shows an instantaneous biotin-ele- vation (at 16 mo), but her biotin concentration of serum becomes to normal level after the healing of bronchitis (at 16.1 mo); i.e., her serum biotin changes from 6.09 μg/mL (total biotin) and 0.737 μg/mL (free biotin) to 2.57 μg/mL (total biotin) and 0.141 μg/mL (free biotin) after the healing, respectively (Table 1). Total- and free-biotin may be increased by the inflammation due to increment of the fetal/inflammatory-type thiol-BIN/LIP; i.e., *V* of her exasperated serum thiol- BIN/LIP activity (196 pmol/min/mg of serum protein) is returned to ordinary value of 123 pmol/min/mg after the healing. This indicates that serum biotin and thiol-BIN/LIP activity is positively correlated during inflammation; i.e., the increase of fetal/inflammatory-type serum- thiol-BIN/LIP seems to be due to the increased gene expression, whose response seems to be prompt to the signal of inflammation. Although biotin has not determined, similar instantaneous elevation of thiol-BIN/LIP activity in the cerebrospinal fluid (CSF) by the *Staphylococcus aureus* meningitis due to subacute sclerosing panencephalitis (SSPE) caused by the measles virus in a girl (11.5 y) has also been reported [52]. In this case, concentration of the sialyl-glycoprotein of CSF-thiol- BIN/LIP may be increased, since free-form N-acetylneuraminic acid (NANA) in the CSF is not changed [52]. Therefore, the possibility of immediate increment of encephalin-hydrolysis rate in early phase of meningitis in CSF-CNS *via* increased the fetal/inflammatory-type thiol-BIN/LIP, which also may have an amino-exopeptidase activity, has been already proposed in this case [52].

With respect to the inflammation, fetal/inflammatory-type thiol-BIN/LIP has also been induced in the serum of common-cold baby (12 mo, female; due to possible infection by a coronavirus of avian infectious bronchitis virus (Avian IBV)), although the amount of ordinary serum thiol-BIN/LIP proteins in her serum is not changed [22]. Furthermore, unique serum-marker of ceruloplasmin-fragment (from 214; DREFVV-; M_r 97,800) for the inflammation is increased in this patient's and in the biotin-deficient patients' sera [22]. The increased serum biotin concentration seems to be associated with the invasion of microbes, and induces immunological reaction and fetal/inflammatory-type thiol-BIN/LIP (Table 1, 2). Biotin deficiency also has induced inflammation (Table 1, and [22]). Similarly, the deficiency of essential amino acid of tryptophan has also been shown to be effective to deteriorate the immunity and to apply the prevention of the spontaneous abortion and/or the rejection after the transplantation [53], though side effects may be expected to occur. In this regard, it is also noteworthy that maize is scanty of tryptophan, and severe disease of pellagra is induced, whereas American native-Indian treated the corn by alkali (called as "tortilla") and is safe. Therefore, adequate and moderate supply of biotin and other nutrient such as tryptophan seems to be of importance to prevent the microbial invasion which causes inflammation.

Since the glycochain-abnormality in thiol-BIN/LIP of biotin-deficient sera (0 – 1 y; during the lactating period) has also been suspected to occur, several glycochain-compounds are tested *in vitro* whether some polysaccharides recover these glycochain disease (biotin deficiency) or not. Heparin, Okinawa-fucan, Noto-fucoidans (from Ishi-Mozuku and Silky-Mozuku) at 0.20 mg/mL are not effective to the serum of healthy baby (with gastroenteritis; 8 mo female) and healthy adult at all as expectedly (Table 3). Heat labile and large K_m of baby patients (3-mo male, 4-mo male, and 1-y female) are all normalized by the Okinawa-fucan, and heat labile *A*mo of one baby (1-y female) is cured by the Okinawa-fucan. On the other hand, the heat labile K_m and *A*mo of the child patient (3-y female) is only normalized by the Noto-fucoidan from Silky-Mozuku (Table 3). Noto-fucoidan induces high K_{ip} in 3-mo male baby, and it seems that the Okinawa-fucan is more suitable to treat the babies' biotin deficiency (Table 3). Heparin seems not effective on the baby and child patients' K_m except one baby (1-y female). Further, heat instable K_m of the adult biotin-deficient patients' sera seem to be adequately healed by only the Noto-fucoidan (Table 3). These observations indicate that biotin deficiency in baby is able to be cured by the Okinawa-fucan instead of using dangerous oral administration of free biotin. It is also noteworthy that the fucan effects onto the K_{ip} of the poorly differentiated HCC and baby's HuH-6 hepatoblastoma cells [23].

Therefore, Okinawa-fucan may become a good drug against the babies' cancer and poorly differentiated cancer *via* normalization of the undifferentiated-type of glycochains, although the Noto-fucoidan is surely effective to the well-differentiated HCC, adult's HepG2 hepatocarcinoma cells [12], and chemically-induced rat's HCC [54] *via* normalization of the well-differentiated-type of glycochains.

II-III. ADULT'S BIOTIN DEFICIENCY ASSOCIATED WITH GAIT DISORDER

The sera of adult biotin-deficient patients with gait disorder together with the optic atrophy (32 y female and 46 y male) have been analyzed for biotin. As is shown in Table 1, these two patients have low concentration of serum total-biotin. The free/total-ratio of biotin of a male patient (46 y) is normal at 3.56%, but that of a female patient (32 y) is high of 8.33% (Table 2). The fresh enzyme C_{ap} (s^{-1}; specific enzyme capacity) of both these adult patients is low (Table 2). Healthy adults' sera show significantly lower total biotin concentration (median; 1.95 μg/mL, range; 1.80 - 2.05 μg/mL) than healthy and actively-growing children's sera (median; 3.11 μg/mL, range; 2.57 – 3.22 μg/mL: $P = 0.01$, Mann-Whitney's *U* test, two-tailed test) (Table 1). The adult female patient (32 y) possesses a heat-instable K_m of thiol-

BIN/LIP; i.e., K_m for biotin-amide substrate is changed from 4.45 μM to 38.2 μM (8.6-fold increase) before and after the heat treatment at 37 ℃ for 4 h, respectively, but has a heat-stable K_{ip} (Table 3 and [22]). The other adult's male-patient (46 y) also possesses a relatively heat-labile thiol-BIN/LIP; i.e., his K_m is changed from 6.33 μM to 9.17 μM (1.5-fold increase) before and after the heat treatment, respectively, and his K_{ip} is also increased from 353 μM to 795 μM (2.25-fold increase) before and after the heat treatment, respectively (Table 3). Therefore, the adult biotin-deficient patients have a relatively low capacity C_{ap} (s^{-1}) in the fresh serum, on the other hand biotin-deficient children have high enzyme capacities (Table 2). Thus, the diagnosis of biotin deficiency by using kinetics of serum thiol-BIN/LIP should carefully be performed and analyzed between child and adult patients.
Further, it is noteworthy that father and grandfather of the female patient (32 y) have been died of gall-bladder cancer and of Parkinson's disease, respectively. The K_m of her healthy mother (52 y) is heat stable as expectedly; i.e., her mother's K_m is not changed at all from 5.96 μM to 5.95 μM before and after the heat treatment at 37 ℃ for 4 h, respectively. But the K_m of her younger sister (22 y) with a slight gait disorder also has a heat labile K_m; i.e., her sister's K_m is changed from 6.83 μM to 17.2 μM (2.52-fold increase) before and after the heat treatment, respectively (un- published result).

Furthermore, the father of the other male patient (46 y) has been died of the common-bile-duct cancer (+HCV) with diabetes at the father's age 49. His son (20 y) and daughter (17 y; with the optic atrophy) also possess considerably heat labile thiol-BIN/LIP; i.e., the son's K_m is changed from 8.16 μM to 12.8 μM (1.6-fold increase) before and after the heat treatment, and the daughter's K_m is also changed from 7.58 μM to 9.62 μM (1.3-fold increase) before and after the heat treatment, respectively (unpublished result). On the other hand, an unrelated healthy volunteer (33 y, male) possesses heat stable thiol-BIN/LIP as expected; i.e., K_m is not changed at all from 5.94 μM to 5.95 μM before and after the heat treatment, respectively (Table 3). Therefore, the father and the grandfather of these two severe gait-disorder-patients seem to have the altered glycochains, which may be transmitted from father to descendants.

Adult female patient (32 y) is treated with foodstuffs of Yakult (fermented beverage from cow's milk; Yakult Honsha Co., Tokyo) and Ebios (dried beer yeast; Tanabe Pharmaceuticals Co., Osaka). This patient has partially been improved the symptoms by the foodstuffs' treatment; i.e., serum biotin has increased from 1.14 μg/mL (total biotin) and 0.0406 μg/mL (free biotin) before, and 2.57 μg/mL (total biotin) and 0.287 μg/mL (free biotin) after for 1.5 y of treatment, respectively.

This biotin therapy with two foodstuffs has been devised by K. Deguchi with me; i.e., the mixture of Ebios (contains high bound-form biotin) [11] and Yakult (contains high biotinidase activity of *Lactobacillus casei*) [16] may liberate much amount of free-form biotin in the gut. Since it is recognized that the free-biotin overdose for long period may induce the symptoms of excessive biotin, this biotin therapy with two foodstuffs is performed for 1.5 y without any side effects.
The specific activity (V) of bacterial biotinidase (BIN/LIP in the case of *Lactobacillus casei*) is surely heat labile since bacterial enzyme has no glycochain; i.e., heat treatment at 60℃ for 10 min reduced the activity to 8% of the original [8]. On the other hand, that (V) of human serum thiol- BIN/LIP purified from stored healthy blood for transfusion is heat stable; i.e., heat treatment at 60℃

For 10 min reduced the activity to 42% of original [55]. This difference in heat stability between humans and bacteria is considered to be due to presence and absence of the N-linked glycochains in human's and bacterial enzyme, respectively. Further, it is found that neuraminidase (sialidase) and/or fucosidase treatments on diluted biotin-deficient patients' sera change thiol-BIN/LIP kinetics, and N-glycanase (peptide-N4-(N-acetyl-β-D-glucosaminyl) asparagine amidase, EC 3.5.1.52) treatment on purified healthy human-serum enzyme totally inactivates the enzyme activity (unpub-lished observation). Therefore, heat instability observed in serum thiol-BIN/LIP of biotin- deficient patients is again considered to be due to the changed glycochain structures.

Similar biotin-deficient patient (15 y male) with bilateral optic neuropathy has been reported by Ramaekers et al. [56]. This patient has been suffered from influenza-like illness ('flu'-like ill- ness), and the acute loss of vision has occurred. This patient's gait is stiff and shuffling, but is not ataxic. His serum thiol-BIN/LIP shows two K_m values (normal and large K_m). After 6 months of treatment at 10 mg/day biotin, he has recovered the peripheral visual fields, and after the 8 months treatment by biotin, his pyramidal signs in the lower limbs have disappeared [56]. The presence of two K_m values might be possible, since it has been found that the heat treatment on serum thiol-BIN/LIP of biotin-deficient patients all exhibited larger K_m possibly due to the changed glycochain structure in the patient's enzyme.

Since the gait disorder of the female biotin-deficient patient is not completely improved by the biotin therapy using foodstuffs, LA deficiency has also been suspected since her thiol-BIN/LIP is heat labile. Therefore, LA therapy using LA-rich foodstuffs such as royal jelly, natto (a Japanese food made from fermented soybeans), spinach, komatsuna (*Brassia rapa* var. *pervidis*), and coffee [42], is now ongoing by K. Deguchi and me. Other intractable neurodegenerative Parkinson's disease is also improved by the oral administration of LA (50 mg/kg/day) in the motor performance in rat brains [57]. Parkinson's disease is partially healed by coffee (caffeine plus biotin) and tobacco (nicotine and/or vitamin B_3 to support NADH production), and adequate supply of biotin and LA may be helpful *via* normalization of membrane biosynthesis. Further, the serum thiol-BIN/LIP and/or brain-LIP [30] of Parkinson's disease patients might also be heat

labile. Porcine brain-LIP is similar to nicotinic acetylcholine receptor (nAchR) of *Electrophorus electricus* with respect to the amino acid composition [30], and to acetylcholinesterase (AChE; from electric eel, Type V-S) with respect to enzyme kinetics [58]. Therefore, the putative responsible enzyme for the Parkinson's disease may be the brain-LIP, and the importance of LA in the brain neuronal membrane-biosynthesis is also expected.

II-IV. CHANGES IN THE THIOL-BIN/LIP ACTIVITY OF ADULT'S SERUM AMONG THE GASTROENTEROLOGICAL DISEASES

It is reported that both serum thiol-BIN/LIP activity and serum total-biotin level in severe liver disease is significantly low, and that serum thiol-BIN/LIP activity and total-biotin level of patient's serum may be positively correlated [59]. Further, serum thiol-BIN/LIP activity of gallbladder cancer and cholangiocarcinoma (bile duct cancer) is also significantly low [60]. Organs of LEW rat show also positive correlation; i.e., total-biotin 6.71 μg/g wet weight and thiol-BIN/LIP's C_{ap} 31.9 s^{-1} for the kidney, and total biotin 4.82 μg/g wet weight and C_{ap} 11.1 s^{-1} for the liver, respectively (unpublished observation). Thus, the patients of severe liver diseases may be in the state of biotin deficiency [59]. Therefore, cancer patients of HCC, cholangiocarcinoma, and gallbladder cancer should be supplemented with an adequate amount of biotin even if they show no symptoms of biotin deficiency.

On the contrary, it is recently found that the content of biotin in the HCC tissue is higher than the surrounding LC tissue, and the kinetic parameter (K_{ip}) of the liver thiol-BIN/LIP is increased [23]. Since serum thiol-BIN/LIP and biotin of HCC patient is low and liver thiol-BIN/LIP and biotin is high, it is again concluded that the serum thiol-BIN/LIP is not produced in the liver of humans. Although purified serum- and milk-thiol-BIN/LIPs have no glucose and no gangliosides [61], enzyme kinetics of purified thiol-BIN/LIP are surely influenced by the externally added gangliosides *in vitro* (unpublished result). Then, observed changes in serum thiol-BIN/LIP activity in the pancreatic-, gallbladder-, bile-duct- cancers [60], and HCC [59] may be due to the appearance of such glycochain-molecules as monosialo-gangliosides (glycosphingolipids) of CA19-9 (carbo- hydrate antigen 19-9; sialyl-Lewis^a) and SLX (sialyl-Lewis), and glycoprotein of CEA (carcino- embryonic antigen; M_r 200,000) in these patients' sera.

Although human organs are not yet fully studied, *cerebrum, testis,* and *kidney* show highest thiol-BIN/LIP activity (V) in male LEW rat, and *cerebrum, kidney,* and *large intestine* show highest activity in female LEW rat, but *liver, small intestine,* and *kidney* show highest activity in male C57BL/6 mouse [16]. Thus, human organ resembles more to rat organ than mouse organ with respect to thiol-BIN/LIP distribution. However, it must be fully alerted for the serum thiol-BIN/LIP activity; i.e., V of LEW rat serum is extremely high of 151 pmol/min/mg protein, and Vs of mouse and humans are 15.2 and 86.6 pmol/min/mg protein, respectively [16]. This species differences may be due to the extremely low cellular-immunity in the LEW rat, which has been frequently used as the recipient of transplantation experiment.

Serum thiol-BIN/LIP activity (V) of pancreatic cancer is significantly high [58], but K_m is also large, and the resultant enzyme capacity (C_{ap}) becomes to normal level (unpublished observation). However, serum total-biotin concentration is still high among pancreatic cancer and primary biliary cirrhosis (PBC) patients. The sera of two PBC adult-female patients possess the thermo-labile thiol-BIN/LIP; i.e., K_m increases from 6.77 to 19.8 μM and from 7.45 to 13.5 μM by the heat-treatment for 4 h at 37 °C, respectively. On the other hand, healthy male adult enzyme is heat-stable as indicated in section II-III. Since thiol-BIN/LIP handles equally biotin-amide and lip amide, the free LA concentrations are compared between serum of PBC patients ($n_1 = 5$) and healthy control ($n_2 = 4$) using HPLC-affinity chromatographic method [42]. Median value of free LA in the healthy adults' sera is 8.67 μg/mL (range; 6.95 - 11.3 μg/mL), on the other hand median of free LA in the PBC patients' sera is 3.60 μg/mL (range; 2.22 - 4.43 μg/mL). This 2.4-fold median difference is found to be significant; i.e., $P < 0.02$ (Mann-Whitney's U test; two-tailed test). Therefore, thiol-BIN/LIP of the PBC patients' sera is thermo-labile, and the median level of free LA is lower than that of healthy control. Thus, PBC patients will possibly be healed by the oral administrating of free LA.

It has also been found that the affinity of ester-LIP to lipoyl-amide substrate in LEW rat is highest in the liver and pancreatic-head with respect to A_{mo} (s^{-1} x M^{-1}) [16]. Liver's A_{mo}s are 54.1 for male and 119 s^{-1} x M^{-1} for female, respectively, and pancreatic-heads' A_{mo}s are 19.5 for male and 16.3 s^{-1} x M^{-1} for female, respectively [16]. There is no rat ester-LIP activity in ovary, bone marrow, abdominal skin, and thigh muscle [16]. Although the reason why such a large differences in gene-expression is occurred among rat organs and between genders is not clearly understood, liver and pancreatic head are the most active organs for LA metabolism. Then, ester-LIP of PBC and pancreatic cancer patients may also be heat instable. Therefore, free LA should also be studied in the patients of pancreatic cancer, even if they show no apparent symptoms of LA deficiency. Insulin-dependent diabetes mellitus (IDDM type 1; juvenile-onset IDDM) is a famous pancre- atic disease due to cell death of β-cells in the islets of Langerhans. Biotin-deficient state in IDDM patients has already been suggested by Coggeshall et al. [62].

Diabetic KK mice, moderately hyperglycaemic and insulin resistant, are treated biotin for 10 weeks, and the biotin treatment lowers the post-prandial glucose level, and improves tolerance to glucose and insulin resistance [63].In streptozotocin-induced diabetic SD rat, oral administration of biotin-added feed (0.02%) for 7days also significantly has decreased the blood glucose level, and the substrate specificity of serum thiol-BIN/LIP is significantly changed [64].

Koutsikos et al. have already reported that oral biotin-administration is effective to prevent the diabetic peripheral neuropathy in humans [65]. Furukawa et al. also have reported that biotin deficiency using avidin (hen's egg white) has induced the IDDM *via* inhibiting insulin excretion into the blood, and free biotin is effective to adequately excrete insulin from the islets of Langerhans possibly effecting onto the transport mechanism of insulin from the β-cells [66]. We also have found that urinary thiol-BIN/LIP and alanine excretions increase in patients with IDDM type 1, suggesting the changes in kidney-membrane metabolism are already arisen in the early stages of diabetes [67].

Vitamin H (D-biotin) [68] and an amino acid L-alanine [69] are actively transported into the bacterial cells. The energy source of glucose is metabolized and is supplied to NADH production through the electron-transport chain, since the respiration inhibitor (1 mM KCN) and the uncoupler (0.1 mM carbonylcyanide *m*-chlorophyll hydrazine C*l*CCP) inhibit the active transport remarkably [69]. Recently, it is also found that fucoidan is actively transported through monolayer of colon cancer cells (Caco-2); i.e., 10 mM sodium azide (respiration inhibitor) and 0.05 mM FCCP (carbonyl cyanide-*p*-trifluoromethoxyphenylhydrazone; uncoupler) inhibit the active transport slightly but significantly [70]. The differences in the inhibition of uncouplers of FCCP and C*l*CCP on the inhibition of the active transport may due to the differences in chemical nature of membrane com- ponent between eukaryotic and bacterial cells [69, 70]. Although active transport system in kidney membrane has not been studied yet, biotin re-uptake system of kidney may already have been injured in the kidney membrane of IDDM type 1 patient [67].

Thus, IDDM type 1 patient is surely in the state of biotin deficiency, and oral-biotin- administration to the patient is expected to be recovered from diabetes and protecting the kidney together with fucoidan [64]. On the other hand, IDDM type 2 (non insulin-dependent diabetes mellitus or adult-onset diabetes) patient is in the state of excess biotin due to excessive body weight and due to few exercise, and sole oral fucoidan-administration with moderation in the eating of nutrition of biotin and LA may be effective in the case of IDDM type 2 patient.

III.EXCESSIVE OF BIOTIN

III-I. EXCESS BIOTIN AND IMMORTAL CELLS

Comparative biochemical studies among the human liver tissues from senile HCC patients (biopsy and autopsy) and the liver cell lines (immortal cells) indicate that the following characteristics are unique points in the immortal cells and tissues; i.e.,
① the occurrence of biotin synthesis [71], ② the increase of thiol-BIN/LIP kinetic constant (K_{ip}) [23], ③ the ppearance of D-aspartic acid [27], ④ the decreased ratio of membrane-proteins / soluble-proteins in the tissue and cellular homogenates [12], ⑤ the increase of proteins of +ssRNA virus [12], and ⑥ the increased production of histone H3.1 [72, 73]. Furthermore, comparative studies on cellular proteins using fucoidan (from edible brown alga of Ishi-Mozuku (*Sphaerutrichia divaricata*)) to the HepG2 cells indicate that (α) histone, (β) ankyrin, (γ) biotinidase, and (δ) membrane active-transport proteins are not changed at all in the three days of fucoidan treatment [12]. Histone H3.1 (⑥) is not detectable in the mortal liver tissue of pseudo-cancer as expectedly, although it is highly expressed (7.3 μg/mg total homogenate protein) in the LC tissue of leprosy patient. HCC tissue of alive-male patient possessed 1.7 μg/mg of histone H3.1, but his LC tissue possessed only histone H1.0 and H1.1 at 0.83 μg/mg. This suggests that histone H3.1 is a marker for poor differentiation. Thus, extremely high content of histone H3.1 of 65 μg/mg in hepato- blastoma HuH-6 and retinoblastoma Y79 are detected [73]. Undifferentiated fetal liver cell (Hc) also contains high amount of histone H3.1 (55.2 μg/mg) [12]. Hepatocarcinoma HepG2 cells also have considerable amount of histone H3.1 at 10.6 μg/mg and histones 27.1 μg/mg [12], and this result suggests that HepG2 may be the moderately differentiated cells (not the well-differentiated cells). Noto-fucoidan, which retards the growth of Hep-G2 cells, unexpectedly does not reduce gene expression of histone H3.1 at all; i.e., histone concentration of non-treated and fucoidan-treated HepG2 cells are similar (2.7% without fucoidan, and 2.6% with fucoidan, respectively) [12].

Therefore, fucoidan does not influence onto the characteristics of ⑥ within three days of treatment (α), however more duration-period for contact with the fucoidan would reduce this marker histone H3.1 protein *via* reducing biotin concentration and reducing thiol-BIN/LIP K_{ip} activity.

Ankyrin (β) is also essential for the cell viability through lateral membrane biogenesis [74], but ankyrin is not present in the HCC tissue with PBC, LC tissue with leprosy, and normal liver tissue with pseudo-cancer, but only one HCC liver (LC and HCC tissues) contains the erythrocyte ankyrin (unpublished result). Therefore, ankyrin is detected at frequency of 25%, and ankyrin may not generally be related to the cancer, but may be related to the immortality.

Edible fucoidan also does not change the amount of biotinidase (γ) and membrane active-transport proteins (δ) [12]. Membrane active-transport proteins are constitutively expressed hydrophobic membrane-proteins in order to alive cells through taking up the nutritional amino-acid using NADH as an energy source [69], although such an exception of the inducible lactose-transport proteins, which is usually absent in glucose-containing medium of *Escherichia coli*, is also present [75]. Constitutive outer-membrane mercury-binding protein is also discovered, which seems to protect the essential intracellular components (such as respiratory system and biotin synthesis system) against the strong heavy-metal toxin (mercuric ion) [76].Thus, essential and constitutively expressed thiol-BIN/LIP and membrane active-transport

proteins are important for the viability and growth of the cells. The putative inducible cell-death mechanism of cancer cells (or the induction of apoptosis) *via* DNA metabolism may not be true, since the effect of fucoidan is not due to inducible gene expression system at all [12]. Then, this is the reason why edible fucoidan is the completely safe drug without side effect on the normal cells and organs.

D-Aspartic acid (D-Asp, D-D) is not usually found in the normal and mortal human organs, but is abundantly present in brain and reproductive organ of testis and ovary [77], the regenerating tissues such as dental root, eye lens, hair roots, dermis, intestinal epithelium, and knee cartilage [78], and immortal liver cell lines [27]. Interestingly, biotin also shows some degrees of similar distribution pattern to D-aspartic acid; i.e., *kidney, liver, testis,* and *brain* in LEW rat [11]. Kidney and liver seem to be not the growing tissue, but these two organs recycle biotin actively by using thiol-BIN/LIP. Biotin in kidney and liver may be working as the controlling molecule for the membrane biosynthesis [79].

Biotin is biosynthesized from aspartic acid in plants, fungi, and some biotin synthesizing bacteria; i.e., 1st essential amino acid of methionine is synthesized from aspartic acid and cysteine, 2nd alanine and *S*-adenosylmethione (SAM) give the NH_2 residues into the pimeloyl-CoA (or pimeloyl- ACP) to produce dethiobiotin (DTB), and 3rd SAM gives the sulphur atom to DTB by biotin synthetase [80]. Then, the growing and regenerating animal tissues must continue the gene expression of the putative biotin synthetic ability (possibly *via* biotin synthase operon) since the fetus age of life. Therefore, D-aspartic acid may be strongly linked to the immortality of the animal regenerating cells and cultured cell lines [27] *via* the synthesis of biotin. However, direct proof to induce the growth retardation in mammalian cultured cells by decreasing the intracellular concentration of D-aspartic acid has not been successful yet. Our test of direct addition of D-aspartic acid (10 mM) in the DMEM medium of cultured rat liver cells (RLC-16), which require L-glutamine (10 mM) in the medium, has been unsuccessful [27]. Extremely high concentration of D-aspartic acid might have inhibited the aspartate racemase reaction (inhibition by the product), or D-aspartate transport system might not be present in the membrane of RLC-16 cells. In order to occur intracellular biotin synthesis, sufficient energy supply might be necessary to displace L-glutamine (L-Gln, L-Q) [81]; i.e., D-aspartic acid (10 mM) in the advanced DMEM medium (containing 0.45% glucose) may be effective. Nevertheless, the direct demonstration in the bacterium *Mycobacterium tuberculosis* about the direct dependence on the biotin for the bacterial growth is recently reported [80]. Biotin is the essential growth factor for all the living cells, and cancer cells may require more amount of biotin than the surrounding non-cancer cell. It has been found that the sexual difference of thiol- BIN/LIP activity (V) is present in the LEW rat [16], and interestingly this finding is coincided to the sexual difference of the occurrence of cancers in humans. Cancers in human male occur highly in *stomach, colon, lung, testis, and liver*, and thiol-BIN/LIP activity (V) in male LEW rat is high in *testis, stomach, colon, liver,* and *lung*. Cancers in human female occur in *(breast), colon, stomach, ovary,* and *lung*, and thiol-BIN/LIP activity in female LEW rat is high in *colon, stomach, lung,* and *ovary*. Although thiol-BIN/LIP activity of mammary gland in the rat has not been measured yet, it might also be high in the female rat. Liver cancer occurs highly in the male as compared to the female humans. Further, the organ distributions of thiol-BIN/LIP activity also show species difference between mouse and rat [16], and human urinary thiol-BIN/LIP activity shows distinctive racial difference [82]. Although sexual biotin-difference has not yet been studied, biotin and thiol-BIN/LIP distributions seem to be strongly correlated. Thus, biotin and thiol-BIN/LIP dis- tributions reflect precisely to the risk of occurrence of cancer. Thiol-BIN/LIP activity with respect to product handling (K_{ip}, R_{ep}, and C_{ap}) is significantly exasperated in the HCC tissues (n = 14) as compared to normal tissues (n = 5) (Fig 1 and Table II in [23]). Okinawa-fucan reduces significantly the aggravated enzyme kinetics of poorly differentiated HCC tissues. Interestingly, kinetic parameter of K_{ip} of thiol-BIN/LIP in the LC tissues is smaller than the normal tissues (Table II in [23]). These observations indicate that fucan gives the measure or the happy mean to the thiol-BIN/LIP of undifferentiated-type liver with respect to K_{ip}.

Further, it is noteworthy that small molecular weight fucan (M_r less than 1,000) increased the growth rate of urinary-bladder cancer cells of T24 (81 y, female, Caucasoid) and of 5637 (68 y, male, Caucasoid in USA) as assessed by MTT assay ($P < 0.05$; Mann-Whiteney's U test, two-tailed test). On the other hand, poorly-differentiated urinary-bladder cancer cell of JMSU1 (75 y, man, Japanese) shows no growth effect by small molecular weight fucan, but is surely inhibited the growth by the high molecular weight or natural fucan. High molecular weight or natural fucan is no effect onto the growth of well-differentiated type T24 and 5637 cells. Thus, the long structure (high mole- cular weight) is important to show the growth inhibition to poorly-differentiated cancer cells, and the short structure fucan inversely accelerate the growth rate of well-differentiated cancer cells. Therefore, small molecular weight fucan is a very dangerous drug for the cancer therapy. How- ever, the short structure fucan may become the growth-promoting additive, as biotin and LA, in the medium of cell culture *in vitro*.

The measure (or median) values of the biotin concentration are expected to be given *via* fucoidan, although direct comparison of biotin concentration between HCC and LC tissues are not yet performed. This difficulty of comparison of biotin is due to the prompt invasion of microbes and consumption of free-form biotin by them after the death in the autopsy or necropsy. High amount of free biotin in the HCC tissue in LEW rat disappears immediately (3 h) after the death at room temperature at 23 ℃(unpublished observation). Since fucoidan may give the measure of biotin in both HCC and also LC, both HCC and LC might be expected to be healed by the edible fucoidan treatment. Further, fucoidan effect onto the viral infection and co-infection (indicated as ⑤) is also the important point to heal the HCC; i.e., co-

infection of HIV and HCV is prevented by the fucoidan treatment [12]. Then, it has been re-examined my results of the PDMD method at this time. Co-infection of HIV (retrovirus) and HCV-HBV has occurred in all the 8 liver specimens; i.e, Hc cells (1.1% of total cellular protein), HepG2 cells (before fucoidan, 2.26%, and after fucoidan, 0.29%; finally deceased), survived male pseudo-liver-cancer (2.0%), female HCC-leprosy (1.5%; finally deceased), female HCC-PBC (7.6%; finally deceased), and survived-HCC man (LC portion,

3.6%; HCC portion, 14.9%), respectively. However, co-infection in the serum occurs very few; i.e., only an excess-biotin patient (1y female) is co-infected by Murine Leukemia virus (MuLV)/ HCV-HVA (1.1% of total serum protein) out of 12 serum samples inspected. Therefore, the state of co-infection ratio larger than 1.5% may be an indicator of HCC, although one pseudo-cancer patient exceptionally shows 2.0%.

Then, the reason of the death of two female patients may be due to another occurrence of co- infection; i.e., one female patient with PBC has died directly of the co-infection with human adeno- virus (HAdV, ds DNA) and Newcastle disease virus (NDV; -ssRNA; expressing haemagglutinin- ***neuraminidase***), and another female patient with leprosy has died of the co-infection by HAdV and feline infectious peritonitis virus (FIPV), and the parvovirus of silk worm denso virus (BmDNV; linear ssDNA; expressing ***phospholipase A2***-like protein) and influenza B virus (FluB; -ssRNA; expressing ***neuraminidase***). Similarly, co-infection of HAdV and NDV (expressing haem- agglutinin-***neuraminidase***) has been occurred in HepG2 cells (from HCC liver of diseased 15 y male Caucasian in USA), and this co-infection has been healed *in vitro* by the fucoidan derived from the Noto-Ishi-Mozuku *via* disappearance of NDV [12]. HepG2 cells also have influenza A and C viruses (FluA and FluC), but these viruses seem not the cause of death of this patient. The reason why neuraminidase of NDV and Flu B, which is not so lethal to healthy persons, becomes especially lethal to HCC cancer patients may be due to the changes in glycochain structure occurred in the total body of cancer patients.

Interestingly, all the HCC patients (5/5) inspected possess HAdV, but non-cancer persons and one normal fetal-liver cells (Hc) possess only 7% (1/14), and the presence of HAdV may be another marker of HCC. Infection rate of HCoV (human coronavirus; +ssRNA) is about 50% in both HCC patients (3/5) and non-cancer specimens (7/14). Edible-fucoidan derived from the Noto- Ishi-Mozuku reduces HAdV + HCoV for 5.8-fold (from 9.3 to 1.6 μg/mg of total cell-protein) in HepG2 cells [12]. Therefore, edible-fucoidan may heal PBC *via* normalization of LA, and also heal leprosy *via* reduction of invaded *Agrobacterium tumefaciens* [12]. Edible-fucoidan further cures co-infection of HIV (retrovirus)/HCV-HBV (preventing tumor growth of the HCC tissue), and also heals lethal co-infections of HAdV/NDV-FluB (inhibiting the production of virulent and lethal sialidase (neuraminidase) in the body of HCC patients). Thus, edible Noto-fucoidan could have the ability to rescue these three sadly-deceased-HCC patients.

III-II. EFFECT OF EXCESS AND SHORTAGE OF BIOTIN ONTO THE PSYCHIATRY

Biotin and D-aspartic acid have another role than growth factors in the brain (CNS) such as neurotransmitter; i.e., D-aspartic acid works as a potential endogenous ligand for N-methyl-D- aspartate (NMDA) receptor [83], and a regulator of glutamate receptor [84], and the biotin-respon- sive depression is surely present [85]. Interestingly, the excess intake of free biotin for a long period has been shown to induce the mania and the cardiac pain (personal communication from J. Oizumi), the infection of microbes into human body [22], and the ALS (amyotrophic lateral sclerosis) [86]. It is also found that both soluble- and membrane- type thiol-BIN/LIP are present in the pig cerebrum [34], and human serum thiol-BIN/LIP also has the amino-exopeptidase activity to opioid neuropeptides such as dynorphin A (YGGFLR), encephalin (YGGFL, YGGFM), δ-sleep- inducing peptide (9-mer; WAGGDASGE), and neuromedin B (10-mer; GNLWATGHFM-amide),but does not hydrolyze γ-Glu-Gln (γ-E-Q), substance P (RPKPQQFFGLM-amide), LH-RH (pyro-ELYENKPRRPYIL), and angiotensin II (DRVYIHPF) [87]. Brain-LIP from pig brain membranes also shows the multiple-hydrolase character; i.e., it hydrolyzes such neuropeptides and neuro- transmitter of γ-E-Q (8.5 nmol/min/mg), acetylcholine (2.1 nmol/min/mg), liver cell growth factor (GHK; 2.7 nmol/min/mg), *Hu*-ras^{HA} (GAGGVGKS; 1.4 nmol/min/mg) [58], and it also has the de-anchoring activity [88]. It is interesting that both thiol-BIN/LIP and brain-LIP can hydrolyze kyotorphin (YR), RF-amide, FMRF-amide, and proctolin (RYLPT) [87, 58], but cannot hydrolyze or liberate the LA from mitochondrial lipoyl-proteins (bovine heart pyruvate dehydrogenase and α-ketoglutarate dehydrogenase) [89]. However, difference is found between thiol-BIN/LIP and brain-LIP; i.e., brain-LIP can hydrolyze aspartame (DF-OMe), γ-E-Q, and glutathione (γ-ECG; highest A_{mo}), but thiol-BIN/LIP cannot. Thiol-BIN/LIP hydrolyzes shorter peptide in higher rate; i.e., Des-Met/Leu-enkephalin (YGGF) at 34.7 nmol/min/mg, dynorphin A (6-mer; YGGFLR) at 28 nmol/min/mg, dynorphin A (7-mer; YGGFLRR) at 2.8 nmol/min/mg, and dynorphin A (17-mer; YGGFLRRIRPKLKWDNQ) at 0.84 nmol/min/mg, respectively, but it hydrolyzes the dynorphin A (6-mer) at the highest affinity (A_{mo}) of 242 s^{-1} x M^{-1} and biocytin at lower affinity (A_{mo}) of 59.4 s^{-1} x M^{-1} [87]. Further, thiol-BIN/LIP activity is activated by Zn^{++} [87], on the other hand ferric- and ferrous-EDTA compounds inhibit the brain-LIP [90]. Thus, these two brain amidases work uniquely and independently in the brain membranes and central nervous system (CNS). Therefore, adequate biotin and LA intakes (moderation in eating biotin and LA) become the important issues to prevent the biotin- and LA- related various tragic brain-diseases.

One of the tragic disease of Down's syndrome is associated with cerebellar hypoplasia having a predisposition to progeria (Hutchinson–Gilford progeria syndrome) [91]. Porcine brain thiol- BIN/LIP activity is two-fold higher in the cerebellum than in the cerebrum [34].Biotin-related metabolites (pyruvate, β-lactic acid, hydroxyl-isovalerate, and leucine) in amniotic fluid are low in Down syndrome [92]. Therefore, biotin deficiency may be occurred in the mother of the Down syndrome patient during fetal brain development. Thus, the pregnant mother should avoid to take chicken egg-white (containing avidin), and should not go to swimming (having a risk to be infected by the marine mycobacteria such as Streptomyces avidinii excreting streptavidin) [93], and should take adequate foodstuffs containing abundant biotin [11, 50] and LA [42] in order to prevent the biotin deficiency during the pregnant period.

The appetite is a typical psychiatry phenomenon which seems to depend upon insulin and leptin. An anorexia and lose-weight case (44 w after the over-dose of biotin therapy at 10-30 mg/ day, 1 y 11 mo female) has expressed the insulin-sensitive sequence DNA-binding protein 1 in her serum, but other 2 sera at before and after 13 w of therapy do not contain insulin and/or leptin related proteins. HepG2 cells contain insulin receptor substrate 2 (IRS-2) and leptin receptor over-lapping transcript-like 1 before the fucoidan treatment, and contains insulin-like growth factor II re- ceptor (IGF-II receptor) after the fucoidan therapy, respectively [12]. Normal fetal liver cells (Hc cells) have no proteins related to insulin and leptin [12]. Therefore, cancer cells are in the elevated level of insulin, and state of cancer is the condition of anorexia. Thus, excess biotin in CNS may signal *via* metabolism of insulin/leptin and membrane receptors to recognize as the sensation of fullness. Furthermore, it is recently estimated when is the true-state of "hara-hachibun-me (moderation of eating)"; i.e., the intake of edible Japanese Mozuku in Sanbai-Zu during eating at the dinner and/or lunch is able to give the signal to the stomach and/or gut, and gives the CNS of the feeling of fullness (unpublished observation).

IV. Biotin and morphogenesis

The cleft lip is a tragic morphological disease enrolling the patient and patient's family, and is considered to be due to biotin deficiency in pregnant mother [94, 95]. This disease occurs highly in the human fetal male (2: 1, male to female), and experimental data on mice also indicates that cleft palate occurs mainly in the male fetal mice [96]. The K_m of thiol-BIN/LIP in the upper labial skin of LEW rat is the smallest in the male (0.75 μM in male, and 1.18 μM in female) [16]. Healthy bloods of baby and adult have heat-stable thiol-BIN/LIP K_ms (except during healing-period in acute bronchitis), but one male adult with cleft lip has a heat-labile thiol-BIN/LIP K_m; i.e., a unique change in reduction after the heat-treatment is occurred. The K_m of the serum of an adult cleft lip patient shows the heat-instability in thiol-BIN/LIP; i.e., K_m decreases from 6.93 to 3.76 μM (46% decrease of original) by the heat-treatment for 4 h at 37 ℃. On the other hand, healthy male adult's enzyme is heat-stable; i.e., K_m is not significantly changed from 5.94 to 5.95 μM (Table 3). Further, the R_{ep} (specific repulsion; k_{cat}/K_{ip}) of the serum of an adult male cleft-lip's patient also shows the heat-instability in thiol-BIN/LIP; i.e., R_{ep} decreases from 48.7 to 19.7 s^{-1} x M x 10^{-3} (59% decrease of original) by the same heat-treatment. On the other hand, healthy male adult enzyme is heat-stable; i.e., R_{ep} is not significantly changed from 59.3 to 74.4 s^{-1} x M x 10^{-3} (125% of the original) (Table 3). Serum total-biotin is 1.93 μg/ml in this patient's blood at 58 y, which is similar to the healthy median value of 1.97 μg/ml (range; 1.80 - 20.5 μg/ml, Table 1). Then, glycochain of thiol-BIN/LIP in cleft-lip's patient may be chemically different and labile, and patient may be suffered from the mother's mal-nutritional state of biotin and LA during the most developing fetus-period of his upper labial skin [22].

Frequency of occurrences of cleft lip is also depended on the racial differences; i.e., the high- EST frequency is observed in the native Americans (3.74/1000), and also high in Japanese and Chinese. These Mongoloid-races may take foodstuffs of low nutrition with respect to biotin and LA; i.e., African takes adequate amount of these vitamins from various African beans such as Coffee bean, Lens bean, Bambara groundnut, and Chickpea (Egyptian bean), even though they Africans may live in poor countries be economically poor. However, Japanese takes "Tofu" made from filtrated extract of powdered soy bean, and the obtained debris of nutrition-rich Okara (a byproduct of soy milk production) is not so frequently eaten. As a principal food, Japanese takes boiled polished rice, which is scanty of nutrition due to the absence of the rice bran. Native Americans also takes mainly meat, which contains scarce amount of biotin and LA, as the staple food. Therefore, Asian's and native American's pregnant-women should take the stewed African beans and coffee, and should not take dangerous chicken egg-white containing biotin/LA-binding protein of avidin, which is heat stable, alcohol and trypsin resistant glycoprotein [11].

Recently, it has been found that fucan derived from Okinawa-Mozuku is actively transported through gut (Caco-2 cells), and is able to attain wholly in the human organs [70]. Further, it is also have found that biotin (0.005 mg/mL) in the medium is the growth promoter of Caco-2 cells (derived from the colon cancer), and this vitamin promotes the active transport activity of fucan [70]. Since the gene expression of the proteins of active transport system is not changed by biotin, biotin seems to promote the incorporation of the hydrophobic active-transport proteins adequately into the membrane compartment. Therefore, fucoidan or fucan gives the measure of biotin and LA *via* interacting with the glycochain of thiol-BIN/LIP and yet safe to the fetal baby, and the pregnant women should better to take the edible Japanese-Mozuku (brown alga) in order to prevent this morphological or developmental disease.

Biotin and LA are surely important in the developmental morphogenesis. It has previously been identified that the *rodA* gene (determining rod shape of *Escherichia coli*) is present at the map position of 14.3 min on the chromosome, where

also is present the *lip* gene (lipoic acid synthase) [97]. Sulphur inserting enzyme of LA synthetase (*lip*) is similar to biotin synthetase (*bioB*) [98]. The gene product protein of the *rodA* gene has been recognized to be the prokaryotic hydrophobic membrane-protein which aggregates even after boiling in sodium dodecyl sulfate (SDS) [99]. Although membrane glycoprotein of porcine brain-LIP (hydrophobicity; 0.530) has been isolated by using 0.5% (v/v) of non-ionic detergent of Nonidet P-40 [32], the extremely hydrophobic membrane-protein of *rodA* (hydrophobicity; 0.726) has not yet been clearly isolated; i.e., other similar extremely hydrophobic membrane-protein of *FeT*-gene (Fe^{++} transporter, hydrophobicity; 0.748) has not yet been isolated (personal communication from Dr. M. Tanokura, The University of Tokyo). Biotin deficiency reduces the activity of biotin-dependent enzymes of acetyl-CoA carboxylase and propionyl-CoA carboxylase, and can cause alterations of lipid metabolism which lead to the impaired biosynthesis of membranes [63, 100]. Further, it has also been recently found that the membrane proteins such as ultrahigh-sulphur keratin associated protein (UHS-KAP, hydrophobicity; 0.597) and olfactory receptor (OR, hydrophobicity; 0.637) are excreted into the bloods or sera from the biotin-deficient alopecia-patients' skins [22].Furthermore, a girl alopecia- patient (3y female) with GSD1b shows the seizure (epileptic seizure) due to hypoglycemic encephalopathy and/or biotin deficiency [22]. It has also been detected in her blood that the brain membrane-proteins of ion-transporting proteins (vacuolar proton pump subunit H, potassium voltage-gated channel subfamily H member 5, calcium-binding mitochondrial carrier protein SCaMC-1, sodium bicarbonate transporter-like protein 11, and brain calcium channel II) appear before biotin therapy, but all these ion-transporting membrane-proteins disappear after the biotin therapy [22]. In this case, adequate concentrations of free-biotin might have normalized the membrane biosynthesis, and her seizure is healed by free biotin. Therefore, the clarifying for the metabolism of membrane protein is again an important issue for the analysis of developmental membrane-based morphogenesis in humans.

Thus, the study for the incorporation mechanisms of membrane proteins into the membrane compartment becomes to be the important issue; i.e., the incorporation of the membrane protein requires adequate metabolic state (the measure) of biotin concentration as firstly reported by J.C. Collins et al. [79]. Furthermore, the detachment of anchored membrane-protein requires the action of the brain-LIP, which is regulated by LA [88]. Therefore, the immortality of the cultured cell-lines, cancer tissues, fetus, and also the morphologic development in normal organs might be strongly under the control of biotin and/or LA [12, 22, 28, and 79]. Thus, it is presumed that these rapidly growing organs are able to biosynthesize both biotin and LA through the aspartic acid as biochemical source [12, 27, 65].

Both high concentration of biotin (total-biotin 35.2 μg / g, and free-biotin 31.7 μg / g; i.e., 90% is free-form biotin) [47] and high activity of thiol-BIN/LIP [12] has been found in chicken egg-yolk, which indicates that both free form biotin and thiol-BIN/LIP supply is essential for the growth of fetal chick. Further, the importance of the direct supply of essential thiol-BIN/LIP [61] and ester-LIP [28], and free biotin [50] to promptly growing mammalian baby by the human breast milk has already been suggested. In the human breast milk obtained from the mother at 9 mo after the gestation, lipoic acid is surely rich; i.e., 0.0626 mg/mL of total LA and 0.0132 mg/mL of free LA (free/total ratio; 21.1%) (Unpublished observation). This human breast milk contains high thiol- BIN/LIP activity (41.0 pmol/min/mg; about half activity of serum) and also high concentration of free-biotin; i.e., 0.209 μg/mL (the ratio of free/total biotin is 56%) as compared to 0.122 μg/mL of human serum (the ratio of free/total biotin is 7%) [50]. Cow's milk also contains relatively high thiol-BIN/LIP [101] and high free biotin (0.248 μg/mL, the ratio of free/total biotin is 68%) [50].Therefore, direct supply of free-form biotin and LA together with thiol-BIN/LIP and ester-LIP is essential for the adequate growth of developing baby and fetus. Fucoidan is expected to give the measure of biotin for the growth of tumors of cancer *via* controlling state of metabolism of membrane compartment. Further, fucoidan seems to have the direct effects *via* glycol-biological and immunological mechanisms together with the reduction of HIV and HCV virus on HepG2 cells [12], the experiment for the therapy or prevention of chemically induced HCC (enlargement of tumors) by edible fucoidan from Silky-Mozuku (*Nemacystis decipiens*) in the rat has already been performed [54]. It has been found that the successful pre- vention is observed only in the SD rat (outbred strain: constitutively possessing the normal immunity), but the tests with the LEW rat (inbred strain: without constitutively possessing the im- munity genetically and generally used in the transplantation experiment) have been all in vain. In- versify, fucoidan may give also the measure in biotin to developing fetus and may prevent tragic morphological diseases such as the Down syndrome and the cleft lip, since edible Japanese fucoidan is expected to be also the fully safe-drug in the pregnant women and the fetal babies. Thus, both Japanese edible fucoidan and fucan shows no effect on the normal fetal liver's Hc cells as assessed by the differences in the cell-growth (by the wet volumes and wet weights), but inedible Ireland's fucoidan killed and detached the Hc cells from the plastic culture plate [12]. Further, it has been found in Japan that Japanese people in the Jomon and Yayoi periods (BC 1,000 - AD 300) has already taken seaweeds and sea algae; i.e., the pottery or earthenware of Jomon and Yayoi, which is adhered by seaweed and sea algae, has been dug up. Then, Japanese has long safely taken the Japanese Mozuku without no descriptions of the side effects. Furthermore, N. Abe et al. recently has reported that the rats fed with edible fucoidan of Japan in long-time and in excess amounts show no symptoms of the side effects [102]. This experiment also indicates that the Japanese edible-fucoidans and Mozuku are the safe drugs/foodstuffs.

Furthermore, there is likely a connection between stress and illness, and alopecia areata, depression, and cleft lip may also be due to psychological stress. Stresses due to economic pover- ty poverty, being reduced to poverty, getting bullied, and harassment from a position of power and/ or a rich person, sometimes cause depression, loss of appetite, and suicide in

psychologically in- stable people. Thus, it is usually said that poverty dulls the conscience. Therefore, pregnant women and bullied persons should firstly have an attitude of ataraxia (freedom from worry and fear) of Greek philosophers of Pyrrho (BC 360 - 270) and/or Epicurus (BC 341 - 270).Then, the good appetite and adequate intake of nutrition would be re-obtainable in these sick persons, and prevention of the illnesses as described is soon healed. Therefore, the spiritual strength and the nutrition (biotin and LA) are strongly linked as expectedly.

V. APPENDIX; MODERATION OF THE MEMBRANE BIOSYNTHESIS BY THE FOODSTUFF OF FUCOIDAN (EDIBLE JAPANESE MOZUKU)

Edible fucoidan or brown alga Noto-Silky-Mozuku has recently been indicated as the moderating drug by normalizing the membrane biosynthesis *via* modulating the metabolism of vita- min H (biotin) [12, 22]. Furthermore, edible fucoidan has an annihilation power against some viruses (+ssRNA viruses, dsDNA virus of HAdV, and -ssRNA virus of NDV) and bacteria [12]. Then, the virulence onto the kidney by *Escherichia coli* O-157 may be healed by fucoidan *via* normalization of kidney membranes; i.e., fucoidan heals the bacterial virulence caused by the detergent-like membrane-proteins of the acid stress chaperone *hde*A proteins (mature chain; ADAQKAADNK KPVNSWTCED FLAVDESFQP-; mixture of 1st major from N-terminal amino acid M_r 9,741, 2nd major from 2nd amino acid from N-terminal M_r 9,670, and 3rd minor from 3rd amino acid from N-terminal M_r 9,555) and β-lactamase TEM fragment (from 24th amino acid of mature chain; HPETLVKVKDA EDQLGARVGY IELDLNSGKI-; M_r 28,907), which are kindly purified and donated to me by M. Tarawa.

Another tragic membrane-morphological diseases due to +ssRNA virus such as poliomyelitis (injured spinal cord's grey matter induced by poliovirus) and congenital rubella syndrome (injured heart, eye, and inner ear induced by rubella virus) may be healed by the fucoidan treatment through giving the happy mean of the ratio of membrane compartment [12]. Further, other severe diseases due to +ssRNA virus such as middle east respiratory syndrome (MERS-coronavirus; MERS-CoV), severe acute respiratory syndrome (SARS-coronavirus; SARS-CoV), dengue fever (Dengue virus), hepatitis C (HCV), yellow fever (yellow fever virus), and West Nile fever (West Nile virus) can also be cured promptly [12]. Further, it seems to me that the co-infection phenomenon is the important issue as stated in the section 3.1; i.e., the recent pandemic in Korea of MERS-CoV seems to be co-infected with canine parvovirus (linear ssDNA) and/or avian influenza A virus (-ssRNA), which increase the virulent power to induce the immediate death of co-infected patients.

Although fucoidan has not yet showed a remarkable annihilation power against the -ssRNA virus within the three days of therapy [12], -ssRNA may also be annihilated with more duration of therapy period. Then, other serious and tragic diseases such as IDDM type-1 (injured pancreatic β-cells induced mainly by measles virus (-ssRNA), diabetic nephropathy (injured kidney membrane induced by IDDM type 1, and requires painful ***dialysis***) [67], Ebola virus disease (-ssRNA), and influenza (the flu; due to influenza virus; -ssRNA) [103] may also be prevented and healed by the edible Japanese fucoidan. In relation to the lethal degenerating disease in nerve cells in CNS, such prion diseases as bovine spongiform encephalopathy (BSE) and human Creutzfeldt-Jakob disease (CJD) may also be prevented by fucoidan, if these diseases are due to some unknown viruses. Otherwise, fucoidan may have the ability on the membrane protein of prion protein (PrP) *via* normalizing the membrane protein metabolism by biotin or normalizing the anchoring/de-anchoring reaction by LA/brain-LIP on the brain membranes.

Fucoidan may also be effective for other serious diseases due to the low and/or labile thiol-BIN/LIP such as Rett syndrome (with scoliosis of the spine) [104], Coffin-Siris syndrome (with hypoplasia of the fifth fingers and toe-nails) [105]. Changed biotin metabolism is reported in the erythroderma [106], and changed glycochain (increased fucosylation of the glycochain of haptoglobin) in the psoriasis has also been reported [107]. Therefore, edible fucoidan of Japanese Mozuku may also heal the erythroderma and psoriasis through changing the glycochains in glyco- proteins (including thiol-BIN/LIP) through giving the measure of biotin.

VI.CONCLUSION

Both biotin and lipoid acid is important regulatory vitamin for the membrane biosynthesis. Further, biotin and lipoid acid are adequately biosynthesized during fetus period of human life in order to grow suitably. It is estimated that excess of these vitamins induces many diseases.Further, edible Japanese fucoidan is found to normalize the ratio of membrane component *via* moderation of the biotin concentration in the human body.Therefore, moderate and adequate intake of these two vitamins and also fucoidan in humans is recommended in order to elongate the life span, to prevent cancer, ageing, and cognitive impairment, and to maintain the healthy, happy, and good quality of human life.

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Table 1: **Biotin concentrations in the human serum.***

Biotin deficiency		Moderation amount		Excessive of biotin	
Child patient (Alopecia)		Healthy child		Child patient	
Total	Free	Total	Free	Total	Free
2.03	0.0355 (1 y, girl)[#]	2.57	0.141 (16.1 mo, girl)	7.25	0.0959 (2 y, girl)[f]
2.31	0.535 (4 mo, boy)[a,#]	2.76	0.197 (10 mo, boy)	6.09	0.737 (16 mo, girl)[g]
1.87	0.525 (3 mo, boy)[##]	3.22	0.804 (3y, boy)[c]		
3.08	0.00 (3 y, GSD1b)[b]	3.01	0.164 (15 mo, boy)		
		3.21	0.180 (8 mo, girl)[d]		
		3.22	0.659 (11 mo, girl)[e]		
		3.11 **	*0.189*	***6.67*** **	*0.416*
Adult patient		Healthy adult		Adult patient	
Total	Free	Total	Free	Total	Free
1.14	0.0406[h]	1.80[k]	0.122	3.14[n]	ND
1.32	0.110[i]	2.05[l]	0.323	2.88[o]	ND
0.748	ND[j]	1.97	0.0439 (20 y, male)		
		1.93[m]	ND		
1.14 **	*0.0753*	***1.95*** **	*0.122*	***3.01***	*ND*

*Total and free biotin in the serum were expressed in μg/mL. Biotin was measured as in [11]. [a]; Biotin deficiency with heat labile thio-BIN/LIP. [b]; GSD-1b with heat labile thio-BIN/LIP. [c]; Elder brother of "a" with heat stable thio-BIN/LIP. [d]; Gastroenteritis. [e]; Common cold. [f]; Anorexia. [g]; Bronchitis. [h]; Gait disorder with optic atrophy (32 y, female). [i]; Gait disorder with optic atrophy (46 y, male). [j]; HCC (+HCV; 56 y, male). [k]; Heathy adult (33y, male). [l]; Mother of patient "h", may slightly be biotin deficient. [m]; Cleft lip but healthy (58 y, male). [n]; Pancreatic-tail cancer (76 y, male). [o]; Pancreatic-head cancer (64 y, female). ND; not yet determined. Bold italic; median value. [#]; Given the powdered milk of Milfy (Meiji). [##]; Given the powdered milk of New MA-1 (Morinaga), and this boy has a large fresh K_m of 14.1 μM. ** Significant between the two median values ($P < 0.05$, Mann-Whitney's U test; one-tailed test).

Table 2: Biotin balance (Free/Total ratio) and enzymatic performance of fresh thio-BIN/LIP in the human serum.*

Biotin deficiency		Moderation amount		Excessive of biotin	
Child patient		Healthy child		Child patient	
Free/Total (%)	C_{ap} (s^{-1})	Free/Total (%)	C_{ap} (s^{-1})	Free/Total (%)	C_{ap} (s^{-1})
1.75[#]	41.0	5.49	37.5	1.32[f]	58.2 (Anorexia)
23.2[a,#]	46.3	7.14	33.2	12.1[g]	52.0 (Bronchitis)
28.1[##]	52.3	25.0[c]	27.2 (may be cold)		
0.00[b]	36.6	5.45	38.1		
		5.61[d]	46.5 (Gastroenteritis)		
		20.5[e]	27.9 (Common cold)		
Median (range)		***5.55(5.45-7.14)***	***37.5(33.2-38.1)***		
Adult patient		Healthy adult		Adult patient	
Free/Total (%)	C_{ap} (s^{-1})	Free/Total (%)	C_{ap} (s^{-1})	Free/Total (%)	C_{ap} (s^{-1})
3.56[h]	22.4	6.78	40.7	ND	ND
8.33[i]	28.7	15.8[l]	41.1		
		2.22	45.2		
		ND[m]	29.7 (Cleft lip)		
Median (range)		***4.50 (2.22-6.78)***	***40.9 (40.7-45.2)***		

Total and free biotin in the serum were expressed in μg/mL. Biotin was measured as in [11]. [a]; Biotin deficiency with heat labile thio-BIN/LIP. [b]; GSD-1b with heat labile thio-BIN/LIP. [c]; Elder brother of "a" with heat stable thio-BIN/LIP. [d]; Gastroenteritis. [e]; Common cold. [f]; Anorexia. [g]; Bronchitis. [h]; Gait disorder with optic atrophy (32 y, female). [i]; Gait disorder with optic atrophy (46 y, male). [l]; Mother of patient "h", may slightly be biotin deficient. [m]; Cleft lip but healthy (58 y, male). ND; not yet determined. Bold italic; median value. [#]; Given the powdered milk of Milfy (Meiji). [##]; given the powdered milk of New MA-1 (Morinaga), and this boy has a large fresh K_m of 14.1 μM.

Table 3: Recovery effect on the kinetic parameters of the glycoprotein enzyme of thiol-BIN/LIP by the addition of polysaccharides *in vitro*.*

Serum	*Amo*	*V* max	*Km*	*Kip*	*Rep*	*Cap*
	$s^{-1} \times M^{-1}$	pmol/min/mg	μM	by biotin (μM)	$s^{-1} \times M \times 10^{-1}$	s^{-1}
Healthy baby control (8 mo, female; this control is the same as [d] of Table 1 and 2; Gastroenteritis)						
Fresh	**22.2**	**156**	**8.91**	**494**	**97.6**	**46.5**
Heat treated control	**26.2**	**185**	**8.93**	**586**	**118**	**55.6**
Okinawa-fucan	**22.3**	**167**	**9.46**	330	69.6	39.4
Noto-Ishi-fucoidan	**33.6**	**211**	**7.94**	471	126	65.1
Noto-Silky-fucoidan	**31.5**	**217**	**8.73**	643	177	74.6
Heparin	**35.2**	**217**	**7.81**	346	95.1	57.9
Biotin-deficient baby patients						
Baby patient A (4 mo, male; this patient is the same as [a] of Table 1 and 2)						
Fresh	25.5	182	**9.02**	**365**	83.9	46.3
Heat treated control	18.6	181	**12.3**	**917**	**210**	62.5
Okinawa-fucan	37.6	157	**5.29**	617	123	68.0
Noto-Silky-fucoidan	33.6	143	**5.39**	760	138	68.1
Heparin	26.3	253	**11.2**	765	**225**	76.9
Baby patient B (3 mo, male)						
Fresh	22.4	249	**14.1**	386	**122**	52.3
Heat treated control	21.4	196	**11.6**	378	93.8	44.8
Okinawa-fucan	29.9	137	**5.81**	441	76.6	47.0
Noto-Ishi-fucoidan	30.4	142	**5.92**	**736**	**133**	56.3
Heparin	18.2	260	**13.9**	**688**	**174**	56.3
Baby patient C (1 y, female)						
Fresh	35.9	112	**3.95**	331	46.9	41.0
Heat treated control	**10.1**	124	**15.6**	**1310**	**206**	45.6
Okinawa-fucan	**16.8**	116	**8.73**	424	62.2	32.3
Heparin	10.3	122	**9.17**	**1020**	158	40.7
Healthy adult control (33 y, male; this is the same person as [k] of Table 1)						
Fresh	**27.9**	**131**	**5.94**	**359**	**59.3**	**40.7**
Heat treated control	**26.0**	**122**	**5.95**	**481**	**74.4**	**44.0**
Okinawa-fucan	**32.2**	**154**	**6.06**	576	112	60.1
Noto-Ishi-fucoidan	**31.7**	**179**	**7.14**	810	183	76.2
Heparin	**26.9**	**132**	**6.21**	280	46.8	35.5
Biotin-deficient child and adult patients						
Child patient with GSD1b (3 y, female; this patient is the same as [b] of Table 1 and 2)						
Fresh	**19.9**	132	**8.38**	**404**	67.4	36.6
Heat treated control	**7.95**	147	**23.4**	478	88.9	26.6
Okinawa-fucan	**10.7**	63.3	**7.46**	774	61.3	25.7
Noto-Silky-fucoidan	**21.5**	98.0	**5.78**	1410	175	61.3
Heparin	13.0	139	**13.5**	202	35.5	21.5
Adult patient (32 y, adult female; this patient is the same as [h] of Table 1 and 2) (gait disorder with optic atrophy)						
Fresh	**18.4**	63.6	**4.45**	342	27.7	22.4
Heat treated control	16.6	300	**38.2**	265	168	52.8
Okinawa-fucan	17.3	125	**9.13**	689	96.2	40.8
Noto-Silky-fucoidan	23.3	201	**10.9**	1010	257	77.4
Heparin	21.8	155	9.01	277	34.4	34.4
Adult patient (46 y, adult male; this patient is the same as [i] of Table 1 and 2) (gait disorder with optic atrophy)						
Fresh	**19.2**	96.2	6.33	353	42.9	28.7
Heat treated control	15.7	114	**9.17**	705	114	42.3
Okinawa-fucan	**33.9**	97.6	**3.65**	861	107	60.2
Heparin	21.0	119	7.19	709	107	47.4

*Kinetic parameters of serum thiol-BIN/LIP were expressed according to [16, 50]. Heat treatment at 37 °C for 4 h of the diluted serum was performed as [50], then this heat treated serum was immediately used for the enzyme assay. Thus, direct recoveries of the kinetic parameters were determined by directly adding polysaccharide to the reaction mixture for thiol-BIN/LIP assay at 0.20 mg/mL, and compared to the control without the addition of polysaccharide. Heparin was derived from porcine intestine. Okinawa-fucan, and Noto-Ishi- and Noto-Silky- fucoidans were derived from the brown algae of *Cladosiphon Okamuranus*, *Sphaerotrichia divaricata,* and *Nemacystis decipiens*, respectively.