

Function of ATP-binding cassette transporter A1 (ABCA1) in apoA-I-containing HDL generation of astroglia.

Jin-ichi Ito*

Department of Nutrition, Faculty of Health and Nutrition, Shubun University, Japan

Abstract

Apolipoprotein A-I (apoA-I) is one of the most abundant apolipoproteins along with apoE in the cerebrospinal fluid (CSF). It is thought that apoA-I produced by brain capillary endothelial cells in the brain, while apoE is mainly done by astrocytes. Astrocytes generate not only apoE-containing high-density lipoprotein-like particle (apoE/HDL) using endogenous apoE but also apoA-I/HDL through the interaction with exogenous apoA-I. Astrocytes generate both apoA-I/HDL and apoE/HDL dependently on a transmembrane protein, ATP-binding cassette transporter A1 (ABCA1). ABCA1 is widely localized in both caveolin-1-rich fraction containing lipid raft domains and non-caveolin-1-fraction of the plasma membrane in rat astrocytes unlike peripheral cells. Exogenous apoA-I binds to ABCA1 localized in the non-caveolin-1-fraction with slightly higher densities than the caveolin-1-rich fraction of plasma membrane but not in the complete non-caveolin-1-fraction with highest density, while the endogenous apoE is associated with ABCA1 in the caveolin-1-rich fraction of rat astrocytes. After the association with ABCA1, exogenous apoA-I stimulates the phosphorylation of ABCA1-associated phospholipase C γ (PL-C γ) in the intracellular site and promotes the translocation of phosphorylated PL-C γ to the cytosolic lipid-protein particles (CLPPs) in the cytosol fraction of rat astrocytes. The CLPPs are intracellular HDL-like lipid-protein complexes with densities of 1.09-1.16 g/ml and diameters of 17-18 nm, and composed of lipids such as cholesterol, sphingomyelin, and phosphatidylcholine with proteins such as caveolin-1, protein kinase C α (PK-C α), and cyclophilin A in astrocytes. Furthermore, the newly synthesized cholesterol is translocated along with sphingomyelin and phosphatidylcholine from the endoplasmic reticulum/Golgi apparatus (ER/Golgi) to the CLPPs in the cytosol and is transported to the plasma membrane through the interaction with microtubules. Thus, the newly synthesized cholesterol is transferred to exogenous apoA-I in the extracellular space dependently on the association of apoA-I with ABCA1 and the series of intracellular reactions in the CLPPs.

Keywords: ABCA1, Astrocytes, apoA-I, apoE, HDL, Cytosolic lipid-protein particle (CLPP).

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Introduction

Cholesterol is abundantly distributed in the plasma membrane as an important lipid component to regulate physicochemical features of plasma membrane [1], to construct lipid raft structure as a platform to promote signaling through the plasma membrane [2,3], and to function as a precursor of cholesterol derivatives such as steroid hormones with physiological activities. Cholesterol is generally biosynthesized via complicated metabolic processes and hardly catabolized in mammalian cells because of its sturdy structure due to steroid skeleton. This means that cholesterol is too important lipid component for living animals not to need to be digested. However, the human placed in a good food situation tends to ingest excess cholesterol from foods. Therefore, excess cellular cholesterol must be removed from the cell surface to the extracellular space using some kinds of apolipoproteins to regulate cellular cholesterol level. Elucidation of the mechanism underlying removal of cellular cholesterol through the interaction with lipid-free apolipoproteins is a very important research subject to understand cholesterol homeostasis.

As neurons and astrocytes have complicated morphological shape with wide cell surface and oligodendrocytes construct myelin sheath for myelination in the central nervous system (CNS), neural cells such as neurons and glia own a large area of plasma membrane. Accordingly, it can be said that the brain is a most cholesterol-rich organ in the body. The human brain indeed accounts for about 25% of total body cholesterol [4]. Therefore, the brain is an organ to require cholesterol at high level also. The CNS in vertebrates, however, is segregated by the blood-brain barrier (BBB) from the systemic circulation, resulted that plasma lipoproteins are unable to enter to the brain from the blood [5]. Accordingly, the cholesterol supply for neural cells in the brain is thought to depend greatly on de novo cholesterol biosynthesis within the brain, and there must be many unique features to regulate intercellular cholesterol transport and cholesterol metabolism in the brain.

It is one of important characteristics of brain lipoproteins that some kinds of HDLs but not low-density lipoprotein (LDL) and very low-density lipoprotein (VLDL) are identified as lipoproteins of the CSF. The brain HDLs contain not only major apolipoproteins such as apoE and apoA-I but also minor apolipoproteins such as apoJ and apoD [6]. As apoE/HDL and

apoA-I/HDL generated by astrocytes are most abundant lipoproteins in the CSF [7], astrocytes must have great effects on metabolism and intercellular transport of cholesterol to preserve the cholesterol homeostasis in the brain. These HDLs are thought to contribute not only to supply cholesterol to cholesterol-poor neural cells but also to remove excess cholesterol from cholesterol-rich neural cells. Interestingly, the original apoE phenotype of the recipient remains unchanged in the brain even after liver transplantation, which results in the change of plasma apoE phenotype to that of the donor [8]. This finding suggests that the apoE in CSF is produced in the CNS and un-exchangeable with the plasma lipoproteins-associated apoE. The observation showing apoE accumulation in damaged lesions of the CNS [9] draws the idea that apoE and/or apoE/HDL may play the role of wound healing. On the other hand, there are four kinds of apoE receptors such as LDL receptor, VLDL receptor, apoE receptor 2, and LDL receptor-related protein (LRP) in the brain [10]. Astrocytes have three kinds of apoE receptors beside of apoE receptor 2, while neurons own all four kinds of apoE receptors. Thus, these apoE receptors are thought to have each role to regulate functions of apoE/HDL in the brain.

It is known that apoA-I produced by hepatocytes in the liver and secreted into the blood removes excess cholesterol from the cell surface of peripheral cells to generate a nascent apoA-I/HDL through the interaction with the plasma membrane-integrated ABCA1, followed by the maturation of apoA-I/HDL through esterification of cholesterol in the HDL mediated by the plasma lecithin-cholesterol acyl transferase (LCAT) [11]. ApoA-I in the brain is thought produced and secreted by the brain capillary endothelial cells [12]. The apoA-I/HDL generated by astrocytes is cholesterol-poor and phosphatidylcholine-rich HDL, while the apoE/HDL is cholesterol-rich one [7]. This suggests that apoA-I/HDL has different function from apoE/HDL in the brain. Although apoA-I is one of major apolipoprotein in the CSF, it is less characterized. It is an important subject whether an apoA-I in the CSF functions to remove excess cellular cholesterol from neural cells like the blood apoA-I that removes cholesterol from peripheral cells.

How cholesterol level is regulated in the brain? How is cholesterol biosynthesis controlled in the brain? How is excess cholesterol removed from the brain? Cholesterol homeostasis in the brain is not well understood. Excess cholesterol is reportedly converted into oxidized 24(S)-hydroxycholesterol (24(S)-OH-Cho) in the brain. 24(S)-OH-Cho is apparently transported to the peripheral circulation through the BBB [13]. Interestingly, 24(S)-OH-Cho functions as an agonist for liver X receptor (LXR) [14], which enhances mRNA expression of ABCA1 and apoE. Accordingly, 24(S)-OH-Cho may promote cholesterol circulation to remove excess cholesterol and oxidized cholesterol from the brain through the activation of LXR and the enhancement of levels of ABCA1 and apoE [15]. It is not so much understood how exogenous apoA-I participates in HDL generation of astrocytes through the interaction with ABCA1 also. In this review, the mechanism underlying HDL generation through the interaction between

ABCA1 and apoA-I in the brain is discussed focusing on our findings.

General function of ABCA1 in apoA-I/HDL generation

ABCA1 is thought to be a most important apoA-I-binding protein on the plasma membrane of peripheral cells. It was reported by several laboratories in 1999 that a loss of HDL in the Tangier disease is caused by gene mutations and dysfunction of a transmembrane protein, ABCA1 [16-18]. ABCA1 is a 12-times membrane-penetrating transmembrane protein with molecular weight of 250 kDa and the mRNA expression of ABCA1 is regulated by a LXR and a retinoid X receptor (RXR) activated by specific ligands such as 22(R)-hydroxycholesterol and 9-cis-retinoic acid [19]. ABCA1 is especially abundant in liver and macrophages [20]. An ABCA1-knockout mouse shows very few HDL in the plasma as well as the case of Tangier disease [16]. ABCA1 deficiency enhances formation of foamed macrophages, resulted in increasing the outbreak of cardiovascular disease [21]. On the other hand, overexpression of ABCA1 is protective to atherosclerosis, accompanied with increasing HDL levels in the plasma [22]. These findings indicate that ABCA1 greatly participates in removal of cholesterol from the plasma membrane of peripheral cells and HDL generation in the plasma.

There are some reports showing that ABCA1 is generally distributed in a non-lipid raft fraction of plasma membrane in peripheral cells. Randry et al. reported that apoA-I preferentially associates with non-raft membranes in ABCA1-expressing cells [23]. They found that ABCA1 induces redistribution of not only cholesterol and sphingomyelin but also caveolin from rafts to nonrafts. ABCA1 was initially accepted as a phosphatidylserine floppase to enhance flip-flop of phosphatidylserine from the inner leaflet to the outer leaflet of plasma membrane to change phospholipid distribution [24]. Such ABCA1 is thought to induce unstable localization of cholesterol in the plasma membrane and then to enhance cholesterol efflux. We observed that exogenous lipid-free apoA-I binds to the membrane fraction with slightly higher densities than that of the caveolin-1-rich fraction containing lipid raft domains in rat astrocytes [25]. A phosphatidylserine floppase activity of ABCA1 appears to remodel lipid-bilayer structure of plasma membrane to facilitate apoA-I-mediated cholesterol efflux.

A unique hypothesis was proposed by Vedhachalam et al. in respect to mechanism underlying apoA-I-mediated cholesterol efflux through the interaction between exogenous apoA-I and membrane ABCA1 [26]. They presented a hypothesis that three step processes provoked by the interaction of apoA-I with ABCA1 are required for apoA-I-mediated HDL generation. ApoA-I-mediated cholesterol efflux begins at the high affinity binding of small amounts of apoA-I to the cell surface ABCA1 as a first step. This step induces next the translocation of net phospholipid to the plasma membrane exofacial leaflet through the binding with ABCA1, followed by formation of unequal lateral packing densities in the phospholipid bilayer as the

second step. The stress of resultant membrane strain is relieved by bending formation of exo-vesiculated lipid domains, which promotes high affinity binding of apoA-I to these domains. Third step is the release of discoidal nascent HDL particle by apoA-I-mediated solubilization of exo-vesiculated domain. This particle appears to contain 2 – 4 molecules of apoA-I, a complement of membrane phospholipid classes, and some cholesterol molecules. This hypothesis supposes that a nascent HDL is generated at once through the formation of bending lipid bilayer and ripping off the exovesiculated domain when exogenous apoA-I binds to ABCA1 and then leaves the plasma membrane. This hypothesis, furthermore, denied the gradual progression of lipid release and HDL development mediated by the interaction between apoA-I and ABCA1. However, it is unclear in this hypothesis how ABCA1 removes other plasma membrane components such as many transmembrane proteins, glycolipids, and cell surface proteins from the phospholipid components of membrane lipid bilayer for bending lipid bilayer and ripping off exovesiculated domain. The physicochemical evidences and explanations to demonstrate formation of exovesiculated domain are required to understand this hypothesis. There are some questions in this hypothesis to explain the apoA-I-mediated generation of nascent HDL through the interaction between ABCA1 and apoA-I.

Tangier disease fibroblasts accumulate both cholesterol and sphingomyelin in the late endosomes. Neufeld et al. observed that ABCA1 is internalized and transported into the late endosomes in ABCA1-GFP-transfected Tangier disease fibroblasts. Exogenously added apoA-I is internalized into the late endosomes via the early endosomes also and enhances cholesterol efflux [27]. These findings suggest the possibility of apoA-I lipidation in the late endosome through internalization of the complex of apoA-I and ABCA1 into the late endosomes after the association of apoA-I with ABCA1 on the cell surface. This observation views things from a different angle from a hypothesis of Vedhachalam et al. However, it is difficult to explain whether lipidated apolipoproteins are generated in the late endosomes. It is thought that more additional research is required to define whether apoA-I/HDL is generated in the late endosomes or on the cell surface.

It is recently believed that apoA-I/HDL is generated via two steps of interaction between exogenous apoA-I and ABC proteins in the plasma membrane, that is, a lipid-free apoA-I generates first a discoidal pre β -HDL through the interaction with ABCA1 and then gradually a matured HDL through the interaction with ABCG1 to stimulate cholesterol transport from the cell surface to nascent HDL [28,29]. Duong et al. showed that the discoidal pre β -HDL particles with diameter of 7.5 ± 0.4 nm are generated as nascent lipid-poor lipoproteins composed of a single apoA-I molecule, three to four phospholipid molecules, and one to two cholesterol molecules through the interaction between ABCA1 of human skin fibroblasts with lipid-free apoA-I [30]. Strangely, the pre β -HDL particles of Duong et al. are biochemically and morphologically different from the nascent discoidal HDL of Vedhachalam et al. As apoA-I is an activator of LCAT [31], the LCAT activated by the apoA-I contained in pre β -HDL also must promote maturation of HDL to enhance esterification of cholesterol.

Furthermore, there are several observations showing that a lipid-free apoA-I up-regulates the cellular level of ABCA1 in peripheral cells. It was observed that the treatment with exogenous apoA-I of human THP1 macrophages protects ABCA1 from thiol protease attack [32]. Wang et al. showed that A PEST sequence in ABCA1 suppresses degradation of ABCA1 attacked by calpain proteases and rises the ABCA1 level in the plasma membrane after the interaction with apoA-I [33]. Thus, it was established that ABCA1 in the plasma membrane is an important apoA-I-binding protein that promotes apoA-I/HDL generation in peripheral cells. However, it remains unknown how apoA-I/HDL is generated dependently on the interaction between apoA-I and ABCA1.

ABCA1 in brain

It was shown by in site hybridization experiments that the mRNA expression of ABCA1 is most abundant in the olfactory bulb, hippocampus, cerebellar cortex, and choroid plexus of mouse and rat brain [34]. Kim et al. were found that the brain expresses five kinds of ABC subfamily-A transporters such as ABCA1, ABCA2, ABCA3, ABCA7, and ABCA8 [35]. ABCA1 is generally distributed in neurons and glia such as astrocytes, oligodendrocytes, and microglia, especially neurons and microglia, in the brain [35]. They stated that the expression of ABCA1 in astrocytes is only under 25% of neuronal expression level [29]. The function of ABCA1 in the brain, however, is not so much understood as compared with that in the peripheral tissues. It is thought that ABCA1 contributes to cholesterol homeostasis in the brain to enhance intercellular cholesterol transport between neural cells through the export of cellular cholesterol to extracellular lipid-free apolipoproteins such as apoE and apoA-I.

It was reported that the total amounts of apoE are drastically reduced by 80% in the cortex and 98% in the CSF in ABCA1-knockout mice [36]. This finding suggests that ABCA1 is very influential in production and/or secretion of apoE in the brain. Hirsch-Reinshagen et al. reported also that deficiency of ABCA1 results in a 65% decrease in apoE levels in whole brain and 75-80% decrease in hippocampus and striatum *in vivo* [37]. However, the apoJ level is hardly decreased in an ABCA1-knock out mouse. These findings suggest that ABCA1 is functional to control specifically apoE levels in the brain. We also observed that astrocytes prepared from ABCA1-knockout mouse decrease cellular level and secretion of apoE in comparison with wild-type astrocytes [38]. Furthermore, the CSF obtained from ABCA1-knockout mouse reduces cholesterol level and contains lipid-poor apoE/HDL [36]. On the contrary, neural cells enhance apoA-I- or apoE-mediated cholesterol efflux, when the ABCA1 expression is enhanced by LXR/RXR activation.

Lutjohann et al. reported that the brain contains approximately 80% of the 24(S)-OH-Cho in the human body [39]. The conversion of excess cholesterol into 24(S)-OH-Cho is metabolized by brain-specific enzyme CYP46A1 in neurons [4]. Generated 24(S)-OH-Cho functions to enhance ABCA1 expression as a ligand of LXRs, resulted in the enhancement of cholesterol transport to lipid-free apoE and apoA-I. Thus,

24(S)-OH-Cho in the brain is a cholesterol oxide with a physiological activity to up-regulate removal of excess brain cholesterol through the enhancement of ABCA1 expression. 24(S)-OH-Cho is reportedly removed as much as 6–7 mg/24 h from the BBB [13]. The removal of 24(S)-OH-Cho from the brain is thought to contribute to the control of cholesterol level in the brain. Saint-Pol et al. reported that 24(S)-OH-Cho influences cellular cholesterol efflux from brain pericytes to HDL, lipid-free apoE or apoA-I via ABCA1-dependent pathway [40]. Astrocytes generate several kinds of HDLs using endogenous apoE and apoJ and exogenous apoA-I in the brain, suggesting that astrocyte is one of most significant neural cells for brain cholesterol homeostasis. Although neurons are able to synthesize cholesterol like glial cells, neurons reportedly exhibit high dependency on cholesterol of glia-derived apoE/HDL for synaptogenesis [41]. It is not understood how cholesterol or cholesteryl ester is incorporated from extracellular apoE/HDL or apoA-I/HDL by neurons in the brain. The HDLs generated by astrocytes are thought to have roles not only to deliver cholesterol between neural cells but also to protect neural cells from injury and stress in the brain [42,43]. An ABCA1 is an important transmembrane protein to mediate efflux of lipids such as cholesterol and phospholipids from the astroglial cell surface for the generation of HDL. It, however, is not exactly known how ABCA1 participates in the generation of lipoproteins such as apoE/HDL and apoA-I/HDL in astrocytes also.

Function of ABCA1 in apoA-I/HDL generation in astrocytes

The plasma apoA-I enhances efflux of cholesterol and phospholipids from the cell surfaces of many kinds of peripheral cells to generate HDL through the interaction with ABCA1 in the non-raft domain [23,44]. ApoA-I with a molecular weight of 27 kDa is reportedly produced and secreted by brain capillary endothelial cells [12] and partly derived also from plasma through the BBB in the brain [10]. The difference of cholesterol contents in apoA-I/HDL and apoE/HDL generated by astrocytes is thought to exhibit different roles in the brain. We studied the mechanism underlying apoA-I/HDL generation mediated by exogenously added apoA-I through the interaction with cellular ABCA1 in astrocytes in order to understand cholesterol homeostasis in the brain.

We found that the newly synthesized lipids such as cholesterol, sphingomyelin, and phosphatidylcholine are transiently translocated from the ER/Golgi to the cytosol fraction in rat astrocytes treated with exogenous human apoA-I for 90 min. The newly synthesized cholesterol was recovered with CLPPs (cytosolic lipid-protein particles) in the cytosolic fraction along with other phospholipids. The CLPPs are plasma HDL-like particles with diameters of 17-18 nm and densities of 1.09-1.16 g/ml [45,46]. A CLPP contains caveolin-1, cyclophilin A, and protein kinase C α as protein components. A cyclophilin A-specific inhibitor, cyclosporin A, suppresses not only the translocation of newly synthesized lipids to the cytosol but also the cholesterol efflux in apoA-I-treated rat astrocytes,

suggested that cyclophilin A participates in the CLPP function and cholesterol efflux [45].

We demonstrated that exogenously added human apoA-I bind to ABCA1 in rat astrocytes like peripheral cells. While the endogenous apoE was associated intracellularly with the ABCA1 in the caveolin-1-rich fraction of rat astrocytes, exogenously added apoA-I was bound to the ABCA1 distributed in the non-caveolin-1-fraction with higher densities than the caveolin-1-rich fraction of rat astrocytes. After the incubation of rat astrocytes with apoA-I, the ABCA1 that bound covalently with apoA-I using a cross-linker was isolated by anti-apoA-I antibody-bound protein G and the ABCA1-bound apoA-I was also done by anti-ABCA1 antibody at the position of protein with molecular weight of over 260 kDa [25]. The ABCA1 in solubilized membrane fraction of rat astrocytes, furthermore, was bound to apoA-I-immobilized Affi-Gel 15. A LXR agonist, To 901317, enhanced the binding of apoA-I to ABCA1, accompanied with the increase in cellular ABCA1 level. We found that the intracellular PL-C γ is associated with ABCA1, suggesting that the extracellular domain of ABCA1 binds with exogenous apoA-I and the intracellular domain is associated with PL-C γ . The PL-C γ is tyrosine-phosphorylated in rat astrocytes stimulated with apoA-I for 5 min and then translocated to the CLPP, followed by the increase in diacylglyceride production in the CLPP fraction [47-49]. The SiRNA of ABCA1 suppressed not only the association of PL-C γ to ABCA1 but also the apoA-I-induced tyrosine phosphorylation of PL-C γ . The translocation and activation of PK-C α to/in the CLPP were also continuously enhanced in apoA-I-treated rat astrocytes [47]. The suppression of either PL-C γ or PK-C α inhibits apoA-I-mediated cholesterol release and apoA-I/HDL generation. These findings indicate that the stimulation with exogenous apoA-I evokes signal transductions through the interaction between apoA-I and ABCA1 in the plasma membrane and then through the translocation of phosphorylated PL-C γ to the CLPP. It is suggested that the CLPP has roles as an intracellular cholesterol transport vehicle and as an intracellular platform for apoA-I-induced signal transduction.

We showed using a cell free-microtubule-reconstituted system in vitro that the CLPP is associated with microtubules through the CLPP-associated caveolin-1. Exogenously added apoA-I enhanced the association of CLPP and microtubules in rat astrocytes. The interaction between CLPP and microtubules promotes intracellular cholesterol transport to the plasma membrane for the apoA-mediated cholesterol efflux through CLPP delivery in rat astrocytes. We, furthermore, found that apoA-I enhances the phosphorylation of α -tubulin [50]. This phosphorylation was suppressed by a protein kinase C inhibitor, bisindolylmaleimide 1 (BIM), which suppressed not only cholesterol translocation to the cytosol from the ER/Golgi but also apoA-I-mediated cholesterol efflux. This implies that PK-C participates in intracellular cholesterol transport and cholesterol efflux through the phosphorylation of α -tubulin. It is thought that the ABCA1 associated with exogenous apoA-I in the plasma membrane activate PL-C γ to phosphorylate PL-C γ , followed by translocation of activated PL-C γ and production of diacylglyceride to/in the CLPP and finally

activation of PK-C α in the CLPP. The caveolin-1-associated α -tubulin is phosphorylated by the caveolin-1-associated PK-C α in the CLPP. The phosphorylation of α -tubulin sited in the microtubules that are associated with CLPP induces the dissociation of α -tubulin from caveolin-1, and the phosphorylated α -tubulin is never associated with caveolin-1 in the CLPP. These findings suggest that apoA-I enhance the association of microtubules with CLPP, followed by the phosphorylation of α -tubulin and the dissociation of CLPP from microtubules. The dephosphorylation of α -tubulin makes this association possible again, suggesting that the phosphorylation/dephosphorylation of α -tubulin regulates intracellular cholesterol transport to the plasma membrane through the transport of CLPP for cholesterol release and apoA-I/HDL generation [50]. These findings indicate that the binding of exogenous apoA-I to the membrane ABCA1 as a first step for apoA-I-mediated cholesterol efflux is important to promote the intracellular reaction in the CLPP as a secondary reaction to enhance intracellular cholesterol transport.

Conclusion

Intercellular cholesterol transport in the brain is generally mediated by HDLs produced by the brain neural cells but not by LDL and VLDL. As both apoE/HDL and apoA-I/HDL are most abundantly distributed in the CSF, it is thought that these lipoproteins regulate intercellular cholesterol transport and cholesterol metabolism in the brain. Astrocytes generate these lipoproteins through the interaction of ABCA1 with endogenous apoE and exogenous apoA-I. Thus, astrocytes are most significant neural cells for transport and metabolism of cholesterol in the brain. The mechanisms underlying generation of apoA-I/HDL and apoE/HDL in astrocytes are very interesting subjects in order to understand brain cholesterol homeostasis.

The mechanism for apoA-I/HDL generation in the brain is poorly studied as well as that for apoE/HDL generation through the interaction with ABCA1. It was explained focusing on our findings obtained by in vitro experiments that apoA-I/HDL is generated through the interaction between ABCA1 and exogenous apoA-I in astrocytes. We have no idea whether CLPP functions in intracellular cholesterol transport in other cell strains. Much more studies are required in order to understand the mechanism underlying apoA-I/HDL generation.

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***Correspondance to**

Jin-ichi Ito,

Department of Nutrition,

Faculty of Health and Nutrition,

Shubun University, Japan

Telephone: +81-52-853-8140,

FAX, +81-52-841-3480

E-mail: jitoh@med.nagoya-cu.ac.jp