る。老人性認知症はおもにアルツハイマー型認知症と脳血管性認知症との二つに分けられるが，近年，欧米の研究者から縦断的疫学調査や5～10年にわたる大規模な追跡調査の結果が相次いで発表され，認知症の発症に食品成分が密接に関与することが明らかにされた[1,2]。我が国でも，平成13年度から厚生科学研究費補助金による「認知症の予防・治療と食事栄養」に関する疫学調査が開始され，その結果がしだいに明らかになりつつある[3]。これら国内外の疫学調査結果を集約すると，アルツハイマー型認知症の発症を予防しうる食品としては，欧米と日本との区別なく，魚油，野菜，果実が有力視されている。そのなかでもとくに，魚油の主成分であるドコサヘキサエン酸（DHA，22：6 n-3）が注目され，最近，DHAと脳機能に関する研究成果が多分野から相次いで発表されている。

DHAはα-リノレン酸（18：3 n-3）から合成される必須不飽和脂肪酸であり，我々動物はDHAが多く含まれる魚介類や，α-リノレン酸が比較的多く含まれるシソ油やナタネ油などの植物油などから，食事栄養としてとらなければならない。DHAは生体内ではとくに，大脳皮質，網膜，および精子に多く含まれ，とくに，大脳皮質・灰白質のリン脂質脂肪酸の約30～40%を構成している[4,5]。そのため，DHAは，成熟ラットの脳と網膜ではそれぞれ総脂肪酸の17%，33%以上を占める[6]。その脳の神経細胞では主に，シナプトゾーム膜，シナプス顆粒，ならびに軸索に最も多く含まれている[7]。このようにDHAは脳に豊富に含まれることから，従来からDHAは記憶・学習機能に重要な役割を担っていることが推察されていた。動物実験により，奥山らのグループ[8]は，n-3不飽和脂肪酸欠乏食ラットでは，脳内DHA含量の低下と共に学習機能が低下することを明らかにした。その後，著者ら[9,10]と鈴木ら[11]のグループから，DHAを摂取したラットやマウスでは，脳内DHA量の増加とともに，学習能がいずれも向上することが相次いで報告され，DHAの脳機能に対する必須性が明らかとなった。しかるにDHAによる脳機能改善効果に関する分子メカニズムは未だ不明である。

Fig. 1には最近までに明らかにされている脳の神経科学領域でのDHAの役割が示されている。この総説の目的は，脳組織でのDHAの有効性を著者らの最近の研究成果をも交えながら紹介することである。この解説が脳でのDHAの有効性の分子メカニズムを明らかにする研究のきっかけになるばかりではなく，この解説によりDHAが神経疾患の重要な治療手段になり得ることをよりよく理解されることを希望する。なお，DNAと脳機能に関する研究動向は，最近の総説[12-15]をもぜひ参照にされたい。

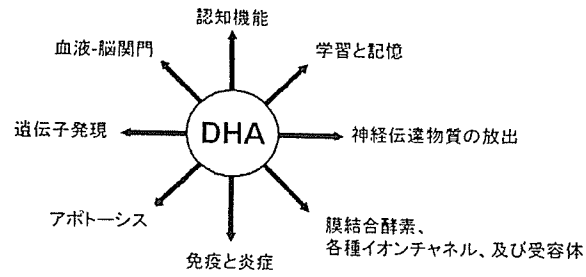

Fig. 1　神経科学領域におけるドコサヘキサエン酸（DHA）の役割。文献12）の原図から改変した。

2　DHAと脳

2·1　食餌性DHAの脳内移行

脳内DHA量は，胎児期と出生後の脳の発達期に著明に増加する。なぜなら，その時期には脳が大きくなるばかりではなく，相対的にもDHA量が増加するからである[5]。その脳の発達期では，脳組織はニューロンの細胞膜をつくるために多くのDHAを必要とする。ニューロンには，DHAやアラキドン酸（AA，20：4 n-6）を合成するために必要な酵素が欠損していることから，これらの脂肪酸は直接食餌から，あるいは肝臓においてこれらの前駆物質であるα-リノレン酸やリノール酸（18：2 n-6）から合成され，脳に転送される。脳循環血中のDHAを含めたn-3不飽和脂肪酸は，血液－脳関門（blood-brain barrier）の構成細胞である血管内皮細胞とグリア細胞に取り込まれ，脱飽和化と逆転写反応を介してDHAが合成され，その後遊離されてニューロンに取り込まれる[16,17]。なお，アストロサイトは延長化と脱飽和化反応を介して，18-，20-，22-炭素n-3化合物や24-炭素化合物からDHAを合成することが出来る[18,19]。しかし，ラットでは脳のリン脂質に結合したDHAの2～8%が，毎日，血漿遊離脂肪酸プールのDHAと置き換わること[20]を考慮すると，アストロサイトから合成されるDHAは，血液から取り込まれる量と比べると極めて少量である。Table 1には，DHAを長期投与した若齢ラットの血漿と脳組織におけるDHA・AA量と過酸化脂質量が示されている。

2·2　DHAの代謝

Fig. 2にはDHAの構造と膜リン脂質への取込み，ならびにその代謝が示されている。細胞外のDHAはacyl CoA synthetasesの作用によりDHA-CoAとなり，ATP-依存性acyltransferaseにより，細胞膜リン脂質のグリセロールの2位の炭素に結合するが，DHAは主に，アミノリン脂質であるphosphatidylethanolamine，plasmenylethanolamine，およびphosphatidylserineの*sn*-2

Table 1　若齢ラットの血漿と脳における脂肪酸と過酸化脂質に及ぼす食餌性DHAの影響

	AA, 20：4(n-6)	DHA, 22：6(n-3)	DHA/AA	過酸化脂質
血漿				
Vehicle	54.8 ± 1.9	8.0 ± 0.3	0.1 ± 0.0	N.E.
DHA	30.6 ± 1.6*	18.3 ± 0.7*	0.4 ± 0.0*	N.E.
大脳皮質				
Vehicle	36.1 ± 1.5	41.4 ± 1.8	1.1 ± 0.0	2.1 ± 0.4
DHA	36.5 ± 1.4	48.2 ± 2.0*	1.4 ± 0.0*	1.2 ± 0.1*
海馬				
Vehicle	29.3 ± 1.1	38.4 ± 1.4	1.3 ± 0.0	3.2 ± 0.3
DHA	32.5 ± 2.7	49.7 ± 4.0*	1.6 ± 0.0*	3.7 ± 0.2
小脳				
Vehicle	23.5 ± 1.2	45.1 ± 2.1	1.9 ± 0.1	4.0 ± 0.1
DHA	23.1 ± 0.6	47.9 ± 1.3	2.1 ± 0.1	4.4 ± 0.3

n-3系不飽和脂肪酸欠乏飼料で3世代飼育した若齢Wistar系雄ラット（5週齢）に，DHA（300 mg/kg/day）の経口投与を12週間行ない測定した。脂肪酸はガスクロマト法で測定し総脂肪酸量として，過酸化脂質量はチオバルビツール酸反応生成物（TBARS）価として各々表されている。各値は平均値±標準偏差で表され，脂肪酸はmg/dL（血漿）とμg/mgタンパク（組織），過酸化脂質はnmol/mgタンパク（組織）で各々示している。AA，アラキドン酸; DHA，ドコサヘキサエン酸; DHA/AA, molar ratio of DHA and AA; DHA：DHA投与群; Vehicle：対照群; $P<0.05$ vs Vehicle群; N.E.：未測定，文献9）を改変した。

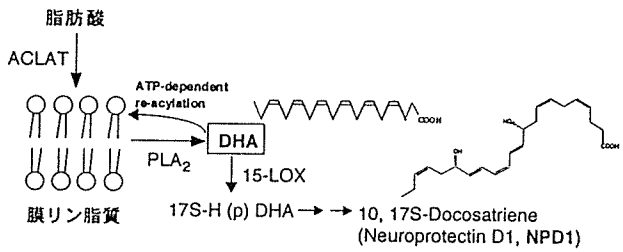

Fig. 2　ドコサヘキサエン酸（DHA）の膜リン脂質への取り込みと代謝

ACLAT, Acyl-coenzyme A: lyso-phospholipid acyltransferase; PLA_2, phospholipase A_2, DHA, docosahexaenoic acid; 15-LOX, 15-lipoxygenase.

位に結合し，コリンリン脂質であるphosphatidylcholineにはほとんど結合していない。結合したDHAの代謝には，docosahexanoyl-sensitive phosphlipase A_2（プラズマローゲン感受性PLA_2）とacyltransferaseが関与する脱アシル化と再アシル化サイクルが関係する[21]。このサイクル反応は，ニューロン膜の正常機能に必要なグリセロリン酸の脂肪酸成分を確保するための反応である[22-24]。ニューロン膜にn-3とn-6脂肪酸は拮抗的に取り込まれるので，膜のDHA・EPAが増加するときにはＡＡレベルは減少する[25]。ＡＡの代謝産物であるエイコサノイドには脳の虚血・再還流時に産生され脳障害をきたす物質も含まれ[26]，ＤＨＡはこのエイコサノイドの産生を抑制することから，間接的にＤＨＡによるニューロンへの保護作用が推察される。事実次章で述べるように，DHAはニューロンでの酸化ストレスを保護する。

2·3　DHAの脳内抗酸化作用

DHAは6個の二重結合をもつ高度多価不飽和脂肪酸（PUFA）であり，このようなPUFAは空気中では非常に速く脂質過酸化を受け，過酸化脂質が増加し活性酸素やフリーラジカルを生じることから，脳内DHA量の増加は過酸化脂質の生成を促し神経細胞障害を増大させ認知機能障害をもたらす，と推察されていた[27]。PUFAによる生体内酸化作用は，TBARSあるいは共役ジエンの生成の増加によって証明される。また，生体内酸化作用は，食事中にビタミンＥを添加することで防ぐことができる。この酸化作用の記述とは反対に，最近の研究では，DHA入りミルクを与えた母親から生まれた新生児ラットの脳では，シクロオキシゲナーゼや一酸化窒素合成酵素を介した酸素利用が高まることにより脂質過酸化が低下することが見出されている[28]。同様に筆者らは，n-3 PUFA欠乏飼料で飼育した100週齢の老齢ラットに，DHA（300 mg/kg/day）を12週間投与すると，大脳のDHA量が増加するにもかかわらず過酸化脂質量は低下し[29]，大脳の抗酸化酵素（カタラーゼ，グルタチオンペルオキシダーゼ）の活性化を見出した。さらに，これらの酵素活性とDHA/AA比との間にはいずれも正の相関が認められることから，大脳のDHA/AA比は脳内抗酸化作用の指標になり得ることを推察している[30]。DHAやエイコサペンタエン酸（EPA, 20：5 n-3）はAAやリノール酸にくらべ，酸素の少ない水溶液中では酸化を受けにくく安定であることから[31]，DHAやEPAは生体内では脂質過酸化をあまり受けることなく細胞膜構成脂質として存在するものと思われる。これらの結果から，DHAが多量に存在する大脳皮質・海馬領域では，DHAは脂質過酸化を受け神経細胞死を惹起するのではなく，むしろ酸化ストレスから神経組織を保護し，ニューロンの正常な機能を維持する神経保護作用を発揮するものと思われる（Fig. 3）。

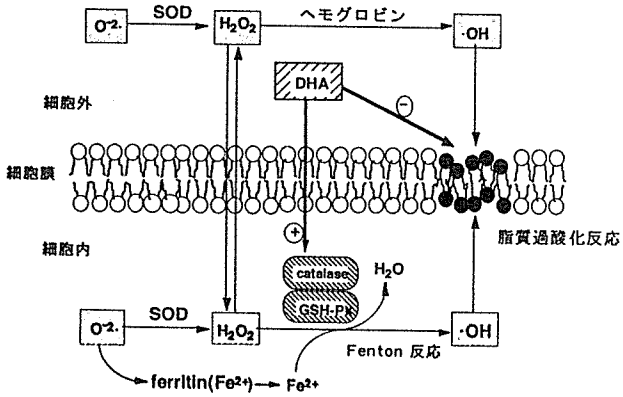

Fig. 3　ドコサヘキサエン酸（DHA）による脳内抗酸化機序
GSH-Px: Reduced glutathione peroxidase, SOD: Superoxide dismutase, H_2O_2: Hydrogen peroxide, O^{-2}: Superoxide anion, ·OH: Hydroxyl radical.

3　ニューロンでのDHAの機能性

DHAの摂取はヒトを含めた哺乳類の脳を正常に保つために多くの有益な効果をもたらすことから[32,33]，食餌によるニューロンの膜の成分変化は，作用や伝達などのニューロンの機能に影響を及ぼすと思われる。この作用とは神経の信号（インパルス）が神経細胞体から軸索にそって神経末端まで伝わることであり，伝達とはシナプスを経由して，一つのニューロンから次のニューロンに電気信号が送られることである[32]。これら両方の過程は，DHA摂取によりもたらされるニューロンの膜の組成変化により影響をうけると思われる。

ニューロンの細胞膜流動性は，神経のシグナル伝達に影響を及ぼす。ニューロン膜に取り込まれたn-3とn-6不飽和脂肪酸バランス（n-6/n-3比）の偏りは，膜流動性を変える[25]。また，コレステロールが膜に過剰に存在する時には膜が硬くなるが，PUFAの摂取はニューロンの膜結合型コレステロール量を減少させる[34]。また，血管内皮細胞[35]や肝臓の胆管上皮細胞[36]などの非神経細胞でも，食餌性のDHAは膜流動性と脂肪酸不飽和度に影響を及ぼす。そのため，DHAの脳機能への作用は，ニューロン膜の物理化学的性質を変えるだけではなく，シグナル伝達に関連する多くの酵素蛋白の遺伝子発現の調節に関与することにも起因するものと思われる[37-40]。この章では，ニューロンでのDHAの機能性，とくに，神経化学と遺伝子，さらにはシナプスの可塑性への影響について解説する。

3·1　DHAによる神経伝達系への作用

魚油の摂取はラットの前頭皮質のドーパミン作動性ニューロンに影響を及ぼす[41-43]。この作用は神経核のドーパミン代謝だけではなく，ドーパミン受容体やそのトランスポーターの性質をかえる。n-3 PUFA欠乏ラットではn-3 PUFA負荷ラットに比べて，ドーパミン量は多いがドーパミンの代謝産物量が少なく，学習能も低下する。この学習能の低下はn-3 PUFAを負荷させることにより改善する。そのため，皮質のドーパミン作動性ニューロンの伝達系の変化はn-3 PUFA欠乏ラットの学習能と関連する。その他に関連する神経伝達物質とその関連物質としてはコリンとアセチルコリンがある。DHAを負荷した脳卒中易発症型高血圧自然発症ラット（SHRSP）の大脳皮質のコリンとアセチルコリン量は，n-3 PUFA欠乏食飼育SHRSPにくらべて40%増加する[44]。

DHAは，NMDA（N-methyl-D-aspartate）によりもたらされる神経毒性から前脳のコリン作動性ニューロンの抵抗性を増強し，保護作用を発揮する[45]。このことは，ニューロン膜のリン脂質に取り込まれたDHAは，NMDAにより誘発される神経障害にたいしてニューロンの抵抗性を高めることを示唆している。またDHAは，抑制性の神経伝達物質であるGABA（gamma-amino butyric acid）の反応性を抑制することにより，AAと同様にNMDAの反応性を増強する[6,46]。これらの結果から，食餌性DHAは，ニューロンの膜リン脂質に取り込まれ，ノルアドレナリン作動性あるいはセロトニン作動性ニューロンの神経伝達系に影響を及ぼし，脳機能や認知行動を向上させるものと思われる。

3·2　DHAによる遺伝子発現への作用

DHAによる遺伝子発現への修飾作用は転写レベルでおこなわれるが，その修飾作用は，標的遺伝子上のcis型の調節領域（regulatory elements）と結合するいくつかの転写因子によって調節されている。DHAやAAはこれらの転写因子を活性化する。この作用様式は，ステロイドホルモン受容体，ビタミンD受容体，ならびにレチノイン酸受容体の場合と同じであり[47]，また，これらPUFAと反応する転写因子の特性は，PPARs(peroxisome proliferator activated receptors）で報告されている転写因子と同一である[48]。PPARファミリーに加えて，他の転写因子（α, β-liver X受容体，hepatic nuclear factor-4，ステロール調節エレメント結合蛋白など）も同定されている。その他に興味あるところでは，DHAがPPARsの重要な活性化因子であるばかりではなくペルオキシゾームの増加促進因子である，との報告がある[49]。しかし，DHAはpalmitoyl Co A oxidase活性，あるいはペルオキシゾームの増加に関連する他の生化学的パラメーターに影響を及ぼさないとの報告もある。

食餌性DHAは，転写の最重要因子の活性を調節するホルモンと同様な作用を発揮する[47,50]。マイクロアレイ法による研究によると，魚油負荷ラットの脳では55種

類の遺伝子の発現促進と，47種類の遺伝子の発現抑制が報告されている。これらの遺伝子には，シナプスの可塑性，細胞骨格と膜関連蛋白，シグナル伝達，イオンチャネル，エネルギー代謝，などを調節している蛋白の遺伝子が含まれている[37-40]。また，DHA投与若齢ラットの海馬では，最早期遺伝子の一つであるFos蛋白の発現が増加し，さらにその増加は放射状迷路法で評価される参照記憶エラー数（報酬餌のない走路を選択した回数）と負の相関を示すことから，最早期遺伝子が空間認知機能の向上に関連することが示唆される[51]。これらの研究から，食餌性DHAにより誘導されたニューロンの遺伝子の過剰発現は，脳機能の改善に関係していると思われる。

3·3　DHAによる記憶形成への作用

記憶・学習のメカニズムは未だ不明ではあるが，良く知られている細胞内メカニズムの一つにシナプスの可塑性がある。反復学習により，あるニューロンの活動が繰り返されたとすると，その細胞の神経終末は成長し他のニューロンとのシナプス結合が増加したり（シナプスの発芽），反対に今まで使われていたシナプスが消失したりする。その結果，学習により新たな神経回路が出来上がる。このように，シナプスの可塑性により神経回路網における信号の伝わり方が変わることが学習であり，記憶とは，神経の可塑性により組み直された神経回路網ができ上がることであると考えられている[52]。

シナプスの可塑性には長期増強（long-term potentiation：LTP）と長期抑制（long-term depression：LTD）に代表される電気生理的機能変化があり，これらは記憶・学習の形成に関連した主要な細胞内メカニズムである[53]。これらのメカニズムはシナプス後部膜の脱分極に関係し，NMDA受容体により誘導されるCa^{2+}イオンの流入とphospholipase A_2（PLA_2）活性化を伴う。PLA_2阻害剤は海馬スライス標本でのCA1領域のLTP形成を阻止する。AAとその代謝産物はシナプス後部から遊離されシナプス前部受容体に作用し，神経伝達物質の遊離とLTPの形成を調節する重要な伝達シグナル（逆行性メッセンジャー）として作用する。加齢により記憶は低下するが，このメカニズムの一つとして，ニューロン膜のAA含量とPLA_2活性が加齢に伴い減少し，その結果LTPの形成が低下することが示唆されている[54]。このAA量の低下とLTP形成の障害は，食餌性のγ-リノレン酸により回復する[55]。

DHAはLTP形成に重要であるばかりではなく，テタヌス刺激により遊離される内因性のDHAはLTP形成の引き金となることから，DHAはLTPの誘導には必須である[56]。また，DHA存在下では，幼若ラットの大脳皮質錐体細胞のNMDA受容体応答が増強され[57]，海馬CA1領域のスライス標本でのLTDの誘導が抑制される[58]。このような結果とは反対に，DHAは海馬CA1領域でのLTPを抑制するとの報告もある[59]。神経細胞のモデル細胞として汎用されているラット褐色細胞腫由来のPC12細胞にDHAを添加すると，神経突起の形成と共に細胞内Ca^{2+}濃度の増加が認められる[60]。LTPの誘導にはシナプス後部細胞へのCa^{2+}の流入が引き金となることから，DHAによる神経細胞活性化機序の一つとして，神経細胞内Ca^{2+}流入機構への関与が推定される。しかしながら，これらの報告はいずれも，培養神経細胞，あるいは脳のスライス標本にDHAを直接作用させた際に得られた結果である。より生体反応に近い検討としては，DHA投与ラットの海馬では，加齢により減少したAA量や低下したLTPの反応性や神経伝達物質の遊離能が若齢ラットレベルにまで回復する，との報告がある[61]。これらの結果から，DHAは記憶・学習の形成に関連し，脳の正常機能を維持するために主要な役割を担っていることが推察されるが，今後，生体反応をより正確に反映した研究成果が待たれる。

4　DHAと記憶・学習機能

4·1　脳の発達期とDHA

脳の発達とDHAとの関連性について最初に注目したのは英国脳栄養研究所のCrawford教授であり，同博士は日本人の子供の高い知能指数（IQ）と魚肉を中心とした日本型食生活習慣との関連性を指摘した。その後，数多くの未熟児・正常出産児での研究成果が報告され，子供の脳の発達や，脳機能の維持・向上にはDHAが不可欠であるとの結論に至っている。たとえば，DHA入り人工乳を与えた早産児の方が与えない早産児に比べて8歳でのIQが高く[62]，DHA入り人工乳で育った未熟児では，DHAの入らない人工乳で育った未熟児と比べて脳の発達や機能が良く，また，乳児の脳内DHA量は妊婦の魚油摂取量の増加に伴い増加し，脳機能の発達に有利であることが明らかにされている[63]。動物実験では最近，DHAが正常レベルの母獣から生まれた新生仔でも，授乳期でのn-3 PUFAの摂取不足は脳機能障害に陥ることが報告されている[64]。これらの結果から，脳の発達期にはDHAの供給が不可欠であると思われるが，しかし，脳の発達期でのDHAの効果について，従来報告されている動物実験やヒトでのデーターを再検討した結果，その評価方法によりDHAの効果が異なる，との最近の総説もあり[14]，今後，より高度な検討方法の導入による再検証が望まれる。

4·2　成熟・老化脳とDHA

大脳皮質・海馬でのDHA量の増加は，成熟ラットの記憶・学習機能に影響を及ぼすであろうか？　n-3 PUFA欠乏飼料で飼育した若齢ラットにDHAの経口投与（300 mg/kg/day，10週間）を行うと，大脳皮質・海馬でのDHA量とDHA/AA比の増加と共に，放射状迷路法により評価される参照記憶エラー数が有意に低下し，さらにエラー数とDHA/AA比との間には有意な負の相関が認められる（Fig. 4）[9]。DHAとAAは共に海馬でのLTPの形成に関与しシナプスの可塑性に強く影響をおよぼすことから（3·3の章を参照）[57]，この両者間での相関関係が認められることは，DHAとAAの中枢での各役割を考えるうえで興味がもたれるところである。

老化により認知機能は低下する。老化への過程でフリーラジカルが発生し，ニューロンの膜DHAレベルは低下する[5,33]ことから，老化による記憶・学習能の低下は部分的に膜のDHAレベルの低下が関与するかもしれない。この低下は，加齢に伴う脳内phosphatidylethanolamine, ethanolamine plasmalogens, そしてphosphatidylserine量などの低下と一致する[65,66]。加齢ラットでの脳内DHA量の低下は，海馬のコリン作動性ニューロンの機能低下を引き起こすかもしれない[66,67]。食餌性DHAは，脳内のDHA量を若齢ラットのレベルまで戻すばかりではなく，脳内のコリンやアセチルコリン量を増加させ，さらにはコントロールラットとくらべて低下した脳卒中易発症型高血圧自然発症ラット（SHRSP）の受動的回避行動機能を改善する[44]。

n-3 PUFA欠乏飼料で飼育した老齢（100週齢）ラットでは，若齢ラットでの場合と同様に，DHA投与により参照作業記憶エラー数が低下し，さらに海馬では過酸化脂質量が減少し，この過酸化脂質と参照記憶エラー数との間には有意な正の相関が見出される（Fig. 5）[10]。老齢ラットでは若齢ラットの場合と異なり，海馬での過酸化脂質量の低下が記憶・学習機能を向上させるものと思われる。海馬は酸化ストレスに対して非常に脆弱であり加齢による障害を受けやすい部位であることから，加齢ラット海馬でのDHA量とDHA/AA比の減少，あるいは過酸化脂質量の増加は，加齢に伴ない認知機能が低下する要因の一つであることが推察される。著者らと同様に，DHAのエチルエステル体を加齢ラット[10,68]や加齢マウス[5]に投与すると，それぞれ放射状迷路法で評価される空間認知機能が亢進し，さらにこのとき，加齢マウスのニューロンの樹状突起スパインは増加する，との報告がある。

5　神経疾患とDHA

5·1　アルツハイマー病とDHA

認知機能障害はアルツハイマー病（AD）患者の主要な臨床所見の一つであるが，その障害の程度は患者個々で異なる。AD患者の海馬のDHA量は同年齢者と比べて著明に低下するが[69]，このDHA量の減少は，ミエリンの主要脂質であるプラズマローゲン量がAD患者では

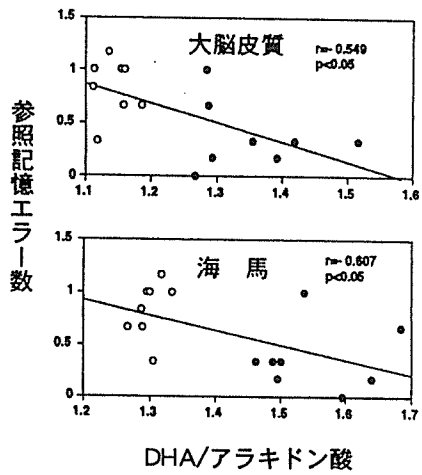

Fig. 4　若齢ラットの大脳皮質と海馬でのドコサヘキサエン酸（DHA）／アラキドン酸比と空間認知機能との関係[9]

n-3不飽和脂肪酸欠乏飼料で3世代飼育した5週齢Wistar系雄ラットにDHA（300 mg/kg/day）を10週間経口投与した後，放射状迷路法により空間認知機能を評価した。参照記憶エラー数は報酬餌のないアームに入った回数であり，長期記憶の指標として用いられている。縦軸の参照記憶エラー数は最終ブロックの数値が用いられた。DHAとアラキドン酸はガスクロマト法で測定した。○：Vehicle群，●：DHA投与群；文献9）の原図を改変した。

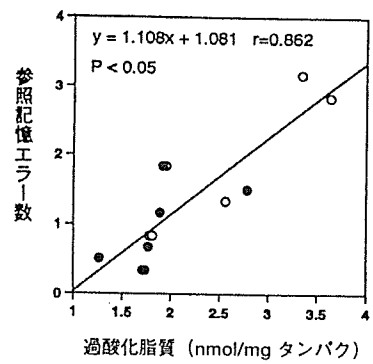

Fig. 5　老齢ラットの海馬での過酸化脂質量と空間認知機能との関係[10]

n-3不飽和脂肪酸欠乏飼料で3世代飼育した100週齢Wistar系雄ラットにDHA（300 mg/kg/day）を12週間経口投与した後，放射状迷路法により空間認知機能を評価した。参照記憶エラー数は報酬餌のないアームに入った回数であり，長期記憶の指標として用いられている。縦軸の参照記憶エラー数は最終ブロックの数値が用いられた。過酸化脂質はチオバルビツール酸反応生成物（TBARS）価で表されている。○：Vehicle群，●：DHA投与群；文献10）の原図を改変した。

低下するとの報告と一致している[70-73]。このプラズマローゲンの低下はプラズマローゲンからDHAを遊離させる酵素であるプラズマローゲン感受性PLA_2の活性化を誘発するばかりではなく，リン脂質の異化代謝産物である過酸化脂質やリン酸モノエステル，リン酸ジエステルの蓄積を伴う。そのため，シナプトゾーム膜に豊富に含まれるDHAやプラズマローゲンの減少は，AD患者でのシナプスの消失や認知機能の障害に関係しているかも知れない。

前述のように，欧米やわが国での大規模な疫学調査研究では，食餌性脂質の種類とADとの関係が明らかにされている[1-3,74]。飽和脂肪酸やコレステロールの摂取過多はADやそのほかの認知症のリスクを高めるとの報告があるが，今日最も注目されているのは，魚の消費とADの発症との関連性である。すなわち，魚の消費が高ければ高い程，加齢やADによる認知機能の低下を抑えることが推察されている[2,3,75]。AD患者に魚油（DHA・EPA）あるいはDHAを与えた時のヒトでの介入試験はほとんど行なわれていないが，5名のAD患者がDHA（1.4 g/日）を6ヵ月間服用すると，全員にやや改善傾向がみられ[76]，また，EPAを服用したAD患者は非服用患者に比べて認知症の臨床症状の改善がみられる，との報告がある[77]。

著者らは，若・加齢ラット共にDHAによる空間認知機能向上効果を明らかにしたことから[9,10]，臨床応用への前段階として，アルツハイマー型痴呆モデルラット（ADモデルラット）を用いて，DHAによるアルツハイマー型痴呆予防・改善効果について検討を行った。ADモデルラットの作製はカニュレーション法で行った。すなわち，ADの発症因子の一つであるβ-アミロイド蛋白（1-40）をラット側脳室に持続的に注入すると一定期間後に空間認知機能が低下する（Fig. 6 A）。n-3 PUFA欠乏飼料で飼育した若齢ラット（20週齢）にDHAをあらかじめ15週間経口投与（300 mg/kg/day）を行った後にADモデルラットを作製し，DHAによる予防効果を検討したところ，あらかじめDHAを投与したラット（DHA＋AD）ではシャトルアボイダンス法による総回避数がADラットに比べて増加し，大脳皮質・海馬でのDHA/AA比と総回避数との間には正の相関が認められる。さらには（DHA＋AD）ラットの大脳皮質の過酸化脂質量と活性酸素種（ROS）量，ならびにアポトーシスの指標であるmono- and oligo-nucleosomes量は，ADラットに比べて低下する[78]。これらの結果から，大脳皮質，海馬での抗酸化能が増強されることにより，食餌性DHAはβ-アミロイド蛋白（1-40）の脳内沈着による空間認知機能障害を予防するものと思われる。

DHAによるAD改善効果を検討するために，ADモデルラットに12週間にわたりDHAを投与すると，海馬の抗酸化能の増加とともにβ-アミロイド蛋白の沈着により低下した空間認知機能が改善する（Fig. 6 B）[79]。このとき，DHAを投与したADモデルラットの海馬β-アミロイド蛋白量は非投与ADラットに比べて有意に減少する（投稿中）。β-アミロイド蛋白前駆体蛋白（APP: amyloid precursor protein）が過剰に発現するトランスジェニックマウス（APPsw）にDHAを投与すると，APPswの空間認知機能が向上し，β-アミロイド蛋白により誘発される後シナプスの蛋白変性が保護され[80]，さらには海馬でのβ-アミロイド蛋白の沈着が著明に減少する[81]。β-アミロイド蛋白はAPPにβ-，γ-セクレターゼが作用して作られるが，DHAはこれら分解酵素を阻害することにより海馬でのβ-アミロイド蛋白の沈着を防ぐことが報告されている[82]。また，マイクロアレイとRT-PCR法による検討では，DHAを摂取した加齢ラットの海馬でのtransthyretin（thyroxin輸送関連蛋白であり，β-アミロイド蛋白へのスカベンジ効果作用をもつ）の遺伝子発現が著明に増加する[40]。

最近，DHAの代謝産物（ドコサノイド）である10, 17 s-docosatriene（neuroprotectin D1：NPD1, Fig. 2

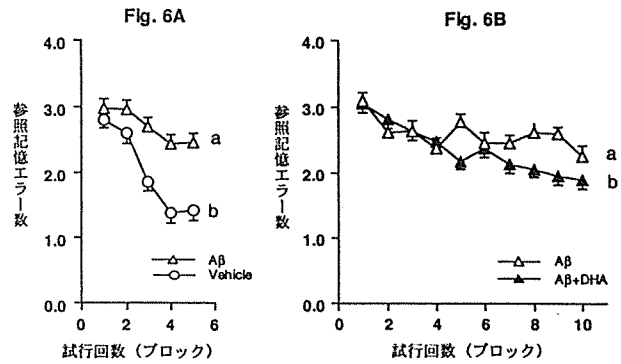

Fig. 6　アルツハイマー型痴呆モデルラットの空間認知機能障害に及ぼすドコサヘキサエン酸（DHA）の影響[79]

Fig. 6Aはβ-アミロイド蛋白（1-40）のラット脳室内注入による空間認知機能の低下を示し，Fig. 6Bはアルツハイマー型痴呆モデルラットの空間認知機能障害へのDHAによる改善効果を示している。
n-3不飽和脂肪酸欠乏飼料で3世代飼育した20週齢Wistar系雄ラットからアルツハイマー型痴呆モデルラットを作製した後，DHA（300 mg/kg/day）を12週間経口投与し，その間，放射状迷路法により空間認知機能を評価した。参照記憶エラー数は報酬餌のないアームに入った回数であり，長期記憶の指標として用いられている。
Aβ：アルツハイマー型痴呆モデルラット群，Aβ＋DHA：DHA投与アルツハイマー型痴呆モデルラット群，横軸は6試行を1ブロックとした時の試行回数を表す。a, bは2群間の統計学的有意差を示す（$p < 0.05$）。文献79）の原図を改変した。

参照）による神経保護作用に関する興味ある報告がなされている[83]。NPD1はPLA_2と15-lipoxygenase様酵素（15-LOX-like）を介してDHAから合成される。マウス脳での虚血・再還流時には，NPD1は抗アポトーシス作用により神経保護作用を発揮し[84]，また，AD患者の海馬では，DHA量とPLA_2と15-LOX-like活性の低下と共にNPD1量が低下する。さらには，NPD1は，可溶性のα-アミロイド蛋白前駆体蛋白（αAPP）により生成が促進され，また，ニューロンのアポトーシスを保護する様々な関連遺伝子の発現を増強することにより，不溶性のβ-アミロイド蛋白（1-42）によりもたらされる脳神経の細胞死を阻止する。これらの結果から，DHAやNPD1生合成代謝のアゴニストやNPD1のアナログは，ADとその関連する神経疾患の新規な機能性物質や治療物質になりうる可能性が示唆される。DHAによるアルツハイマー型認知症の予防・改善効果機序を解明するうえで，脳内でのβ-アミロイド蛋白の合成・代謝過程とDHAとその代謝物との関連性を検討する研究は，今後益々展開されるものと確信している。

5·2　他の神経疾患とDHA

精神活動や精神疾患の症状改善効果とDHAや魚油との関係についても，急速に明らかにされつつある。魚の消費量が多いほどうつ病の生涯有病率が低く[85]，うつ症状が強度であるほど，赤血球膜リン脂質のAA/EPAやn-6/n-3比が高く[86]，さらには，うつ病患者は健常対照者よりも赤血球中の総n-3 PUFA，EPA，ならびにDHAの各濃度が低下する[87]，等の報告から，うつ病とDHAにはなんらかの関係があるものと思われる。また，ヒトの敵意性（他人に対する攻撃性）は，DHA摂取により抑制されること[88]，さらには乳児性Refsum病，新生児副腎脳白質ジストロフィー（ALD），X染色体連鎖ALD，ならび副腎脊髄神経障害などの神経疾患ではDHAが欠乏する，などの報告がある[89,90]。精神活動や精神疾患へのDHAによる症状改善効果の作用機序は明らかではないが，今後，この分野での研究が発展することを期待する。

6　おわりに

最近の脳機能研究の進展には目覚しいものがあるが，とくに注目すべき研究成果の一つとしては，ヒトを含めた成熟動物での神経幹細胞の存在が明らかになり，その細胞機能が解明されつつあることである。つい最近まで，神経細胞は分裂能がないために成体中枢神経は障害を受けると二度と再生しないと信じられていた。しかし神経幹細胞は，神経成長因子存在下では増殖し（自己複製能），神経成長因子非存在下では神経細胞集団である

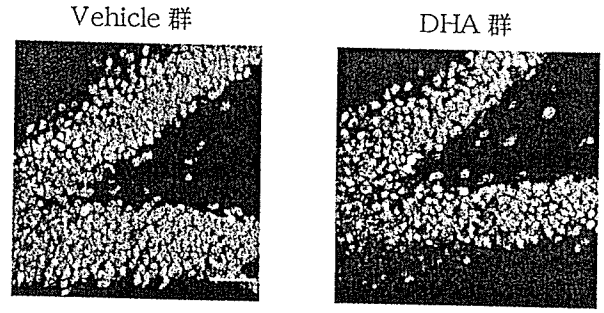

Fig. 7　ラット海馬歯状回でのニューロン新生に及ぼす食餌性DHAの影響

n-3不飽和脂肪酸欠乏飼料で3世代飼育した20週齢の雄ラットに，ドコサヘキサエン酸（DHA, 300 mg/kg/day）を7週間，経口投与したのち，海馬歯状回領域でのニューロン新生を共焦点レーザー顕微鏡で観察・評価した。白い細胞は，BrdU（分裂細胞マーカー：神経幹細胞）とNeu N（成熟ニューロンマーカー）の二重染色細胞であり新生ニューロンを示す。灰色な細胞群は歯状回の顆粒細胞層。スケールは50 μmを表す。DHA群はVehicle群に比べて新生ニューロン数は増加した。（文献92参照）

ニューロン，アストロサイト，オリゴデンドロサイトに分化する（多分化能）など，特異的な性質をもつ未分化な細胞である，と報告[91]されて以来，多くの検討がなされ，最近では，神経幹細胞の機能により神経細胞は再生可能であると考えられている。最近著者らは，神経幹細胞にDHAやEPAを作用させると，その分化能が亢進し，アポトーシスが抑制され，さらには，DHAを投与した成熟ラットでは，海馬歯状回の新生ニューロン数が増加することを見出した（Fig. 7）[92]。神経幹細胞は主に海馬歯状回や側脳質に局在することから，神経幹細胞による記憶・学習のメカニズムへの関与，さらには脳機能の活性化と保護作用，ならびにさまざまな脳機能賦活物質による痴呆改善効果などへの関与が期待される。DHAによる脳機能改善効果機序を解明するためには，ニューロン膜をターゲットとした従来の視点に加えて，

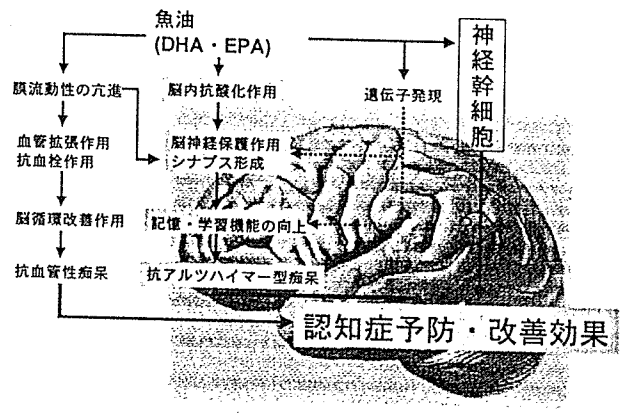

Fig. 8　ドコサヘキサエン酸（DHA）による認知症の予防・改善効果機序

神経幹細胞をターゲットとした研究も今後は展開されるものと思われる (Fig. 8)。

文 献

1) P. Barberger-Gateau, L. Letenneur, V. Deschamps, K. Peres, J.F. Dartigues & S. Renaud, *Br. Med. J.*, 325, 932-3 (2002).
2) M.C. Morris, D.A. Evans, J.L. Bienias, C.C. Tangney, D.A. Bennett, R.S. Wilson, N. Aggaewal & J. Schneider, *Arch. Neurol.*, 60, 940-6 (2003).
3) A. Ueki, M. Otsuka, T. Sato & S. Sasaki, *J. Nutr. Health Aging*, 8, 432 (2004).
4) S.M. Innis, *Pro. Lipid Res.*, 30, 39-103 (1991).
5) L. Lauritzen, H.S. Hansen, M.H. Jorgensen & K.F. Michaelsen, *Orog. Lipid Res.*, 40, 1-94 (2001).
6) H. Hamano, J. Nabekura, M. Nishikawa & T. Ogawa, *J. Neurophysiol.*, 75, 1264-70 (1996).
7) B.L. Scott & N.G. Bazan, *Proc. Natl. Acad. Sci. USA*, 86, 2903-07 (1989).
8) N. Yamamoto, M. Saitoh, A. Moriguchi, M. Nomura & H. Okuyama, *J. Lipid Res.*, 28, 144-51 (1987).
9) S. Gamoh, M. Hashimoto, K. Sugioka, M.S. Hossain, N. Hata, Y. Misawa & S. Masumura, *Neuroscience*, 93, 237-41 (1999).
10) S. Gamoh, M. Hashimoto, S. Hossain & S. Masumura, *Clin. Exp. Pharmacol. Physiol.*, 28, 266-70 (2001).
11) S.Y. Lim & H. Suzuki, *J. Nutr.*, 130, 1629-32 (2001).
12) L.A. Horrocks & A.A. Farooqui, *Prostaglandings Leukot. Essent. Fatty Acids*, 70, 361-72 (2004).
13) J-M. Alessandri, P. Guesnet, S. Vancassel, P. Astorg, I. Denis, B. Langelier, S. Aid, C. Poumes-Ballihaut, G. Champeil-Potpkar & M. Lavialle, *Reprod. Nutr. Dev.* 44, 509-38 (2004).
14) J.C. McCann & B.N. Ames, *Am. J. Clin. Nutr.*, 82, 281-95 (2005).
15) J.P. Marszalek & H.F. Lodish, *Ann. Rev. Cell Dev. Biol.*, 21, 633-57 (2005).
16) S.A. Moore, E. Yoder, S. Murphy, G.R. Dutton & A.A. Spector, *J. Neurochem.*, 56, 518-24 (1991).
17) S.A. Moore, *World Rev. Nutr. Diet.*, 75, 128-33 (1994).
18) S.M. Innis & R.A. Dyer, *J. Lipid Res.*, 43, 1529-36 (2002).
19) D.E. Williard, S.D. Harmon, T.L. Kaduce, Preuss, M. S.A. Moore, M.E.C. Robbins & A.A. Spector, *J. Lipid Res.*, 42, 1368-76 (2001).
20) S.I. Rapoport, M.C.J. Chang & A.A. Spector, *J. Lipid Res.*, 42, 678-85 (2001).
21) A.A. Farooqui, L.A. Horrocks & T. Farooqui, *J. Mol. Neurosci.*, 14, 123-35 (2000).
22) A.A. Farooqui & L.A. Horrocks, *J. Mol. Neurosci.*, 16, 263-72 (2001).
23) A.A. Farooqui, H-C. Yang & L.A. Horrocks, *Brain Res. Rev.*, 21, 152-61 (1995).
24) M. Shikano, Y. Masuzawa, K. Yazawa, K. Takayama, I. Kudo & K. Inoue, *Biochim. Biophys. Acta Lipids Lipid Metab.*, 1212, 211-6 (1994).
25) S. Hossain, M. Hashimoto, T. Shimada & O. Shido, *Life Sci.*, 74, 3009-24 (2004).
26) M. Okada, T. Amamoto, M. Tomonaga, A. Kawachi, K. Yazawa, K. Mine & M. Fujiwara, *Neuroscience*, 71, 17-25 (1996).
27) D.E. Eddy & D. Harman, *J. Am. Geriatr. Soci.*, 25, 220-9 (1977).
28) P. Green, S. Glozman & E. Yavin, *Biochim. Biophys. Acta. Mol. Cell. Bio. Lipids*, 1531, 156-64 (2001).
29) S. Hossain, M. Hashimoto & S. Masumura, *Nurosci. Lett.*, 244, 157-60 (1998).
30) S. Hossain, M. Hashimoto, S. Gamoh & S. Masumura, *J. Neurochem.*, 72, 1133-8 (1999).
31) K. Miyashita, E. Kara & T. Ota, *Biosci. Biotech. Biochem.*, 57, 1638-40 (1993).
32) J.D. Fernstrom, *Lipids*, 34, 161-9 (1999).
33) L.A. Horrocks & Y.K. Yeo, *Pharmacol. Res.*, 40, 211-25 (1999).
34) S. Yehuda, S. Rabinovitz, R.L. Carasso & D.I. Mostofsky, *Neurobiol. Aging*, 23, 843-53 (2002).
35) M. Hashimoto, K. Shinozuka, S. Gamoh, Y. Tanabe, M.S. Hossain, Y.M. Kwon, N. Hata, Y. Misawa, M. Kunitomo & S. Masumura, *J. Nutr.*, 129, 70-6 (1999).
36) M. Hashimoto, S.M. Hossain, T. Shimada, H. Yamasaki, Y. Fujii & O. Shido, *J. Lipid Res.*, 42, 1160-8 (2001).
37) G. Barcelo-Coblijn, K. Kitajka, L.G. Puskas, E. Hogyes, A. Zara, L. Hackler Jr. & T. Farkas, *Biochim. Biophys. Acta*, 1632, 72-9 (2003).
38) T. Farkas, K. Kitajka, E. Fodor, I. Csengeri, E. Lahdes, Y.K. Yeo, Z. Krasznai & J.E. Halver, *Proc. Natl. Acad. Sci. USA*, 97, 6362-6 (2000).
39) K. Kitajka, L.G. Puskas, A. Zvara, L.J. Hackler, G. Barcelo-Coblijin, Y.K. Yeo & T. Farkas, *Proc. Natl. Acad. Sci. USA*, 99, 2619-24 (2002).
40) L.G. Puskas, K. Kitajka, C. Nyakas, G. Barcelo-Coblijin & T. Farkas, *Proc. Natl. Acad. Sci. USA*, 100, 1580-5 (2003).
41) S. Chalon, S. Delion-Vancassel, C. Belzung, D. Guilloteau, A.M. Leguisquet, J.C. Besnard & G. Durand, *J. Nutr.*, 128, 2512-9 (1998).
42) S. Delion, S. Chalon, D. Guilloteau, B. Lejeune, J.C. Besnard & G. Durand, *J. Lipid Res.*, 38, 680-9 (1997).
43) L. Zimmer, S. Delpal, D. Guilloteau, J. Aioun, G. Durand & S. Chalon, *Neurosci. Lett.*, 284, 25-8 (2000).
44) M. Minami, S. Kimura, T. Endo, N. Hamaue, M. Hirafuji, H. Togashi, M. Matsumoto, M. Yoshioka, H. Saito, S. Watanabe, T. Kobayashi & H. Okuyama, *Pharmacol. Biochem. Behav.*, 58, 1123-9 (1997).
45) E. Hogyes, C. Nyakas, A. Kiliaan, T. Farkas, B. Penke & P.G. Luiten, *Neuroscience*, 119, 999-1012 (2003).
46) M. Vreugdenhil, C. Bruehl, R.A. Voskuyl, J.X. Kang, A. Leaf & W.J. Wadman, *Proc. Natl. Acad. Sci. USA*, 93, 12559-63 (1996).
47) D.B. Jump, *Curr. Opin. Lipidol.*, 13, 155-64 (2002).
48) I. Issemann, R.A. Prince, J.D. Tugwood & S. Green, *J. Mol. Endocrinol.*, 11, 37-47 (1993).

49) M. Gottlicher, A. Demoz, D. Svensson, P. Tollet, R.K. Berge & J.A. Gustafsson, *Biochem. Pharmacol.*, 46, 2177-84 (1993).
50) J.M. Ntambi & H. Bene, *J. Mol. Neurosci.*, 16, 273-8 (2001).
51) Y. Tanabe, M. Hashimoto, K. Sugioka, M. Maruyama, Y. Fujii, R. Hagiwara, T. Hara, M.S. Hossain & O. Shido, *Clin. Exp. Pharmacol. Physiol.*, 31, 700-3 (2004).
52) 伊藤正男，脳の不思議，岩波書店，pp. 58-74 (1999).
53) C. Chen & S. Tonegawa, *Ann. Rev. Neurosci.*, 20, 157-84 (1997).
54) M.A. Lynch & K.L. Voss, *Eur. J. Neurosci.*, 6, 1008-14 (1994).
55) B. McGahon, M.P. Clements & M.A. Lynch, *Neuroscience*, 81, 9-16 (1997).
56) S. Fujita, Y. Ikegaya, M. Nishikawa, N. Nishiyama & N. Matsuki, *Br. J. Pharmacol.*, 132, 1417-22 (2001).
57) M. Nishikawa, S. Kimura & N. Akaike, *J. Physiol.*, 475, 83-93 (1994).
58) C. Young, P.W. Gean, L.C. Chiou & Y.Z. Shen, *Synapse*, 37, 90-4 (2000).
59) N. Itokazu, Y. Ikegaya, M. Nishikawa & N. Matsuki, *Brain Res.*, 862, 211-6 (2000).
60) 高畑京也，物部啓一，多田幹郎，脂質生化学, 3, 90 (1994).
61) B.M. McGahon, D.S. Martin, D.F. Horrobin & M.A. Lynch, *Neuroscience*, 94, 305-14 (1999).
62) A. Lucas, R. Morley, T.J. Cole, G. Lister, C. Leeson-Payne, *Lancet*, 339, 261-4 (1992).
63) A.C. van Houwelingen, J.D. Sorensen, G. Hornstra, M.M. Simonis, J. Boris, S.F. Olsen & N.J. Secher, *Br. J. Nutr.*, 74, 723-31 (1995).
64) T. Moriguchi, S-Y. Lim, R. Greiner, W. Lefkowitz, J. Loewke, J. Hoshiba & N. Salem Jr., *J. Lipid Res.*, 45, 1437-45 (2004).
65) S. Favreliere, L. Barrier, G. Durand, S. Chalon & C. Tallineau, *Lipids*, 33, 401-7 (1998).
66) S. Favreliere, M.C. Perault, F. Huguet, D. De Javel, N. Bertrand, A. Piriou & G. Durand, *Neurobiol. Aging*, 24, 233-43 (2003).
67) C.R. Jones, T. Arai & S.I. Rapoport, *Neurochem. Res.*, 22, 663-70 (1997).
68) N. Kotchabhakdi, R. Tipyasang, W. Thangnipon, N. Jutapukdeekun & C. Jindaduangratn, *Soc. Neurosci. Abstract*, Program No. 941.17 (2003).
69) M. Soderberg, C. Edlund, K. Kristensson & G. Dallner, *Lipids*, 26, 421-5 (1991).
70) Z. Guan, Y. Wang, N.L. Cairns, P.L. Lantos, G. Dallner & P.J. Sindelar, *J. Neuropathol. Exp. Neurol.*, 58, 740-7 (1999).
71) X.L. Han, D.M. Holtzman, D.W. McKeel Jr, *J. Neurochem.*, 77, 1168-80 (2001).
72) J.W. Pettegrew, K. Panchalingam, R.L. Hamilton, R.J. MaClure, *Neurochem. Res.*, 26, 771-82 (2001).
73) K. Wells, A.A. Farooqui, L. Liss & L.A. Horrocks, *Neurochem. Res.*, 20, 1329-33 (1995).
74) S. Kalmijn, E.J.M. Feskens, L.J. Launer & D. Kromhout, *Am. J. Epidemiol.*, 145, 33-41 (1997).
75) R.P. Friedland, *Arch. Neurol.*, 60, 923-4 (2003).
76) 宮永和夫，米村公江，高木正勝，臨床医薬，11, 881-9 (1995).
77) 大塚美恵子，植木　彰，*Dement. Jpn.*, 15, 21-9 (2001).
78) M. Hashimoto, S. Hossain, T. Shimada, K. Sugioka, H. Yamasaki, Y. Fujii, Y. Ishibashi, J.-I. Oka & O. Shido, *J. Neurochem.*, 81, 1084-91 (2002).
79) M. Hashimoto, Y. Tanabe, Y. Fujii, T. Kikuta & H. Shibata, O. Shido, *J. Nutr.*, 135, 549-55 (2005).
80) F. Calon, G.P. Lim, F. Yang, T. Morihara, B. Teter, O. Ubeda, P. Rostaing, A. Triller, N. Salem Jr, K.H. Ashe, S.A. Frautschy & G.M. Cole, *Neuron*, 43, 633-45 (2004).
81) G.P. Lim, F. Calon, T. Morihara, F. Yang, B. Teter, O. Ubeda, N. Salem Jr, S.A. Frautschy & G.M. Cole, *J. Neurosci.*, 25, 3032-40 (2005).
82) M.C. de Wilde, I. Leenders, L.M. Broersen,A.A.M. Kuipers, E.M. van der Beek & A.J. Kiliaan, *Soc. Neurosci. Abstracts*, Program No. 730.11 (2003).
83) W.J. Lukiw, J-G. Cui, V.L. Marcheselli, M. Bodker, A. Botkjaer, K. Gotlinger, C.N. Serhan & N.G. Bazan, *J. Clin. Invest.*, 115, 2774-83 (2005).
84) V.L. Marcheselli, S. Hong, W.J. Lukiw, X.H. Tian, K. Gronert, A. Musto, M. Hardy, J.M. Gimenez, N. Chiang, C.N. Serhan & N.G. Bazan, *J. Biol. Chem.*, 278, 43807-17 (2003).
85) J.R. Hibblen, *Lancet*, 351, 1213 (1998).
86) M. Maes, R. Smith, A. Christophe, P. Cosyns, R. Desnyder & H. Meltzer, *J. Affect. Disord.*, 38, 35-46 (1996).
87) R. Edward, M. Peet, J. Shay & D. Horrobin, *J. Affect. Disord.*, 48, 149-55 (1998).
88) T. Hamasaki, S. Sawazaki, M. Itomura, E. Asaoka, Y. Nagao, N. Nishimura, K. Yazawa, T. Kuwamori & M. Kobayashi, *J. Clin. Invest.*, 97, 1129-33 (1996).
89) M. Martinez, E. Vazquez, Garcia-Silva, J. Manzanares, J.M. Bertran, F. Castello & I. Mougan, *Am. J. Clin. Nutr.*, 71, 376 S-85 S (2000).
90) H.W. Moser, *Clin. Biochem.*, 24, 343-51 (1991).
91) B.A. Reynolds & S. Weiss, *Science*, 255, 1707-10 (1992).
92) 橋本道男，川北映輔，片倉賢紀，矢野　崇，原田　剛，ハク・アブドール，水口　清，紫藤　治，脂質栄養学, 14, 154 (2005).

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Docosahexaenoic acid-induced amelioration on impairment of memory learning in amyloid β-infused rats relates to the decreases of amyloid β and cholesterol levels in detergent-insoluble membrane fractions

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Abstract

We investigated the effects of dietary administration of docosahexaenoic acid (DHA; C22:6n-3) on the levels of amyloid β (Aβ) peptide (1–40) and cholesterol in the nonionic detergent Triton 100×-insoluble membrane fractions (DIFs) of the cerebral cortex and, also, on learning-related memory in an animal model of Alzheimer's disease (AD) rats infused with Aβ peptide (1–40) into the cerebral ventricle. The infusion increased the levels of Aβ peptide and cholesterol in the DIFs concurrently with a significant increase in reference memory errors (measured by eight-arm radial-maze tasks) compared with those of vehicle rats. Conversely, the dietary administration of DHA to AD-model rats decreased the levels of Aβ peptide and cholesterol in the DIFs, with the decrease being more prominent in the DHA-administered rats. Regression analysis revealed a significant positive correlation between Aβ peptide and each of cholesterol, palmitic acid and stearic acid, and between the number of reference memory errors and each of cholesterol, palmitic, stearic and oleic acid; moreover, a significant negative correlation was observed between the number of reference memory errors and the molar ratio of DHA to palmitic plus stearic acid. These results suggest that DHA-induced protection of memory deficits in AD-model rats is related to the interactions of cholesterol, palmitic acid or stearic acid with Aβ peptides in DIFs where DHA ameliorates these interactions.

Keywords: Amyloid β peptide; Detergent-insoluble membrane fraction; Docosahexaenoic acid; Learning ability; Alzheimer's disease model rats

1. Introduction

The molecular event of the deposition of amyloid beta (Aβ) peptide (1–40) or Aβ peptide (1–42) in the brains of Alzheimer's disease (AD) subjects and the consequent loss of cognition is not clearly understood. The process is believed to involve complicated actions of β- and γ-secretase on their substrate amyloid precursor protein (APP) in membrane lipid environments; for example, the APP is subjected to sequential cleavage of β- and γ-secretase, a prerequisite to intramembrane events that result in the production of Aβ peptides (1–40)/(1–42) that act as seeds of amyloidogenesis [1]. Recent evidence suggests that the seeding process correlates the amyloid peptides with the cholesterol content of the so-called lipid rafts or detergent-resistant specialized membrane domains [2]. In vitro studies with human neuroblastoma cells have also shown that these specialized membrane domains are highly rich in cholesterol and glycosphingolipids and play an important role in cellular trafficking and signal transduction cascades [3]. Moreover, the decreased prevalence of AD is associated with treatment by 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase inhibitors [4]. Thus, interest has focused on the initial localization of amyloid peptides in cholesterol-rich membrane domains.

The search for agents capable of protecting against amyloidogenesis and associated cognitive loss by dietary intervention has been one of the most interesting aspects of AD research. Therefore, the effects of dietary docosahexaenoic acid (DHA; C22:6n-3) on Aβ peptides and cholesterol in

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detergent insoluble membrane fractions (DIFs) could be of special significance, especially since DHA reduces cholesterol in both neuronal [5] and non-neuronal cells [6]. On the other hand, dietary administration of DHA protects against [7] and ameliorates [8] the impairment of memory learning induced by the infusion of Aβ peptide (1–40) into the rat cerebral ventricle, suggesting that DHA plays an important role in the neurochemical basis of memory. DHA exerts antioxidative effects on brain neurons [7–11], and a variety of antioxidants exhibits anti-AD pathology [12]. Epidemiological studies also suggest that AD is less frequent in populations that consume DHA [13]; however, these mechanisms have remained largely unknown. The aim of this preliminary investigation was, therefore, to investigate whether Aβ peptide (1–40) infusion into the rat cerebral ventricle affects the levels of cholesterol and Aβ peptide (1–40) in DIFs and, also, whether the effects of dietary DHA on these levels are associated with memory-related learning ability in AD model rats.

2. Materials and methods

2.1. Animals and diet

Rats were provided for and killed in accordance with the procedures outlined in the *Guidelines for Animal Experimentation of Shimane Medical University* (Shimane, Japan), compiled from the *Guidelines for Animal Experimentation of the Japanese Association for Laboratory of Animal Science.* Wistar rats (generation 1, G1) (*Jcl*: Wistar; Clea Japan Co., Osaka, Japan) were housed in a room under controlled temperature (23±2 °C), relative humidity (50±10%) and light–dark cycles (light: 0800 to 2000 h: dark: 2000 to 0800 h), and provided with a fish-oil-deficient pellet diet (F-1®; Funabashi Farm, Funabashi, Japan) and water ad libitum. The fatty acid composition of the F-1® is shown in Table 1. The inbred third generation (G3) male rats [n=31; 20 weeks old; 384±5.3 g body weight (BW)], fed the same F1 diet, were randomly divided into 4 groups: a vehicle group (n=7), an Aβ peptide (1–40)-infused group (Aβ group) (n=7), an Aβ+DHA group (n=8) and a DHA group (n=9).

2.2. Surgery for the preparation of Aβ-infused AD model rats

The formation of neurofibrillary tangles and neuritic plaques of amyloid peptides such as Aβ peptide$_{(1-40)}$ and neuronal loss are hallmarks of AD [14], and we [7,8] and others [14–16] have previously reported that the infusion of Aβ peptide (1–40) into the rat cerebral ventricle induces learning impairment, and neuronal and morphological degeneration. The surgical techniques for preparing Aβ-infused rats were, therefore, essentially the same as those described [7,8]. Briefly, each rat was anesthetized with sodium pentobarbital (50 mg/kg BW i.p.). The skull was exposed and two holes (right and left, relative to the bregma; 0.8 mm posterior, 1.4 mm lateral) were drilled according to the atlas of Paxinos and Watson [17] using a stereotaxic frame (Narishige, Tokyo, Japan). A solvent of 35% (v/v) acetonitrile plus 0.1% (v/v) trifluoroacetic acid (pH 2.0) was used as the vehicle for Aβ peptide (1–40) (Peptide Inst., Osaka, Japan). Since a small amount of $AlCl_3$ facilitates the aggregation of Aβ peptide in vitro, and since the method has limited reproducibility without $AlCl_3$, we used 0.5 μg $AlCl_3$ (in 5 μL, intracerebroventricularly, 1 μL/min) before implanting the osmotic pump for continuous infusion of Aβ. This procedure greatly improved reproducibility and reliability in producing an animal model of AD with impaired memory. A mini-osmotic pump (alzet 2002; Durect Co., Cupertino, CA, USA), containing either Aβ peptide (1–40) solution or the vehicle alone was quickly implanted into the backs of the rats. The outlet of the pump was inserted 3.5 mm into the left ventricle and attached to the skull with screws and dental cement. The osmotic pump contained 234±13.9 μL Aβ solution. The infusion rate was 0.56 μL/h, and the total amount infused was approximately 4.9–5.5 nmol/L Aβ.

Table 1
Fatty acid composition (mol%) of a fish-oil-deficient pellet diet

Myristic acid $_{C14:0}$	0.09±0.09
Palmitic acid $_{C16:0}$	15.6±0.53
Palmitoleic acid $_{C16:1,\ n-7}$	ND
Stearic acid $_{C18:0}$	5.41±0.09
Oleic acid $_{C18:1,\ n-9}$	21.1±0.17
Linoleic acid $_{C18:2,\ n-6}$	52.4±0.80
Linolenic acid $_{C18:3,\ n-3}$	4.49±0.13
Arachidic acid $_{C20:0}$	0.14±0.13
Eicosenoic acid $_{C20:1,\ n-9}$	0.33±0.15
Arachidonic acid $_{C20:4,\ n-6}$	ND
Eicosapentaenoic acid $_{C20:5,\ n-3}$	0.06±0.06
Docosapentaenoic acid $_{C22:5,\ n-3}$	ND
Docosahexaenoic acid $_{C22:6,\ n-3}$	ND
Tetracosanoic acid $_{C24:0}$	0.11±0.07

Values are means±S.E.M. of quadruplicate determinations; ND: not detected.

2.3. Radial maze-related memory test

Learning-related behavior was assessed using an eight-arm radial maze (Toyo Sangyo, Toyama, Japan) to test whether memory was impaired as described previously [8,18–20]. Briefly, 4 weeks after the implantation of the mini-osmotic pump, the rats, maintained under a food-deprivation schedule, were trained to acquire a reward (food pellet) at the end of each of four arms of an eight-arm radial maze. The performance involved two parameters of memory-related function, namely, reference memory error (RME), entry into unbaited arms; and working memory error (WME), repeated entry into arms that had already been visited and obtaining the rewards within a trial. Thus, the higher the number of RME, the worse the learning ability, and vice versa. Each rat was given two daily trials, 6 days/week for a total of 2.5 weeks. The DHA and Aβ+DHA groups were then orally fed DHA-95E (300 mg/kg BW/day, an ethyl ester all-*cis*-4,7,10,13,16,19-docosahexaenoate with purity greater than 95%; Harima Chemicals, Tokyo, Japan) gently emulsified in a 5% (w/v) gum Arabic solution in ice-cold water before administration; the vehicle and Aβ groups were fed an equal volume of gum Arabic solution only. The total volume of gum Arabic solution administered with DHA was 0.5–0.8 mL. Seven weeks after starting the administration of DHA, the rats were tested for learning ability using the 8-arm radial maze for a total of 5 weeks, to assess the effect of dietary DHA on the impairment of learning ability.

2.4. Preparation of brains

After completing the behavioral studies, the rats were anesthetized with sodium pentobarbital (65 mg/kg BW, i.p.), and the blood was collected; the cerebral cortex was separated from the whole brain on ice, blotted gently with filter paper to remove blood and extraneous tissue fragments, then flash-frozen with liquid N_2 and stored at −80 °C until use.

2.5. Preparation of detergent insoluble membrane fractions (DIFs)

DIFs were prepared as previously described [21] with minor modifications. Cortical tissues (80–120 mg) were transferred to a capsule precooled in liquid N_2, crushed with an amalgam mixer (UT-1600, Sharp, Osaka, Japan) and suspended in 1.0 mL of ice-cold Tris–saline (50 mM Tris–HCl, pH, 7.6, 0.15 M NaCl) buffer containing 1% (v/v) Triton X-100 and the following protease inhibitors: 1.0 μM phenylmethylsulphonile fluoride, 10 μg/mL leupeptin, 1.0 μg/mL papstatin and 10 μg/mL aprotinin. The homogenate was centrifuged at 100,000×g for 30 min and the supernatant was used for measuring detergent-soluble Aβ peptide (1–40). The pellets were washed with the MES-buffered saline (25 mM MES, pH 6.5 and 0.15 M NaCl) containing 1% (v/v) Triton X-100 and various protease inhibitors (1.0 μM phenylmethylsulphonile fluoride, 10 μg/mL leupeptin, 1.0 μg/mL papstatin and 10 μg/mL aprotinin), and used as DIFs. After washing, the pelleted DIFs were initially suspended by vigorous mixing in a small volume of 6.0 M guanidine hydrochloride in 50 mM

Tris–HCl, pH 7.6 and centrifuged again at 125,000×g for 30 min. The resultant supernatant was diluted to a final concentration of 0.5 M guanidine–HCl and used as DIFs without further purification. The guanidine–HCl-solubilized detergent insoluble fraction (DIF) was subjected to ELISA and/or utilized for the measurement of detergent-insoluble membrane-bound Aβ peptide (1–40), cholesterol and fatty acid profiles.

2.6. Measurement of Aβ peptide (1–40) in the DIFs

The levels of Aβ peptide (1–40) were analyzed with a colorimetric sandwich ELISA kit according to the manufacturer's instructions (Immuno-Biological Laboratories Co., LTD, Gunma, Japan).

2.7. Lipid analyses

The cholesterol levels were measured by gas chromatography on a Model 5890II (Hewlett-Packard, Avondale, PA, USA) equipped with a flame ionization detector and an automatic sampler (Model 7673), as previously described [22] with a few modifications. Briefly, a 50-μL suspension of DIFs containing ~100 μg DIF-protein was mixed with 50 μg α-cholestane (1.0 μg/μL) in ethanol as an internal standard. The mixture was added to 1.0 mL saturated methanolic KOH, incubated at 80 °C for 30 min, cooled, supplemented with 1.0 mL saturated NaCl solution and 200 μL cyclohexane then vigorously shaken and centrifuged at 2000 g. The upper organic layer was directly subjected to gas chromatography (using a 30 m×0.25 mm inner diameter fused silica column coated with a methyl siloxane film 0.25 μm thick; HP-1, Hewlett-Packard), with helium as the carrier gas at a flow rate of 1.5 mL/min and oven temperatures programmed from 180 °C to 280 °C at 20 °C/min then at 280 °C for 10 min, and an injector splitter at 20 min/min at a temperature of 290 °C.

The fatty acid profiles were determined by the one-step analysis of Lepage and Roy [23] using gas chromatography as described previously [7,8]. Protein concentrations were estimated by the method of Lowry et al. [24].

2.8. Statistical analysis

Results are expressed as means±S.E. For intergroup differences, the data were analyzed by one-way ANOVA. ANOVA followed by Fisher's PLSD was used for post hoc comparisons. Correlation was determined by simple regression analysis. The statistical program used was StatView® 4.01 (MindVision Software, Abacus Concepts, Inc., Berkeley, CA, USA). A level of $P<0.05$ was considered statistically significant.

3. Results

3.1. Body weight

No significant differences were observed in body weight before and after surgery among the groups (vehicle: 408±6 vs. 485±7 g; DHA: 409±5 vs. 491±6 g; Aβ: 406±6 vs. 487±8 g; Aβ+DHA: 396±9 vs. 473±9 g).

3.2. Learning-related behavior

The effect of DHA administered to vehicle and Aβ peptide (1–40)-infused rats on reference memory-related learning ability is shown in Fig. 1. The bars represent the average or the mean number of RMEs of six trials for each group, measured at the end of the experiments and before the rats were killed for biochemical analysis. A one-way ANOVA applied to the means of RMEs revealed a significant effect of DHA on the RMEs in the AD model rats. The Aβ-infused group exhibited a significantly higher number of RMEs (by 133%) compared with that of the vehicle group. The dietary administration of DHA, however, significantly decreased the number of RMEs (by 24%) of the Aβ-infused group (Aβ+DHA) compared with that of the Aβ group. Correspondingly, the dietary administration of DHA decreased the number of RMEs of the vehicle-infused group (DHA) to a greater extent (44%) compared with that of the vehicle group, suggesting that the decrease in the number of RMEs of the Aβ+DHA group was attributable to the effects of DHA.

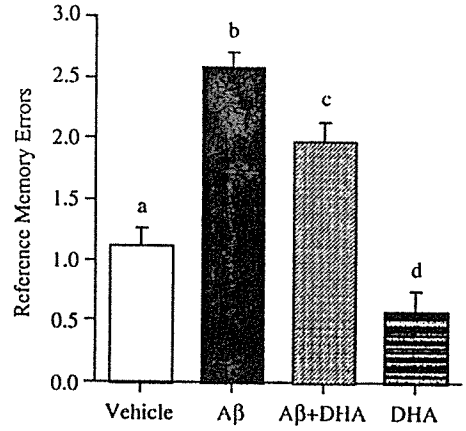

Fig. 1. Effects of administration of docosahexaenoic acid (DHA) on the number of reference memory errors of Alzheimer's disease model rats produced by infusion of amyloid β (Aβ) peptide (1–40). The data were analyzed by one-way ANOVA followed by Fisher's PLSD (Protected Least Square Difference) test for post hoc comparisons. Each bar represents the mean±S.E. of six trials from the final session. Vehicle ($n=7$): rats infused with the solvent (35% acetonitrile plus 0.1% trifluoroacetic acid [pH 2.0]) used for Aβ peptide (1–40); Aβ ($n=7$): Aβ peptide (1–40)-infused rats; Aβ+DHA ($n=8$): Aβ infused DHA-administered rats; DHA ($n=9$): DHA administered vehicle-infused rats. Bars not sharing a common notation $^{(a-d)}$ are significantly different at $P<0.05$.

3.3. Effects of DHA administration on Aβ peptide (1–40) and cholesterol levels in DIFs

The level of Aβ peptide (1–40) in the DIFs of the Aβ group was higher than that of the vehicle, DHA, or Aβ+DHA group ($P<0.05$). In the DIFs of the DHA group, it was significantly lower than that of the vehicle, Aβ ~~or Aβ+DHA~~ group (Fig. 2A). Similarly, the level of cholesterol in the DIFs of the Aβ group was significantly higher than that of the vehicle, DHA, or Aβ+DHA group ($P<0.05$). In the DIFs of the Aβ+DHA and DHA groups, it was significantly lower than that of the vehicle and Aβ groups (Fig. 2B).

3.4. Effects of DHA administration on the fatty acid profile in the DIFs

The levels of palmitic acid, stearic acid and oleic acid in the DIFs were significantly higher in the Aβ group than in the other three groups (Table 2). The level of tetracosanoic acid was significantly higher in the vehicle group than in the other groups. The level of arachidonic acid was significantly lower in the Aβ+DHA and DHA groups than in the Aβ group. The level of docosapentaenoic acid was significantly higher in the Aβ group than in the other groups. The levels of EPA were not significantly

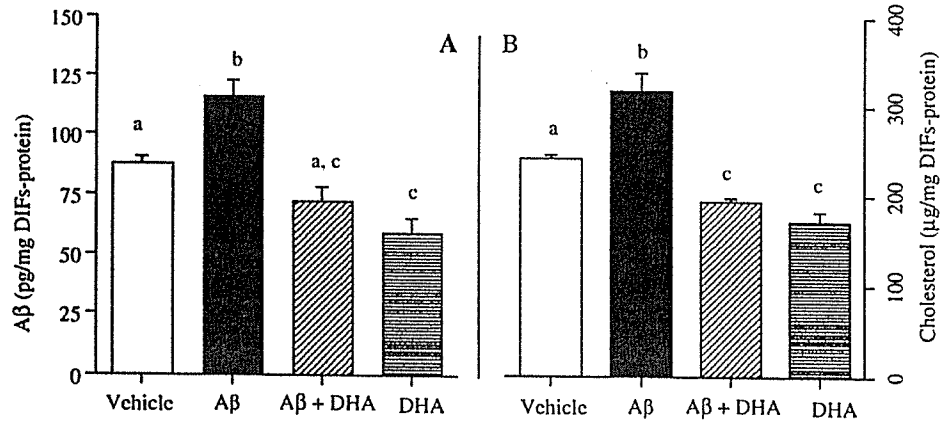

Fig. 2. Effects of administration of docosahexaenoic acid (DHA) on the levels of Aβ peptide (1–40) (A) and cholesterol (B) in the detergent-insoluble membrane fractions. Each bar represents the mean±S.E. (n=7–9). The data were analyzed by one-way ANOVA followed by Fisher's PLSD (Protected Least Square Difference) test for post hoc comparisons. Each bar represents the mean±S.E. of six trials from the final session. Vehicle group: rats infused with solvent (35% acetonitrile plus 0.1% trifluoroacetic acid [pH 2.0]) used for Aβ peptide (1–40); Aβ group: Aβ peptide (1–40)-infused rats; Aβ+DHA group: DHA-administered Aβ rats; DHA group: DHA administered vehicle rats. Bars not sharing a common notation $^{(a-d)}$ are significantly different at $P<0.05$.

different among the four groups. The levels (nmol/mg protein) of DHA did not change in the DIFs of the Aβ+DHA or DHA rats; however, the mol% of this fatty acid increased significantly compared with that of the Aβ rats [Vehicle=10.7±0.50; Aβ=8.7±0.25; Aβ+DHA=10.3±0.50; DHA=11.0±0.40]. The mol% of DHA also increased significantly ($P<0.05$) in the detergent soluble fraction (DSFs) [Vehicle=23.0±0.40; Aβ=20.0±0.70; Aβ+DHA=25.0±1.30; DHA=26.0±1.20]. The mol% of DHA in the DIFs was half of that in the DSFs. In addition, the molar ratio of DHA in DSFs versus DIFs increased significantly in both the Aβ+DHA and DHA rats (data not shown).

Table 2
Effect of dietary administration of docosahexaenoic acid on the fatty acid profile (nmol/mg protin)of detergent-insoluble membrane domains (DIFs)

Fatty acids	Vehicle (n=7)	Aβ (n=7)	Aβ+DHA (n=8)	DHA (n=9)
Palmitic acid $_{C16:0}$	365±25.0^a	443±30.0^b	321±11.0^a	323±22.0^a
Stearic acid $_{C18:0}$	295±8.0^a	362±27.0^b	273±17.0^a	251±17.0^a
Oleic acid $_{C18:1,\ n-9}$	176±17.0^a	216±20.0^b	158±12.0^a	156±16.0^a
Linoleic acid $_{C18:2,\ n-6}$	7.25±0.5	8.0±0.9	6.90±0.8	7.35±0.6
Linolenic acid $_{C18:3,\ n-3}$	0.20± 0.04	0.20±0.04	0.14±0.02	0.14±0.04
Arachidonic acid $_{C20:4,\ n-6}$	65.0±6.0^a	70.5±5.70^a	51.0±3.30^b	47.0±4.25^b
Eicosapentaenoic acid $_{C20:5,\ n-3}$	0.80±0.15	1.0±0.14	0.90±0.05	0.85±0.08
Docosapentaenoic acid $_{C22:5,\ n-3}$	0.80±0.10^a	1.50±0.18^b	0.80±0.09^a	0.70±0.10^a
Docosahexaenoic acid $_{C22:6,\ n-3}$	113±7.0	108±6.0	98.7±7.5	99.0±8.5
Tetracosanoic acid $_{C24:0}$	18.0±2.0^a	13.5±1.15^b	12.5±0.9^b	11.0±0.9^b
Nervonic acid $_{C24:1,\ n-9}$	16.0±1.0	15.5±1.15	14.5±1.25	14.0±1.20

Results are means±S.E. Values in the same row that do not share a common superscript are significantly different at $P<0.05$.

3.5. Correlations between Aβ peptide (1–40) and cholesterol and fatty acids in the DIFs

A significantly positive correlation was observed between cholesterol and Aβ peptide (1–40) in the DIFs (Fig. 3A). Regression analysis also revealed a significantly positive relation between Aβ peptide (1–40) and each of palmitic acid and stearic acid (Fig. 3B and C, respectively).

A highly significant positive correlation was observed between the number of RMEs and each of Aβ peptide (1–40) (Fig. 4A), cholesterol content (Fig. 4B), palmitic acid (Fig. 4C), stearic acid (Fig. 4D), oleic acid levels (Fig. 4E) in the DIFs. A significant negative correlation was also observed between the number of RMEs and the molar ratio of DHA to palmitic plus stearic acids (Fig. 4F).

4. Discussion

We have reported the preventive [7] and ameliorative [8] effects of the chronic administration of DHA on the impairment of spatial cognition learning ability in Aβ peptide (1–40)-infused rats. The present study suggests that the DHA-induced improvement of memory loss in AD model rats is mediated, at least partially, by the decreasing level of Aβ peptide (1–40) in DIFs. Our results are consistent with the reports on nicotine, demonstrating that Aβ peptide (1–40) from DIFs decreases significantly, while the levels of soluble Aβ peptide (1–40) are not affected [21,25]. Thus, the nicotine-induced decrease in detergent-insoluble amyloid is attributable to its inhibitory effect on $A\beta_{1-40}$ aggregation.

In the present study, the dietary administration of DHA significantly decreased the levels of palmitic acid by 28%, of stearic acid by 25% and of cholesterol by 39% in the DIFs of Aβ+DHA rats compared with those in the DIFs of Aβ rats. Many of the raft proteins of DIFs remain in palmitoylated form [26], and palmitoylation recruits membrane-proteins to the raft domains [27]. Raft proteins are not the only ones recruited to palmitoylation, rather other fatty acids may also be involved in

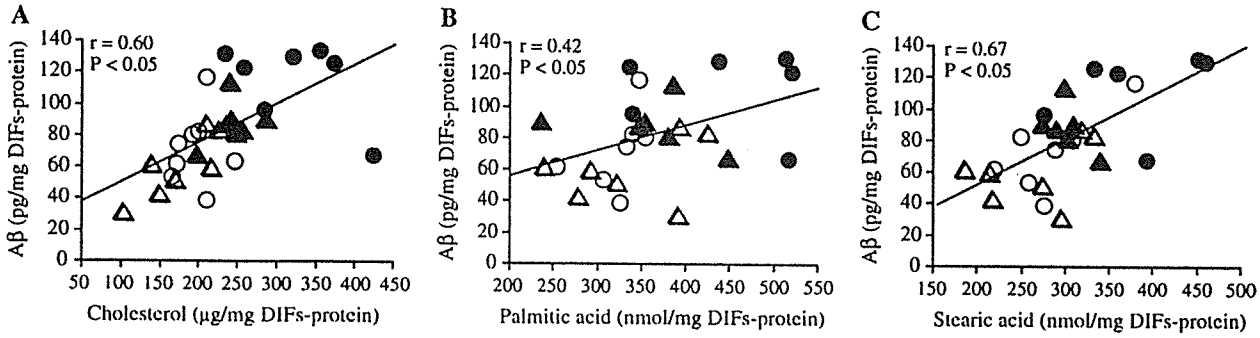

Fig. 3. Correlation between Aβ peptide, on the one hand, and cholesterol, palmitic acid and stearic acid, on the other, in the detergent-insoluble membrane fractions was evaluated by simple regression analysis. ●=Aβ, ○=Aβ+DHA, △=DHA, ▲=vehicle.

their acylation [28]. We are not certain whether Aβ peptide (1–40) remains in DIFs in plamitoylated form or whether it also remains associated (acylated) with stearic acid; as such, it can be speculated that DHA assists in scavenging Aβ peptide (1–40) by decreasing the amount of palmitic acid and stearic acid in DIFs. Thus it can also be assumed that DHA indirectly plays a significant role in disrupting the lipid environment in DIFs by somehow decreasing its stoichiometrically friendly molecules of palmitic or stearic acid with a concomitant reduction in Aβ. We assume that the same mechanism functions also in the DHA-induced decrease of cholesterol in DIFs. In this study, the levels of cholesterol, palmitic acid and stearic acid in DIFs correlated positively with the levels of detergent-insoluble Aβ peptide (1–40), and RMEs also correlated positively with Aβ peptide (1–40), palmitic, stearic and oleic acids in DIFs, suggesting a direct role of DIF cholesterol, palmitic, stearic acid and oleic acid in Aβ peptide (1–40) accumulation and in the amelioration of memory impairment by the DHA-induced elimination of cholesterol and/or by decreases of these fatty acids. This assumption is further supported by the negative correlation between the molar ratio of DHA to palmitic plus stearic acids and RMEs (Fig. 4F) and the concurrent increase in the mol% of DHA in the DIFs. The present findings are also in line with the results of statin-induced reductions of cholesterol, AD prevalence and amyloid peptides [4,29,30]. Our results are qualitatively consistent with the finding in AD-model mice demonstrating that dietary DHA reduces amyloid deposition in the cortex as well as in the hippocampus [31].

The increase of cholesterol in the DIFs of the Aβ rats was accompanied by greater increases of palmitic and stearic acids than those of other fatty acids (Table 2). Usually, membrane-bound cholesterol molecules exhibit higher affinity for saturated fatty acids to be solvated by their acyl chains [32]. It is conceivable, therefore, that the lipid domains of DIFs, which contained more cholesterol, concurrently with Aβ-peptide(1–40), recruited more saturated fatty acids like palmitic and stearic acids in order to solubilize the additional load of cholesterol, leading to an increase of these fatty acids in the DIFs of Aβ rats. These consequences are consistent with the decreases of membrane fluidity [22,33] in the DIFs of Aβ rats. Cholesterol's rigid-planner ring together with its intimately-interacted straight fatty acyl chains of the saturated fatty acids lowers the fluidity of the bilayer membrane [33]. The dietary

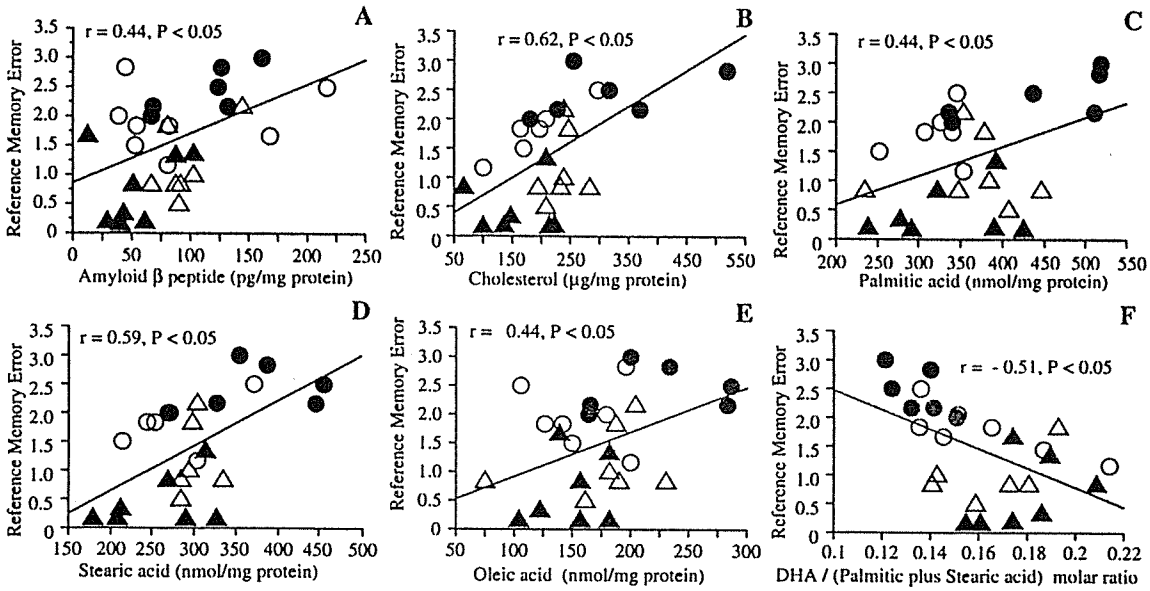

Fig. 4. Correlations between reference memory errors (RMEs) and each of Aβ peptide, cholesterol, palmitic acid, stearic acid, oleic acid and the ratio of DHA to palmitic plus stearic acid in the detergent-insoluble membrane fractions were evaluated by simple regression analysis. ●=Aβ, ○=Aβ+DHA, △=DHA, ▲=vehicle.

administration of DHA, however, reversed the action because DHA decreased both cholesterol and saturated fatty acids of the Aβ+DHA and DHA rats. Stearic acid decreases membrane fluidity [7,34,35], as does arachidonic acid by its high tendency of producing oxidative free radicals [7,36,37]. Therefore, the DIFs of the Aβ+DHA or DHA rats would experience a higher degree of fluidity compared with those of the Aβ rats. This speculation is consistent with that of Stubbs and Smith [38] and of Yeagle [39] demonstrating that membrane fluidity usually increases with unsaturation and decreases with cholesterol. Aβ peptide increases neuronal oxidative stress [7]. Membrane fluidity decreases by oxidative stress [22] and by Aβ peptide [40,41]. DHA not only decreases brain oxidative stress [7,10] but also increases neuronal membrane fluidity [22]. Increased plasma membrane fluidity facilitates both endocytosis [42] and exocytosis [42,43]. It is, thus, speculated that DHA serves a dual function: dietary supplementation of DHA decreases membrane-rigidifying molecules like cholesterol and palmitic, stearic, oleic and arachidonic acids in DIFs and, by so doing, assists in the expulsion of Aβ 1–40 from DIFs, probably by facilitating exocytosis.

The exact mechanism of how DHA actually decreases DIF-cholesterol remains to be clarified because whether brain cholesterol can be manipulated is subject of conflicting views. Brain membranes isolated from AD patients show a significant decrease in the content of cholesterol [44,45], while not only cholesterol content [46], but also mRNA levels of HMG-CoA reductase [47], do not change in AD patients compared with those in age-matched human controls. Rather, AD-related changes in the metabolism of membrane cholesterol are suggested to be restricted to defined membrane pools since total membrane cholesterol levels are mostly unchanged in the AD brain [48]. In contrast, we reproducibly observed that the infusion of Aβ peptide (1–40) into rat ventricles significantly increased cholesterol levels in DIFs, although whether that is an effect or a cause remains to be clarified. One of the effects whereby DHA provides health benefits is the hypocholesterolemic action of inhibiting HMG-CoA reductase activity [49]. The molecular mechanism of the direct effect of DHA on the HMG-CoA reductase of Aβ+DHA rats is, however, not clearly understood. Reduced cholesterol levels are associated with inhibited Aβ production and, subsequently, reduced symptoms of AD pathology, as demonstrated in human populations using statins, inhibitors of HMG-CoA reductase [4,50]. Similarly, animal studies on guinea pigs treated with high doses of simvastatin demonstrate decreased Aβ production [51]. The polyunsaturated fatty acids, including DHA, decrease cholesterol levels of plasma and bile canalicular membranes of rats [52], endothelial cells [6] and neuronal cells [22] after administration of DHA, and the higher the extent of the unsaturation of fatty acids, the greater the inhibiting effect on HMG-CoA reductase [53]. Thus, if a similar effect on the brain HMG-CoA reductase is exerted by the dietary administration of DHA, the decrease of cholesterol in the Aβ+DHA and DHA rats is, at least partially, attributable to the inhibitory effect of DHA on HMG-CoA reductase. Consistent with these data, the DHA-administered rats in our study demonstrated the lowest level of cholesterol in DIFs among the four groups.

The DHA-induced increase in the expression of Fos protein, a transcription factor and functional marker of neuronal activity, is associated with improvement in memory-related tasks [20]; also, DHA increases the expression of transthyretin [54], which binds to Aβ peptides and prevents their aggregation [55]. These data suggest that DHA reduces the level of Aβ peptide (1–40) in DIFs by increasing the expression of these proteins. In addition, dietary DHA increases cortical acetylcholine levels with a concomitant increase in memory-related performance [56]. In contrast, Aβ peptide (1–40) infusion degenerates cholinergic neurons and reduces memory-related performance [16]. The level of DHA in the hippocampus is low in AD patients, compared with that in brain samples from age-matched human controls [57], and cognitive impairment increases with aging [58]. The decreased amyloid peptides in the DHA-treated Aβ rats are compatible with the observation that increased cholinergic transmission reduces amyloid deposition [59]. Infusion of amyloid peptide into brain ventricles increases oxidative stress [7,8]. Thus, DHA may, by decreasing detergent-resistant amyloid peptide, provide antioxidative defense for continued smooth cholinergic neurotransmission and easy memory-related performance.

Numerous lipid rafts rich in cholesterol, sphingolipids and raft-specific marker proteins were contained in the total DIFs isolated for use in the present investigation. These rafts function as floating platforms for β- and γ-secretases [60]. The tight packing of the raft lipids endows them with detergent-insoluble properties. Indeed, the procedure for preparing these specialized lipid-enriched domains and their relevance to rafts in vivo is the subject of ongoing debate [61]. In the present study, the lipid-raft domains of the DIFs were not further isolated and purified by density gradient centrifugation. Thus, the present data would reflect the effects of DHA administration on the amyloid peptides associated with the detergent insoluble total membrane fractions wherein all the lipid rafts are believed to be contained. The reduction in amyloid peptide levels in the DHA-administered rats had an effect on the cognitive performance of the AD model rats. The detergent-insoluble amyloid beta fraction is, therefore, speculated to overlap the rafts-associated lipid fraction. This speculation remains to be clarified, however, after isolating the raft lipids by density gradient centrifugation.

In conclusion, the present study indicates that dietary DHA inhibits the accumulation of Aβ peptide (1–40), partially, by decreasing cholesterol levels in the detergent insoluble membrane domains of the cerebral cortex, and that the DHA-induced decrease in the levels of cholesterol and saturated fatty acids is related to the DHA-induced improvement of memory-related performance. Further research is needed, however, for clarifying this mechanism by isolating the lipid rafts associated with other lipid components, particularly glycosphingolipids.

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References

[1] D.J. Selkoe, The cell biology of β amyloid precursor protein and presenilin in Alzheimer's disease, Trends Cell Biol. 8 (1998) 447–453.

[2] M. Simons, P. Keller, J. Dichgans, J.B. Schulz, Cholesterol and Alzheimer's disease: is there a link? Neurology 57 (2001) 1089–1093.

[3] M. Morishima-Kawashima, Y. Ihara, The presence of amyloid β-protein in the detergent-insoluble membrane compartment of human neuroblastoma cells, Biochemistry 37 (1998) 15247–15253.

[4] B. Wolozin, W. Kellman, P. Ruosseau, G.G. Celesia, G. Siegel, Decreased prevalence of Alzheimer disease associated with 3-hydroxy-3-methyglutaryl coenzyme A reductase inhibitors, Arch. Neurol. 57 (2000) 1439–1443.

[5] S. Yehuda, S. Rabinovitz, R.L. Carasso, D.I. Mostofsky, The role of polyunsaturated fatty acids in restoring the aging neuronal membrane, Neurobiol. Aging 23 (2002) 843–853.

[6] M. Hashimoto, S. Hossain, H. Yamasaki, K. Yazawa, S. Masumura, Effects of eicosapentaenoic acid and docosahexaenoic acid on plasma membrane fluidity of aortic endothelial cells, Lipids 34 (1999) 1297–1304.

[7] M. Hashimoto, S. Hossain, T. Shimada, K. Sugioka, H. Yamasaki, Y. Fujii, Y. Ishibashi, J. Oka, O. Shido, Docosahexaenoic acid provides protection from impairment of learning ability in Alzheimer's disease model rats, J. Neurochem. 81 (2002) 1084–1091.

[8] M. Hashimoto, Y. Tanabe, Y. Fujii, T. Kikuta, H. Shibata, O. Shido, Chronic administration of docosahexaenoic acid ameliorates the impairment of spatial cognition learning ability in amyloid β-infused rats, J. Nutr. 135 (2005) 549–555.

[9] S. Glozman, P. Green, E. Yavin, Intraamniotic ethyl docosahexaenoate administration protects fetal rat brain from ischemic stress, J. Neurochem. 70 (1998) 2484–2491.

[10] M.S. Hossain, M. Hashimoto, S. Gamoh, S. Masumura, Antioxidative effects of docosahexaenoic acid in the cerebrum versus cerebellum and brainstem of aged hypercholesterolemic rats, J. Neurochem. 72 (1999) 1133–1138.

[11] F. Calon, G.P. Lim, F. Yang, T. Morihara, B. Teter, O. Ubeda, P. Rostaing, A. Triller, N. Salem Jr., K.H. Ashe, S.A. Frautschy, G.M. Cole, Docosahexaenoic acid protects from dendritic pathology in an Alzheimer's disease mouse model, Neuron 43 (2004) 633–645.

[12] C. Behl, B. Moosmann, Antioxidant neuroprotection in Alzheimer's disease as preventive and therapeutic approach, Free Radical Biol. Med. 33 (2002) 182–191.

[13] M.C. Morris, D.A. Evans, J.L. Bienias, C.C. Tangney, D.A. Bennett, R.S. Wilson, N. Aggarwal, J. Schneider, Consumption of fish and n-3 fatty acids and risk of incident Alzheimer disease, Arch. Neurol. 60 (2003) 940–946.

[14] A. Nitta, T. Fukuta, T. Hasegawa, T. Nabeshima, Continuous infusion of β-amyloid protein into cerebral ventricle induces learning impairment and neuronal and morphological degeneration, Jpn. J. Pharmacol. 73 (1997) 51–57.

[15] G.V. De Ferrari, N.C. Inestrosa, Wnt signaling function in Alzheimer's disease, Brain Res. Brain Res. Rev. 33 (2000) 1–12.

[16] A. Itoh, T. Akaike, M. Sokabe, A. Nitta, R. Iida, A. Olariu, K. Yamada, T. Nabeshima, Impairments of long-term potentiation in hippocampal slices of β-amyloid-infused rats, Eur. J. Pharmacol. 382 (1999) 167–175.

[17] W.C. Paxinos, G., The Rat Brain in Stereotaxic Coordinates, Academic Press, New York, 1986.

[18] S. Gamoh, M. Hashimoto, K. Sugioka, M.S. Hossain, N. Hata, Y. Misawa, S. Masumura, Chronic administration of docosahexaenoic acid improves reference memory-related learning ability in young rats, Neuroscience 93 (1999) 237–241.

[19] S. Gamoh, M. Hashimoto, S. Hossain, S. Masumura, Chronic administration of docosahexaenoic acid improves the performance of radial arm maze task in aged rats, Clin. Exp. Pharmacol. Physiol. 28 (2001) 266–270.

[20] Y. Tanabe, M. Hashimoto, K. Sugioka, M. Maruyama, Y. Fujii, R. Hagiwara, T. Hara, S.M. Hossain, O. Shido, Improvement of spatial cognition with dietary docosahexaenoic acid is associated with an increase in Fos expression in rat CA1 hippocampus, Clin. Exp. Pharmacol. Physiol. 31 (2004) 700–703.

[21] A. Nordberg, E. Hellstrom-Lindahl, M. Lee, M. Johnson, M. Mousavi, R. Hall, E. Perry, I. Bednar, J. Court, Chronic nicotine treatment reduces β-amyloidosis in the brain of a mouse model of Alzheimer's disease (APPsw), J. Neurochem. 81 (2002) 655–658.

[22] H. Shahdat, M. Hashimoto, T. Shimada, O. Shido, Synaptic plasma membrane-bound acetylcholinesterase activity is not affected by docosahexaenoic acid-induced decrease in membrane order, Life Sci. 74 (2004) 3009–3024.

[23] G. Lepage, C.C. Roy, Specific methylation of plasma nonesterified fatty acids in a one-step reaction, J. Lipid Res. 29 (1988) 227–235.

[24] O.H. Lowry, N.J. Rosebrough, A.L. Farr, R.J. Randall, Protein measurement with the Folin phenol reagent, J. Biol. Chem. 193 (1951) 265–275.

[25] A.R. Salomon, K.J. Marcinowski, R.P. Friedland, M.G. Zagorski, Nicotine inhibits amyloid formation by the Aβ peptide, Biochemistry 35 (1996) 13568–13578.

[26] K.A. Melkonian, A.G. Ostermeyer, J.Z. Chen, M.G. Roth, D.A. Brown, Role of lipid modifications in targeting proteins to detergent-resistant membrane rafts. Many raft proteins are acylated, while few are prenylated, J. Biol. Chem. 274 (1999) 3910–3917.

[27] H. Shogomori, A.T. Hammond, A.G. Ostermeyer-Fay, D.J. Barr, G.W. Feigenson, E. London, D.A. Brown, Palmitoylation and intracellular domain interactions both contribute to raft targeting of linker for activation of T cells, J. Biol. Chem. 280 (2005) 18931–18942.

[28] S.M. Mumby, Reversible palmitoylation of signaling proteins, Curr. Opin. Cell Biol. 9 (1997) 148–154.

[29] K. Fassbender, M. Simons, C. Bergmann, M. Stroick, D. Lutjohann, P. Keller, H. Runz, S. Kuhl, T. Bertsch, K. von Bergmann, M. Hennerici, K. Beyreuther, T. Hartmann, Simvastatin strongly reduces levels of Alzheimer's disease β-amyloid peptides Aβ 42 and Aβ 40 in vitro and in vivo, Proc. Natl. Acad. Sci. U. S. A. 98 (2001) 5856–5861.

[30] R. Ehehalt, P. Keller, C. Haass, C. Thiele, K. Simons, Amyloidogenic processing of the Alzheimer β-amyloid precursor protein depends on lipid rafts, J. Cell Biol. 160 (2003) 113–123.

[31] G.P. Lim, F. Calon, T. Morihara, F. Yang, B. Teter, O. Ubeda, N. Salem Jr., S.A. Frautschy, G.M. Cole, A diet enriched with the omega-3 fatty acid docosahexaenoic acid reduces amyloid burden in an aged Alzheimer mouse model, J. Neurosci. 25 (2005) 3032–3040.

[32] M.C. Pitman, F. Suits, Molecular-level organization of saturated and polyunsaturated fatty acids in a phosphatidylcholine bilayer containing cholesterol, Biochemistry 43 (2004) 15318–15328.

[33] D.C. Mitchell, B.J. Litman, Effect of cholesterol on molecular order and dynamics in highly polyunsaturated phospholipid bilayers, Biophys. J. 75 (1998) 808–896.

[34] T.M. Yau, T. Buckman, A.H. Hale, M.J. Weber, Alterations in lipid acyl group composition and membrane structure in cells transformed by Rous sarcoma virus, Biochemistry 15 (1976) 3212–3219.

[35] A.A. Cader, D.A. Butterfield, B.A. Watkins, B.H. Chung, B. Hennig, Electron spin resonance studies of fatty acid-induced alterations in membrane fluidity in cultured endothelial cells, Int. J. Biochem. Cell Biol. 27 (1995) 665–673.

[36] M.S. Hossain, M. Hashimoto, S. Gamoh, S. Masumura, Association of age-related decrease in platelet membrane fluidity with platelet lipid peroxide, Life Sci. 64 (1999) 135–143.

[37] A. Buczynski, B. Wachowicz, K. Kedziora-Kornatowska, W. Tkaczewski, J. Kedziora, Changes in antioxidant enzymes activities, aggregability and malonyldialdehyde concentration in blood platelets from patients with coronary heart disease, Atherosclerosis 100 (1993) 223–228.

[38] C.D. Stubbs, A.D. Smith, The modification of mammalian membrane polyunsaturated fatty acid composition in relation to membrane fluidity and function, Biochim. Biophys. Acta 779 (1984) 89–137.

[39] P.L. Yeagle, Cholesterol and the cell membrane, Biochim. Biophys. Acta 822 (1985) 267–287.

[40] W.E. Muller, S. Koch, A. Eckert, H. Hartmann, K. Scheuer, β-Amyloid peptide decreases membrane fluidity, Brain Res. 674 (1995) 133–136.

[41] C.M. Yip, E.A. Elton, A.A. Darabie, M.R. Morrison, J. McLaurin, Cholesterol, a modulator of membrane-associated Aβ-fibrillogenesis and neurotoxicity, J. Mol. Biol. 311 (2001) 723–734.

[42] M.S.F. Clarke, M.A. Prendergast, A.V. Terry Jr., Plasma membrane

ordering agent pluronic F-68 (PF-68) reduces neurotransmitter uptake and release and produces learning and memory deficits in rat, Learn. Mem. 6 (1999) 634–649.
[43] S. Urano, Y. Asai, S. Makabe, M. Matsuo, N. Izumiyama, K. Ohtsubo, T. Endo, Oxidative injury of synapse and alteration of antioxidative defense systems in rats, and its prevention by vitamin E, Eur. J. Biochem. 245 (1997) 64–70.
[44] R.P. Mason, W.J. Shoemaker, L. Shajenko, T.E. Chambers, L.G. Herbette, Evidence for changes in the Alzheimer's disease brain cortical membrane structure mediated by cholesterol, Neurobiol. Aging 13 (1992) 413–419.
[45] G.S. Roth, J.A. Joseph, R.P. Mason, Membrane alterations as causes of impaired signal transduction in Alzheimer's disease and aging, Trends Neurosci. 18 (1995) 203–206.
[46] G.P. Eckert, N.J. Cairns, A. Maras, W.F. Gattaz, W.E. Muller, Cholesterol modulates the membrane-disordering effects of β-amyloid peptides in the hippocampus: specific changes in Alzheimer's disease, Dement. Geriatr. Cogn. Disord. 11 (2000) 181–186.
[47] K. Yasojima, E.G. McGeer, P.L. McGeer, 3-hydroxy-3-methylglutaryl-coenzyme A reductase mRNA in Alzheimer and control brain, NeuroReport 12 (2001) 2935–2938.
[48] G.P. Eckert, C. Kirsch, S. Leutz, W.G. Wood, W.E. Muller, Cholesterol modulates amyloid β-peptide's membrane interactions, Pharmacopsychiatry 36 (Suppl. 2) (2003) S136–S143.
[49] L. Froyland, H. Vaagenes, D.K. Asiedu, A. Garras, O. Lie, R.K. Berge, Chronic administration of eicosapentaenoic acid and docosahexaenoic acid as ethyl esters reduced plasma cholesterol and changed the fatty acid composition in rat blood and organs, Lipids 31 (1996) 169–178.
[50] H. Jick, G.L. Zornberg, S.S. Jick, S. Seshadri, D.A. Drachman, Statins and the risk of dementia, Lancet 356 (2000) 1627–1631 (Erratum in: Lancet 357 (2001) 562).
[51] M.D. Ledesma, C.G. Dotti, The conflicting role of brain cholesterol in Alzheimer's disease: lessons from the brain plasminogen system, Biochem. Soc. Symp. 72 (2005) 129–138.
[52] M. Hashimoto, M.S. Hossain, T. Shimada, H. Yamasaki, Y. Fujii, O. Shido, Effects of docosahexaenoic acid on annular lipid fluidity of the rat bile canalicular plasma membrane, J. Lipid Res. 135 (2001) 1160–1168.
[53] M. Kuroda, A. Endo, Inhibition of in vitro cholesterol synthesis by fatty acids, Biochim. Biophys. Acta 486 (1976) 70–81.
[54] L.G. Puskas, K. Kitajka, C. Nyakas, G. Barcelo-Coblijn, T. Farkas, Short-term administration of omega 3 fatty acids from fish oil results in increased transthyretin transcription in old rat hippocampus, Proc. Natl. Acad. Sci. U. S. A. 100 (2003) 1580–1585.
[55] A.L. Schwarzman, L. Gregori, M.P. Vitek, S. Lyubski, W.J. Strittmatter, J.J. Enghilde, R. Bhasin, J. Silverman, K.H. Weisgraber, P.K. Coyle, M.G. Zagorski, J. Talafous, M. Eisenberg, A.M. Saunders, A.D. Roses, D. Gordgaber, Transthyretin sequesters amyloid βprotein and prevents amyloid formation, Proc. Natl. Acad. Sci. U. S. A. 91 (1994) 8368–8372.
[56] M. Minami, S. Kimura, T. Endo, N. Hamaue, M. Hirafuji, H. Togashi, M. Matsumoto, M. Yoshioka, H. Saito, S. Watanabe, T. Kobayashi, H. Okuyama, Dietary docosahexaenoic acid increases cerebral acetylcholine levels and improves passive avoidance performance in stroke-prone spontaneously hypertensive rats, Pharmacol. Biochem. Behav. 58 (1997) 1123–1129.
[57] M. Söderberg, C. Edlund, K. Kristensson, G. Dallner, Fatty acid composition of brain phospholipids in aging and in Alzheimer's disease, Lipids 26 (1991) 421–425.
[58] J.A. Conquer, M.C. Tierney, J. Zecevic, W.J. Bettger, R.H. Fisher, Fatty acid analysis of blood plasma of patients with Alzheimer's disease, other types of dementia, and cognitive impairment, Lipids 35 (2000) 1305–1312.
[59] T.G. Beach, Y.M. Kuo, C. Schwab, D.G. Walker, A.E. Roher, Reduction of cortical amyloid β levels in guinea pig brain after systemic administration of physostigmine, Neurosci. Lett. 310 (2001) 21–24.
[60] D.A. Brown, E. London, Structure and function of sphingolipid- and cholesterol-rich membrane rafts, J. Biol. Chem. 275 (2000) 17221–17224.
[61] E.C. Lai, Lipid rafts make for slippery platforms, J. Cell Biol. 162 (2003) 365–370.

Long-Term Administration of Green Tea Catechins Improves Spatial Cognition Learning Ability in Rats[1]

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ABSTRACT Green tea catechins confer potent biological properties including antioxidation and free-radical scavenging. We investigated the effect of long-term oral administration of green tea catechins (Polyphenon^R E, PE: EGCG 63%; EC 11%; EGC 6%; ECG 6%) mixed with water on the spatial cognition learning ability of young rats. The learning ability of rats administered PE (0%, 0.1%, 0.5%) for 26 wk was assessed in the partially baited 8-arm radial maze. Relative to controls, those administered PE had improved reference and working memory–related learning ability. They also had lower plasma concentrations of lipid peroxides and greater plasma ferric-reducing antioxidation power than controls. Furthermore, rats administered PE had lower hippocampus reactive oxygen species concentrations than controls. We suggest that this improvement in spatial cognitive learning ability is due to the antioxidative activity of green tea catechins. J. Nutr. 136: 1–5, 2006.

KEY WORDS: • *green tea catechins* • *memory learning* • *antioxidants* • *rats*

The free-radical hypothesis suggests that increased production of lipid peroxide (LPO)[3] and reactive oxygen species (ROS), which are produced with free radicals in membrane lipids, causes deterioration of a wide variety of cellular enzymes, subsequently exacerbating the neurodegenerative process (1). Oxidative stress, a condition of cellular prooxidant-antioxidant disturbance in favor of the prooxidant state, also induces the production of ROS, leading to serious functional impairments such as cognitive decline (2). On the other hand, a decrease in hippocampal LPO improves spatial cognition learning memory in aged rats (3), and an increase in antioxidative activity in the hippocampus prevents (4) or ameliorates (5) the impairment of learning ability in rats produced by the infusion of amyloid-β peptide 1–40 into the cerebral ventricle.

Tea is rich in polyphenols contained in the leaves and stems of the tea plant. The main polyphenolic components in green tea are (-)-epigallocatechin gallate (EGCG), (-)-epicatechin (EC), (-)-epigallocatechin (EGC), and (-)-epicatechin gallate (ECG) (6). EGCG, the major and most active component of green tea catechins, acts as an antioxidant in the biological system (7) and is rapidly absorbed and distributed mainly into the mucous membranes of the small intestine and the liver; more interestingly, it can cross the blood brain barrier (8). Moreover, oxidative stress–induced neuronal apoptosis is prevented by EGCG treatment of neuronal cells (7). Therefore, in the present study, we investigated, through radial maze tasks, how long-term (26 wk) administration of water containing green tea catechins affected spatial cognition learning ability in rats and the oxidative status of their plasma and brain.

MATERIALS AND METHODS

Animals. All animal experiment protocols were carried out in accordance with the guidelines for animal experimentation of Shimane University compiled from the guidelines for animal experimentation of the Japanese Association for Laboratory Animal Science. Male Wistar rats (n = 24; 5 wk old; *Jcl*: Wistar; Clea Japan) were randomly divided into 3 groups and orally administered green tea catechins (Polyphenon E, PE: Mitsui Norin) mixed with water, or water alone for 26 wk as follows: a 0.1% group (administered 1 g/L PE; n = 7), a 0.5% group (5 g/L PE; n = 9) and a control group (given water alone; n = 8). The rats were maintained in an air-conditioned animal room with a 12-h dark:light cycle under controlled temperature (23 ± 2°C) and humidity (50 ± 10% relative humidity); the rats had free access to a normal laboratory diet, MF (Oriental Yeast) and tap water with or without PE. The MF diet, a nutritionally adequate and standard solid diet, comprising (in descending order of amount) flour, corn, soybean meal, whitefish meal, yeast, alfalfa meal and soybean oil, included the following (g/kg): 70 water, 240 crude protein, 51 crude fat, 62 crude ash, 32 crude fiber, and 545 nitrogen free extract (>90% starch).

Water containing PE as EGCG (63%), EC (11%), EGC (6%), and ECG (6%) was freshly prepared every other day.

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[3] Abbreviations used: 0.5% PE rats, rats administered 5 g/L PE; 0.1% PE rats, rats administered 1 g/L PE; BW, body weight; EC, (-)-epicatechin; ECG, (-)-epicatechin; EGC, (-)-epigallocatechin; EGCG, (-)-epigallocatechin gallate; FRAP, ferric reducing antioxidation power; LPO, lipid peroxide; PE, Polyphenon^R E; RME: reference memory error; ROS, reactive oxygen species; TBARS, thiobarbituric acid reactive substance; WME, working memory error.

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Radial maze–learning ability. Both 2 and 5 mo after starting the PE administration, the rats' learning ability was tested by an assessment of their behavior in an 8-arm radial maze (Toyo Sangyo) as described (9). Briefly, the rats were trained to acquire a reward (food pellet) at the end of each of the 4 arms of an 8-arm radial maze. The performance involved 2 parameters of memory function: reference memory error (RME), i.e., entry into unbaited arms; and working memory error (WME), i.e., repeated entry into arms that had already been visited in the same trial. Each rat was given 2 trials, 6 d/wk, for a total of 5 wk.

Tissue preparation. After completing the maze task, the rats were anesthetized with sodium pentobarbital (50 mg/kg BW, i.p.), and their blood was collected; the cerebral cortex and hippocampus were then separated as described (4,5). A portion of the frontal cortex (100 mg) was immediately homogenized on ice in 1.0 mL of ice-cold 0.32 mol/L sucrose buffer (pH 7.4) containing 2 mmol/L EDTA, 0.5 mg/L leupeptin, 0.5 mg/L pepstatin, 0.5 mg/L aprotinin, and 0.2 mmol/L phenylmethylsulfonyl fluoride using a Polytron homogenizer (PCU 2–110; Kinematica). The residual tissues were stored at −80°C after flash-freezing in liquid N_2 until use. The homogenates were immediately subjected to the assays described below or stored at −80°C after liquid N_2 flash and bath until use.

Measurements of antioxidative status. The LPO concentration was assessed by the TBARS assay of Ohkawa et al. (10), as described (4,5), with the concentration measured in nanomoles malondialdehyde/mg protein. Malondialdehyde levels were calculated relative to a standard preparation of 1,1,3,3-tetraethoxypropane.

Plasma total antioxidant activity was measured by the ferric reducing antioxidation power (FRAP) assay of Benzic and Strain (11) with slight modification. The working FRAP reagent was prepared by mixing 300 mmol/L acetate buffer (pH 3.6), 10 mmol/L 2,4,6-tripyridyl-*s*-triazine (TP) in 40 mmol/L HCl and 20 mmol/L $FeCl_3 \cdot 6H_2O$ solution. After mixing 3 mL of the working FRAP reagent with 400 μL plasma or standard solution in a test tube, a second reading was taken at 600 nm. A blank reading with only the FRAP reagent was subtracted from the absorbance of the FRAP reagent with a sample to measure the actual FRAP value of each tube.

The levels of ROS were determined as described (4,5). Briefly, 50 μL of freshly prepared tissue homogenate was mixed with 4.85 mL of 0.1 mol/L potassium phosphate buffer (pH 7.4) and incubated with 2′,7′-dichlorofluorescein diacetate (Molecular Probes) in methanol at a final concentration of 5 μmol/L for 15 min at 37°C. The dye-loaded samples were centrifuged at 12,500 × *g* for 10 min at 4°C. The pellet was mixed on a vortex at 0°C in 5 mL of 0.1 mol/L potassium phosphate buffer (pH 7.4) and incubated for 60 min at 37°C. Fluorescence was measured with a Hitachi 850 spectrofluorometer at wavelengths of 488 nm for excitation and 525 nm for emission. The cuvette holder was maintained at 37°C. ROS were quantified from a dichlorofluorescein standard curve in methanol. The protein concentration was estimated by the method of Lowry et al. (12).

Statistical analysis. Results are expressed as means ± SE. Behavioral data were analyzed by a 2-factor (group and block) randomized block factorial ANOVA; all other variables were analyzed for intergroup differences by 1-way ANOVA. ANOVA was followed by Fisher's Protected Least Significant Difference test for post hoc comparisons. Correlation was determined by simple regression analysis. The statistical programs used were GB-STAT 6.5.4 (Dynamic Microsystems) and StatView 4.01 (MindVision Software, Abacus Concepts). A level of $P < 0.05$ was considered significant.

RESULTS

PE intake and body weight. Daily water intake did not differ among the control [27.7 ± 1.7 mL/(rat·d)], 0.1% PE [26.0 ± 1.4 mL/(rat·d)], and 0.5% PE [26.2 ± 1.0 mL/(rat·d)] groups. PE intakes were 26.0 ± 1.4 mg/(rat·d) in the 0.1% PE group and 131 ± 7.0 in the 0.5% PE group). Final body weights did not differ among the groups and were 496 ± 8 g in the control group, 503 ± 10 g in the 0.1% PE group, and 508 ± 11 g in the 0.5% PE group.

Radial-maze learning ability. After 2 mo of PE administration, the scores of RME and WME in block 10 of the radial maze tasks undergone by the 0.5% PE rats were not lower than those of the control and the 0.1% PE rats. Therefore, we reestimated the learning ability (over a period of 6 wk) 20 wk after starting the administration of PE.

The effect of PE administration for 26 wk on reference (Fig. 1*A*) and working (Fig. 1*B*) memory-related learning ability is expressed as the mean number of RME and WME for each group, with the data averaged over blocks of 6 trials (Fig. 1). Randomized 2-factor (block and group) ANOVA, for analyzing the effect of PE (0.1 and 0.5%), revealed significant main effects of both blocks of trials ($P < 0.0001$) and groups ($P < 0.0001$) on the number of RME (Fig. 1*A*), but without a significant block × group interaction. Similarly, a significant main effect of both blocks of trials ($P < 0.0001$) and groups ($P = 0.0002$) was observed on the number of WME (Fig. 1*B*), but with a significant block × group interaction ($P < 0.0001$). Subtest analysis (**Table 1**) of the number of RME showed the effect of 0.1% PE group on control group (blocks of trials and groups, without a significant block × group interaction); the effect of 0.5% PE group on control group (blocks of trials and groups, without a significant block × group interaction); and the effect of the PE dose on PE-administered rats (blocks of trials and groups, without a significant block × group interaction), demonstrating that rats administered 0.1 and 0.5% PE had a lower RME score than the control rats (Fig.1*A*). Similarly, subtest analysis (Table 1) of the number of WME showed the effect of 0.1% PE group on control group (blocks of trials and groups, with a significant block × group interaction); the effect of 0.5% PE group on control group (blocks of trials and groups, with a significant block × group interaction); and the effect of the PE dose on PE-administered rats (blocks of trials, but not groups), without a significant block × group interaction, demonstrating that rats administered 0.1% and 0.5% PE had a lower WME score than the control rats (Fig. 1*B*). These analyses suggested that long-term administration of PE improved reference and working memory–related learning ability of rats.

Oxidative status of rat plasma and brains. Plasma TBARS concentrations were dose dependently decreased in the groups administered PE compared with the control group ($P = 0.0002$, Table 2). The plasma FRAP concentration was higher in the 0.5% PE group than in the control group ($P = 0.007$) (Table 2).

TBARS levels in the hippocampus were reduced in the 0.1 and 0.5% groups, compared with the control group ($P = 0.002$)

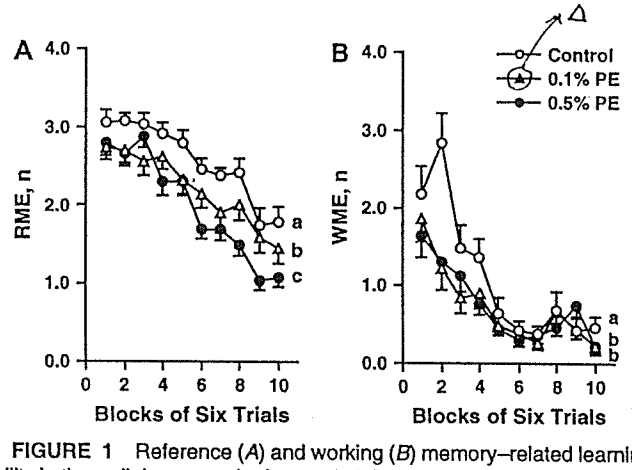

FIGURE 1 Reference (*A*) and working (*B*) memory–related learning ability in the radial maze task of rats administered 0 (control, $n = 8$), 0.1% PE ($n = 7$), or 0.5% PE ($n = 9$) for 26 wk. Values are means ± SEM in each block of 6 trials. Groups without a common letter differ, $P < 0.05$.

TABLE 1

Results of the 2-factor ANOVA and PLSD test conducted on RME and WME data obtained in rats administered 0 (control, n = 8), 0.1% PE (n = 7), or 0.5% PE (n = 9) for 26 wk[1]

	Reference memory error			Working memory error		
Group	Block	Group	Block × Group interaction	Block	Group	Block × Group interaction
0 vs. 0.1% PE	< 0.0001 ($F_{9, 423}$ = 17.55)	0.0003 ($F_{1, 47}$ = 15.18)	NS ($F_{9, 423}$ = 0.99)	< 0.0001 ($F_{9, 423}$ = 23.31)	0.0036 ($F_{1, 47}$ = 9.38)	0.003 ($F_{9, 423}$ = 2.83)
0 vs. 0.5% PE	< 0.0001 ($F_{9, 477}$ = 34.69)	< 0.0001 ($F_{1, 53}$ = 42.91)	NS ($F_{9, 477}$ = 0.15)	< 0.0001 ($F_{9, 477}$ = 24.58)	0.0005 ($F_{1, 53}$ = 13.56)	0.0002 ($F_{9, 477}$ = 3.61)
0.1% PE vs. 0.5% PE	< 0.0001 ($F_{9, 477}$ = 35.05)	0.0254 ($F_{1, 53}$ = 5.29)	NS ($F_{9, 477}$ = 1.86)	< 0.0001 ($F_{9, 477}$ = 25.83)	NS ($F_{1, 53}$ = 17.58)	NS ($F_{9, 477}$ = 0.76)

[1] Data are presented in Figure 1. NS, not significant, $P > 0.05$.

(Table 3). Similarly, the levels of ROS in the hippocampus were reduced in the 0.1 and 0.5% groups, compared with the control group ($P = 0.021$). Cortex TBARS and ROS levels did not differ among the rat groups. These results indicate that PE has antioxidative effects on oxidative status in rat plasma and the hippocampus.

Regression analysis revealed a significant positive correlation ($r = 0.520$, $P = 0.032$) between the hippocampal TBARS levels and the number of RME in block 10 of the radial maze task in control and 0.5% PE-administered rats (Table 4). There was a significant negative correlation between the plasma FRAP levels and the number of RME in block 10 of the radial maze task in control rats and those administered 0.5% PE ($r = -0.570$, $P = 0.017$) (Table 4). Similarly, the number of WME in block 10 of the radial maze task in control rats and those administered 0.5% PE correlated positively with plasma TBARS levels ($r = 0.622$, $P = 0.008$). The hippocampal TBARS levels and the number of WME tended to be positively correlated ($r = 0.480$, $P = 0.051$; Table 4).

DISCUSSION

The present study demonstrated that long-term administration of green tea catechins (PE) improves the performance in radial maze tasks and that the level of LPO in the hippocampus correlates significantly with the RME score. Thus, green tea catechins may be involved in protecting against neuronal degenerative stress and in the accumulation of LPO and ROS.

Green tea catechins comprise EGCG, EGC, ECG, and EC and protect the brain, liver, and kidney from lipid peroxidation injury (13). The relative antioxidant activity among tea catechins is EGCG = ECG > EGC > EC (14) Catechins have a protective effect against age-related neurological diseases associated with ROS (15). In this study, long-term (26 wk) administration of PE decreased the plasma and hippocampal oxidative status. In the process of aging, LPO and ROS accumulate and are constantly involved in some of the pathophysiologic effects associated with oxidative stress in cells and tissues. An increase in the production of LPO exacerbates the neurodegenerative process by deteriorating cellular enzymes (1). Antioxidative enzymes are activated by green tea catechin intake (16), and the antioxidative potency of human plasma increases with continual ingestion of green tea (17). These antioxidative defense systems might also prevent oxidative damage in the brain. Long-term intake of green tea catechins may be important because cells are constantly exposed to oxidative stress.

Aging leads to a decline in spatial memory–related learning ability. Oxidative damage to the brain is associated with age-related cognitive dysfunction (18), and some antioxidants are effective in improving such dysfunction; examples include the effects of a garlic extract on aged SAMP10 mice, a model of brain senescence with cerebral atrophy and cognitive dysfunction (19), and of vitamin E on rats with oxidative stress (20). Catechins are more effective radical scavengers than vitamins E and C (21,22). Long-term administration of green tea catechins to SAMP10 mice also suppressed cognitive dysfunction, as demonstrated by the duration of learning needed to acquire an avoidance response and by the assessment of working memory in the Y-maze (23). Chronic administration of catechins for 3.5 mo improved learning memory in maze behavior of both adult and old mice, although the mechanism of the improvement has not been clarified (24). In this study, to estimate the effects of the administration of green tea catechins on the learning ability of rats, PE was administered for 26 wk starting at 5 wk of age. Therefore, the point in time at which the effect of green tea catechins on the improvement of learning ability becomes apparent may differ among animal species.

The hippocampus and the cerebral cortex are the key structures of memory formation. Because the hippocampus is especially indispensable in the integration of spatial information, a decline in learning ability may be induced by the deterioration of hippocampal function. In this study, both a decrease in TBARS levels in the hippocampus and an increase in FRAP levels in the plasma were related to the acquisition of higher reference memory-related learning ability; in addition, a decrease in plasma TBARS levels was related to the acquisition of higher working memory-related learning ability (Table 4). A decrease in hippocampal LPO levels suggests an improvement in spatial cognitive learning memory in aged rats (3). Furthermore, an increase in the antioxidative effects of docosahexaenoic acid on the hippocampus prevents impairment of

TABLE 2

Plasma oxidative status of rats administered 0, 0.1% PE, or 0.5% PE for 26 wk[1]

		TBARS	FRAP
	n	*μmol/L*	
0%	8	4.02 ± 0.18[a]	223.7 ± 10.8[b]
0.1% PE	7	3.51 ± 0.12[b]	257.2 ± 17.7[ab]
0.5% PE	9	3.00 ± 0.12[c]	270.9 ± 11.4[a]

[1] Values are means ± SEM. Means in a column without a common letter differ, $P < 0.05$.