Creation of Multi-Functional Foods

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BIOREGULATORY FUNCTION OF FOOD COMPONENTS

Foods contain various biologically functional components which contribute to our health. As shown in Table 1, bioregulatory function of food components are classified into five categories. In the enhancement of biodefense system, dietary fibers (DF), unsaturated fatty acids (UFA), antioxidants are effective. These components are also effective in other categories. This means that food components are multifunctional. To maintain our health, effective use of multi-functional activity of food components is essential.

In Japan, foods containing biologically functional factors are called "functional foods". Among them, foods passed the assessment of the Ministry of Health, Welfare and Labor are called "Tokutei Hoken-you Shokuhin" (THS) which means foods designed for special use of human health. So far more than 400 items were permitted as THS to improve intestinal condition, serum lipid and glucose levels and blood pressure, and to enhance mineral absorption especially calcium and iron. However, active substances used in these foods are very limited as shown in Table 1. To obtain the permission for THS, manufacturers should present the evidences on the expression of biological effects in animal, if possible in human. In the case of food components which express their function in our body, it is difficult and expensive to clarify the mechanism for their biological effect expression. Thus, most active components permitted to use in THS act at the outside of our body, i.e. in the digestive tract. In addition, these active substances are widely used for the production of various foods and these foods are in sale without the label of THS. These conditions make food industries hesitative to use their money for the development of functional foods. To cut down the cost, combinational use of known functional factors is important.

FATTY ACIDS

Fatty acids are divided into two groups, saturated fatty acids and UFA. UFA is further divided into mono and polyunsaturated fatty acids (PUFA). In addition, UFA is divided into several groups such as n-3, n-6 and n-9 series dependent on the location of double bond,

Table 1. Bioregulatory functions of food components

Classification	Function (Food components)
Enhancement of defensive system	Enhancement of immune function (DF). Anti-allergic (UFA, Antioxidants) and anti-cancer (DF, UFA, Antioxidants) activities.
Prevention and remediation of diseases	Prevention of diabetes (DF, Antioxidants), hypertension (Peptides), allergy (DF, UFA, Antioxidants).
Regulation of biorhythm	Hormone and anti-hormone activity (Isoflavones, CLA).
Prevention of obesity	Inhibition of digestion (Enzyme inhibitors). Enhancement of energy consumption (CLA, Spices). Low calorie materials (DF, Oligosaccharides, Sugar alcohols).
Suppression of aging	Suppression of oxidation (Antioxidants). Improvement of intestinal flora (DF, Oligosaccharides, Sugar alcohols, Lactobacillus).

DF, dietary fibers; UFA, unsaturated fatty acids; CLA, conjugated fatty acid.

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and biological functions of UFA are highly dependent of the group of the fatty acids. Animal cells can proliferate in the absence of PUFA (1), but these fatty acids are essential for the expression of differentiated functions. In n-6 PUFA, animals demand linoleic acid (LA, 18 : 2n-6) as a starting material of fatty acid metabolism to produce γ-linolenic (GLA, 18 : 3n-6), dihomo-γ-linolenic (DGLA, 20 : 3n-6) and arachidonic (AA, 20 : 4n-6) acids. Oxidation of AA by lipoxygenase produces 4-series leukotrienes (LT) such as LTB_4 and LTC_4 which induce the type I allergy. In the case of n-3 PUFA, α-linolenic acid (ALA, 18 : 3n-3) is metabolized to eicosapentaenoic (EPA, 20 : 5n-3) and docosahexaenoic (DHA, 22 : 6n-6) acids. EPA is a substrate of the synthesis of 5-series LT such as LTB_5 and LTC_5 which inhibit the type I allergy through the competition with 4-series LT.

PUFA exert various biological functions, including the regulation of lipid metabolism (2,3) and immune function (4). Among them, n-3 PUFA exert strong regulatory activities on the lipid metabolism and immune function. As shown in Table 2, oral administration of EPA or DHA ester at the 2% level for 3 weeks significantly decrease serum levels of cholesterol, triglycerides and phospholipids (3). In addition, these esters suppress LTB_4 productivity of rat peritoneal exudate cells (PEC) through the decrease of AA content, and enhance LTB_5 productivity through the increase of EPA content (3). There was no significant deference between EPA and DHA in the regulation of serum lipid levels, but EPA showed much stronger activity than DHA in the regulation of eicosanoid production. This means that these PUFA rich in fish oils exert different biological effect at the same time and that the expression of these activities is component dependent. Though purified EPA and DHA esters were used to clarify the difference between these two PUFA in this experiment, natural oils are preferable for the production of biologically functional foods. When Sprague-Dawley rats were fed EPA-rich or DHA-rich fish oils at the 10% level for 3 weeks, similar results were obtained (2).

CLA is a generic term for the geometric and positional isomers of octadecadienoic acid. CLA exerts various functions such as anti-cancer (5-7), anti-obesity (8-11) and immunoregulatory activities (12-14). As summarized in Table 3, oral administration of CLA at the 1.0% level significantly decreased epididymal adipose tissue weight with a significant increase of liver weight (12). In the rats fed 0.5% CLA, the reducing tendency of adipose tissue weight is observed, but not the increase of liver weight. CLA also affects to eicosanoid production in a tissue-dependent manner. Significant decrease of serum PGE_2 level is observed in the rats fed 1.0% CLA. In addition, significant decrease of LTC_4 productivity is observed even in the rats fed 0.5% CLA in the lung, but not in the spleen. In spleen, CLA feeding decreased LTB_4 level, but not LTC_4 level immunoglobulin (Ig) production-stimulating activity of CLA is not only tissue-dependent, but also dose-dependent. CLA enhanced Ig productivity of MLN lymphocytes, but not that of spleen lymphocytes, in the rats fed at the 0.5 or 1.0% levels (12). On the other hand, it enhanced Ig productivity of spleen lymphocytes, but not that of MLN lymphocytes, in the rats fed at the

Table 2. Dietary effect of EPA and DHA esters on serum lipid levels and eicosanoid releasing activity of peritoneal exudate cells isolated from Sprague-Dawley rats

	Safflower	EPA	DHA
TBARS (nmol MDA/mL)	3.0±0.2	3.4±0.1	3.3±0.4
Liver α-tocopherol (mg/g)	30.3±8.1	19.8±2.7	15.5±2.7
Serum cholesterol (mg/dL)	76.8±5.6^a	57.1±4.4^b	45.1±6.4^b
Serum triglycerides (mg/dL)	57.2±2.5^a	42.8±5.4^b	41.6±5.1^b
Serum phospholipids (mg/dL)	109.8±4.7^a	85.2±4.7^b	75.3±4.5^b
AA content in PEC (%)	19.8±0.6^a	13.3±0.2^b	13.8±0.3^b
LTB_4 release from PEC (ng/10^6 cells)	18.5±0.6^a	8.3±0.3^b	11.4±0.5^c
EPA content in PEC (%)	0^a	5.0±0.2^b	1.3±0.0^c
LTB_5 release from PEC (ng/10^6 cells)	0^a	6.0±0.1^b	0.6±0.1^c

Data are mean±SE (n=5) and values without common letters are significantly different at $p<0.05$. TBARS, thiobarbituric acid-reactive substances; AA, arachidonic acid; PEC, peritoneal exudate cells; LT, leukotriene; EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid.

Table 3. Dietary effect of conjugated linoleic acid on tissue weight, eicosanoid production and serum immunoglobulin levels of Sprague-Dawley rats

	CLA dose (%)		
	0	0.5	1.0
Liver (g/100 g body weight)	4.17±0.09[a]	4.11±0.09[a]	4.54±0.07[b]
Adipose tissue (g/100 g body weight)	1.41±0.07[a]	1.09±0.09[ab]	0.97±0.14[b]
Serum PGE_2 (ng/mL)	23.2±0.9[a]	19.9±1.0[ab]	17.7±0.8[b]
Lung LTB_4 (ng/g)	37.6±11.2	29.5±3.6	24.4±3.7
Lung LTC_4 (ng/g)	34.7±3.2[a]	15.3±2.9[b]	11.1±3.0[b]
Spleen LTB_4 (ng/g)	47.3±2.4[a]	43.0±2.7[ab]	38.2±2.6[b]
Spleen LTC_4 (ng/g)	169±12	187±19	154±18
Serum IgA (μg/mL)	21.5±0.5[a]	24.3±4.1[ab]	30.8±2.9[b]
Serum IgE (ng/mL)	15.9±1.2[a]	16.2±2.7[a]	9.0±1.6[b]
Serum IgG (mg/mL)	1.4±0.3[a]	3.7±1.1[b]	3.0±0.9[b]
Serum IgM (μg/mL)	223±33[a]	401±91[ab]	568±100[b]

Data are means±SE (n=5) and values without common letters are significantly different at $p<0.05$. PG, prostaglandin; LT, leukotriene; CLA, conjugated linoleic acid.

levels lower than 0.5% (13). These results suggest that the expression of biological effect of food components is highly dependent on tissues, dose and biological reaction.

CLA is produced from LA by bacteria in bovine stomach, where 9cis,11trans (9c,11t) isomer is predominantly occurred. Thus, small amount of the isomer is present in milk product. On the other hand, CLA studies have been executed using an artificial CLA preparation, an 1 : 1 mixture of 9c,11t and 10t,12c CLA. Recent studies clarified that biological activities of these two isomers are fairly different. For example, 10t,12c CLA feeding to C57BL/6J mice enhanced IgA production of spleen lymphocytes, but 9c,11t isomer did not (14). On the other hand, feeding of 9c,11t CLA enhanced the production of tumor necrosis factor-α of the lymphocytes, but 10t,12c CLA did not. In the case of cancer cell-specific toxicity, 10t,12c CLA exerts stronger toxicity to rat hepatoma dRLh-84 cells (5). In human mammary cancer MCF-7 cells, 9c,11t CLA exerts cytotoxic effect stronger that 10t,12c CLA in the presence of 1% fetal bovine serum (FBS) (6). When the cells were cultured in the presence of charchol-treated FBS (estrogen-free serum), 10t,12c CLA inhibited the induction of proliferation by estrogen and insulin, but 9c,11t CLA did not. These results suggest the biological effect of CLA is highly structure-dependent and that the dependency varies with biological reactions. In addition to the above hormones, the expression of CLA is modified by monoenic fatty acids. For example, cytotoxic activity of 10t,12c CLA is alleviated by oleic and palmitoleic acids (6). These results indicate that the clarification of the interaction between food components is important to control their biological activities.

Tissue dependency in the biological effects of food components may partly due to the tissue-specific transport and metabolism of these components. Thus, determination of active component in various tissues is important for the clarification of their biological effects. Since CLA is fairly unstable components, its esterification method for gas chromatography should be optimized (15). CLA is absorbed from intestine with a slightly lower rate than LA and distributed to various tissues (16). It tissue levels were low in the brain and high in the lung and adipose tissues. Spleen and kidney have a higher CLA levels than serum, but the levels in heart and liver are as high as serum. Such information on the distribution of food components is useful for the determination of target tissue and the clarification of action mechanism.

Excess feeding of biologically active substances may lead to the expression of side effect. Thus, confirmation of food safety is important. Though CLA enhanced lipid catabolism and decrease adipose tissue weight, it also induces an increase of the liver weight and a morphological change similar to fatty liver in Sprague-Dawley rats fed CLA at the 2.0% level (17). In addition, dietary CLA enhanced the proliferation of transplanted rat hepatoma dRLh84 cells (18,19), though it suppressed the proliferation of the cells *in vitro* (5). Though UFA enhanced IgE production of rat lymphocytes and suppressed IgA, IgG and IgM production at 1 mM (20),

these unfavorable effects were not expressed in feeding experiments (2,3). However, a decreasing tendency of liver α-tocopherol level is marked in the rats fed EPA or DHA esters as shown in Table 2 (3). Appropriate estimation of such data suggesting toxic effect of food components is important for the establishment of food safety.

ANTI-OXIDATIVE COMPONENTS

UFA exerts cytotoxic effect through their oxidation, as well as beneficial effects mentioned above. Thus, the addition of antioxidants is important in the application of UFA for functional foods. Though UFA enhanced IgE production of rat lymphocytes and suppressed other protective Igs, the enhancement of IgE production is cancelled by a lipophilic antioxidant a-tocopherol but not by a water-soluble antioxidant ascorbic acid (20,21). This suggests that the oxidation of UFA in a lipophilic environment such as cell membrane is important in the stimulation of IgE production. In addition, we found that oxidative stress induced by visible light irradiation in mouse splenocytes suppressed their IgM production and the suppression was cancelled by α-tocopherol (22). These results suggest that excessive oxidation is lead to the abnormal immune functions. In addition to the suppression of lipid peroxidation, antioxidants exert anti-allergic effect through the suppression of 4-series LT production by inhibiting lipoxygenase activity (23). These results suggest that antioxidants are effective for the prevention of various diseases induced by lipid oxidation.

α-Tocopherol is a representative lipophilic antioxidant widely distributed in various foods. It suppresses lipid peroxidation more effectively than ascorbic acid and contributes to the elongation of life time through the prevention of various diseases. In the case of antioxidants, synergism plays an important role in the expression of their biological functions. Ascorbic acid could not inhibit lipid peroxidation in lipophilic circumstance (20,21), but enhance the activity of α-tocopherol. Similarly, a sesame derived antioxidant sesamin exerts a strong immunoregulatory activity in the presence of α-tocopherol (24,25). Foods contain various active substances and their functions should be expressed in the presence of other factors. Thus, studies on the interaction between active components are important. Simultaneous use of sesamin and α-tocopherol can decrease the dose of each active substance. This enables us to cut down the production cost of functional foods and the possibility of side effect expression.

Tocotrienols (T3) are tocopherol derivatives with three double bonds in their side chain. Oral administration of T3 decreased lipid peroxide levels in the serum and liver, and LTB_4-releasing activity of PEC without affecting AA level as well as α-tocopherol feeding (26,27). However, tissue distribution of T3 and α-tocopherol is fairly different. The latter was detected in all tissues tested, but α- and γ-T3 were detected only in the MLN and adipose tissue, when Sprague-Dawley rats were killed after an overnight fasting, though these T3 derivatives were detected in all tissues when the rats were killed without fasting (27,28). These results suggest that turnover of T3 derivatives are much faster than α-tocopherol and the turnover rate is tissue-specific.

Tea is an important source of biological active substances, such as caffeine, teanine and polyphenols. Among them, tea polypehnols (TP) exert various biological effects including anti-cancer (29) and anti-allergic activities (4,23,30-33). TP is composed of catechins with diphenolic B ring and gallocatechins with triphenolic B ring. Gallic acid bound to the C3 OH of the A ring to give epicatechin gallate or epigallocatechin gallate. Among them, epigallocatechin gallate (EGCG) is a most abundant TP with the highest acitivity. The triphenolic groups in the B ring and gallate moiety seems to play an important role in the expression of these biological effects. Only triphenolic compounds exerted a strong cancer cell-specific toxicity (29) and suppressed histamine release from rat basophilic leukemia RBL-2H3 cells (31) and from PEC isolated from Wistar rats (33). Since epigallocatechin 3-O-(3-O-methyl) gallate suppressed histamine release from human basophilic KU812 cells as strongly as EGCG, triphenolic structure in the gallate moiety may not be essentiall (34). In addition, both EGCG and methylated EGCG suppressed FcεRI expression in KU812 cells (35, 36). Though various antioxidants with membrane permeability suppressed LT release from rat PEC, EGCG

Table 4. Synergic effect of tea polyphenols and dietary fats on chemical mediator release from peritoneal exudate cells Wistar rats

Dietary fats	TP (%)	Histamine release (ng/10^6 cells)	LTB_4 release (ng/10^6 cells)	Arachidonic acid content (%)
Sufflower	0	611±21[a]	30.0±1.0[a]	20.9±1.3[a]
	1	626±20[a]	11.2±0.9[bc]	14.2±0.8[c]
Perrila	0	573±39[a]	13.6±0.3[b]	8.7±1.0[b]
	1	613±37[a]	8.6±0.8[c]	7.2±0.7[b]
Palm	0	760±15[b]	27.7±1.1[a]	14.1±2.4[c]
	1	827±23[b]	20.1±0.9[d]	14.2±2.0[c]

Data are means±SE (n=3) and values without common letters are significantly different at $p<0.05$. TP, tea polyphenol; LT, leukotriene.

exerted the highest suppressive activity among TP (4). These results suggest that EGCG and related compounds exert their physiological activity through the interaction with multiple cell components. Recent studies showed that the binding of EGCG to the lipid raft is important for the suppression of FcεRI expression (37).

When Wistar rats were fed TP at the 1.0% level, a significant decrease of LTB_4 productivity of PEC was induced, but their histamine releasing activity was not affected (33). As shown in Table 4, PEC isolated from the rats fed perrila oil and/or TP have low arachidonic acid level and LTB_4 releasing activity. Perrila oil contains an n-3 ALA at a high level (53.7%). n-3 PUFAs such as ALA, EPA, DHA strongly suppress 4-series LT production from PEC through the reduction of membrane AA level (2,3,33). Since TP suppress 4-series LT productivity of the PEC through a direct suppression of lipoxygenase activity, perrila oil and TP suppress the activity synergically.

Flavonoids are another group of antioxidants presents in various foods. It strongly suppressed LTB_4 release from rat PEC (4,23). In addition, oral administration of quercetin at the 0.1% level decreased serum triglyceride level, as efficiently as T3 feeding (38). It has also shown that quercetin and luteolin exert a strong cytotoxicity against human mammary carcinoma MCF-7 cells in the presense of endocrine disruptors, as well as isoflavones such as daidzein and genistein (39). Though isoflavones enhanced proliferation of MCF-7 cells through the interaction with estrogen receptor as well as intrinsic estrogens, they exerted cytotoxic effect at high doses (39,40). In addition, these isoflavones exerted IgE production-suppressing activity at the concentrations below 1 μM (41). These results suggest that flavonoids are multi-functional factors as well as other antioxidants. To clarify the regulatory mechanism of these antioxidants, determination of their *in vivo* level is essential. Coulometric array is a sensitive method for the detection of phenolic compounds and affords informations on their structure (42). A high-performance liquid chromatography system equipped with an eight channel coulometric detector enabled us to analyse a mixture of tea polyphenols or a mixture of isoflavone aglycon and glycosides simultaneously (42). The system was useful for the determination of isoflavone levels in various tissues of the rats fed isoflavones (43).

DIETARY FIBERS

DF is divided into two groups, water-insoluble and water-soluble ones. Both exert various biological effects such as improvement of serum lipid parameter and intestinal flora, and prevention of some types of cancers. In addition, we found that DF enhanced Ig and cytokine productivities of rat lymphocytes (44,45). Among them, immunoregulatory effect is related to the incidence or prevention of infectious diseases, allergies and cancers. As summarized in Table 5, water-soluble DF such as guar gum, glucomannan and pectin exert stronger activities on lipid metabolism and immune functions than water-insoluble cellulose. These activities are inducible in young Sprague-Dawley rats from 4 to 8 weeks old (46), but not in aged 8 month-old Sprague-Dawley rats (47). In the aged rats, enhancement of Ig productivity in MLN lymphocytes could be observed, but other activities were not. This means that the effect of water-soluble DF is age-dependent. To establish active immune system, food style in young generation is

Table 5. Dietary effect of water-soluble dietary fibers on lipid metabolism and immune function of young Sprague-Dawley rats

	Cellulose	Guar gum	PHGG	Glucomannan	HM-pectin
Serum Chol (mg/dL)	127±13^{a}	77±7^{b}	89±12^{b}	78±6^{b}	94±8^{b}
Serum TG (mg/dL)	57±8^{a}	28±5^{b}	30±7^{b}	27±4^{b}	40±5^{b}
Serum PL (mg/dL)	77±6^{a}	48±3^{b}	72±8^{a}	46±3^{b}	77±6^{a}
Serum IgA (μg/mL)	91±7^{a}	216±46^{b}	82±21^{a}	225±13^{b}	195±32^{b}
Serum IgG (mg/mL)	2.4±0.3^{ab}	1.7±0.4^{a}	1.8±0.1^{a}	3.3±0.7^{b}	2.4±0.1^{b}
Serum IgM (mg/mL)	0.20±0.02^{a}	0.28±0.03^{ab}	0.25±0.07^{a}	0.41±0.05^{b}	0.30±0.01^{ab}
MLN IgA (ng/mL)	2.7±0.3^{a}	11.2±0.8^{b}	5.4±0.9^{b}	15.9±2.3^{c}	17.0±1.5^{c}
MLN IgG (ng/mL)	8.4±1.0^{a}	38.5±3.1^{c}	22.2±3.2^{b}	52.1±6.1^{d}	27.4±2.9^{b}
MLN IgM (ng/mL)	8.6±0.2^{a}	16.5±1.4^{bc}	11.2±0.2^{ac}	20.3±3.7^{b}	11.6±0.6^{ac}
Spleen IgA (ng/mL)	12.3±1.4^{a}	21.8±1.9^{b}	18.5±3.1^{b}	21.6±0.7^{b}	22.0±1.2^{b}
Spleen IgG (ng/mL)	27.7±1.9^{a}	33.5±2.3^{a}	29.9±3.1^{a}	47.3±2.1^{b}	46.1±1.7^{b}
Spleen IgM (ng/mL)	130±3	142±13	123±11	157±15	160±10

Data are means±SE (n=4 or 5) and values without common letters are significantly different at $p<0.05$. Chol, cholesterol; TG, triglycerides; PL, Phospholipids; MLN, mesenteric lymph node; PHGG, partially hydrolyzed guar gum; HM-pectin, highly methylated pectin.

important.

These results also suggest that the effect of DF is tissue-dependent. Dietary effect of DF is expressed in MLN lymphocytes stronger than spleen lymphocytes in young rats (46). Similarly, oral administration of CLA (12) and T3 (38) enhanced Ig productivity of MLN lymphocytes more strongly than that of spleen lymphocytes. These results suggest that food components can activate Ig production of lymphocytes in a tissue-specific manner. It has been shown that dietary T3 were predominantly accumulated in MLN and some adipose tissue, while α-tocopherol is widely distributed in various tissues (28). Thus, the tissue-specific effect of food components may be partly due to the transportation of active substances.

The enhancement of Ig productivity of water-soluble DF is induced only after feeding experiment. When these lymphocytes were cultured in the presence of these DF or sugar components, stimulation of Ig production is negligible (46). Though water-soluble DF is metabolized to short chain fatty acids such as propionic and butyric acids and exerts various biological effects, there was no evidence on immunoregulatory function in short chain fatty acids. As shown in Table 5, the activity of partially hydrolyzed guar gum is much lower than that of intact guar gum. These results suggest that the dietary effect DF is expressed by the interaction with intestinal flora.

CREATION OF MULTI-FUNCTIONAL FOODS

As described above, food components exert various functions. Our recent efforts are focused on the clarification of the regulatory mechanism of these food components and their interaction with signal transduction system is emerging. Among them, the importance of peroxisome-proliferator activated receptor γ has already been reported (48,49). Foods containing these biologically active components will be useful for the maintenance of our health, if they were used appropriately. However, the activity is higher, the greater the probability of side effect expression, if they were used inadequately. In the use of health supporting foods such as THS, establishment of the food safety is most important. Thus, dose control of the active components in THS has great meaning. For safety, data on the dose-dependency of their biological effects should be acquired in animal and clinical experiments. As shown above, doses of the active components can be cut down by their combinational uses. Such cut down of doses enables us to cut down the production cost and probability of side effect expression. Thus, the development of multi-functional foods through the combinational use of multi-functional factors is prospective target for our health.

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