

恒常性機能解析分野 Division of Analysis of Homeostasis

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研究目的 Aims of the research projects

植物が作り、動物の機能維持に必須な脂肪酸には二種類ある。リノール酸 (n-6) は成長や生殖生理の維持に必須であるが、体内でアラキドン酸に変換されエイコサノイドと呼ばれる種々の生理活性物質 (炎症メディエーター) の前駆体となっている (リノール酸カスケード)。 α -リノレン酸 (n-3) はエイコサペンタエン酸 (EPA) やドコサヘキサエン酸 (DHA) に変換され、EPA はエイコサノイド合成の前駆体として働き、DHA は脳・神経機能の維持に必須の役割をしている。n-6 系と n-3 系は多くの酵素、受容体の段階で競合的であり、そのバランスが身体の恒常性維持 (健康と病気) に重要な因子となっている。

本研究は、摂取食品によって変わるリノール酸 (n-6) 系と α -リノレン酸 (n-3) 系のバランスが、過去半世紀に急増しているアレルギー過敏症、米国型癌、心疾患などに及ぼす影響を、長期投与の結果の解析によって評価することを目的としている (薬食一如)

研究概要 Research projects

I) アレルギー過敏症の体質改善—基礎と臨床

リノール酸カスケードを介して作られる n-6 系メディエーターは、異物 (アレルゲン) の体内への侵入を防ぐ役割を果たしている。しかし、リノール酸の摂取過剰によりアラキドン酸が増えるとメディエーターが過剰に作られ、その防御反応が過剰におこって病的なアレルギー症となる。一方、 α -リノレン酸系の EPA から類似のメディエーターが作られるが、一般に作られる量は少なく活性も弱い。そして、食品を選び、n-6/n-3 の比を低くすると、アレルゲン刺激で作られる炎症メディエーターの産生量と活性を低くすることができる (体質の改善)。動物実験の結果に基づき、「リノール酸を減らし、 α -リノレン酸系を増やす食事療法」が、アトピー性皮膚炎の治療に有効であることを証明しつつある (共同研究)。多くの抗アレルギー薬はリノール酸カスケードを抑えて効果を発揮するので、リノール酸の多い食品の摂取を減らすという服薬指導と同時に、食物油脂の選択による体質改善が勧められる。

II) n-6/n-3 比を下げることによる米国型癌の予防—基礎と臨床

米国で先行しわが国で増えている癌 (肺腺癌、大腸癌、乳癌など) に対し、リノール酸カスケードを抑える n-6/n-3 比の低い油脂、抗炎症薬、遺伝子操作が抑制的にはたらく (動物実験)。リノール酸カスケードを介して作られる過剰のプロスタグランジン、ロイコトリエンなどが炎症を持続させ、炎症細胞からの過剰の活性酸素 (ROS) が遺伝子傷害をひきおこし、細胞増殖的にはたらき、発癌を促進する。また、これらメディエーターは各種転写因子を介して細胞増殖的にはたらくと理解できる。この理解に基づき、n-6/n-3 比を下げることによる大腸腫瘍再発予防の介入試験を継続中である (共同研究)。

III) 紫蘇油 (荳胡麻油) の開発と食用油の安全性評価

蘇子 (紫蘇種子) は漢方では上品に分類され、安全性が高く、長期的に効果を示すとされている。近年工業的に使われていた紫蘇種子の油 (紫蘇油) の安全性と有効性を、長期投与の結果を評価することにより示して、食用に再開された。紫蘇油は癌の化学予防に最も有望であると評価され、脳卒中発症予防効果もある。この過程で、菜種油、オリーブ油などが脳卒中ラットの寿命を異常に短縮することが明らかとなり、その内分泌攪乱作用の本体の探索を続けている。

◇原著 Original papers

- 1) Du. C., Fujii Y., Ito M., Harada M., Moriyama E., Shimada R., Ikemoto A. and Okuyama H.: **Dietary polyunsaturated fatty acids suppress acute hepatitis, alter gene expression and prolong survival of Long-Evans Cinnamon rats, a model of Wilson Disease. J. Nutr. Biochem., 15(5) , 273-280, 2004.**

Abstract: In the Long-Evans Cinnamon rat, copper accumulates in the liver because of a mutation in the copper-transporting ATPase gene, and peroxidative stresses are supposed to be augmented. We examined the effects of dietary fatty acids on hepatitis, hepatic gene expression, and survival. Rats were fed a conventional, low-fat diet (CE2), a CE2 diet supplemented with 10 wt% of lard (Lar), high-linoleic soybean oil (Soy), or a mixture of docosahexaenoic acid (DHA)-rich fish oil and soybean oil (DHA/Soy). Among female rats, the mean survival times of the DHA/Soy and the Soy groups were longer by 17~20% than in the Lar and the CE2 groups. Among male rats, the survival times were much longer than in the females, but no significant difference in survival was observed among the dietary groups. Serum ceruloplasmin levels in female and male rats of all of the dietary groups were similar. Serum transaminase levels of the DHA/Soy group tended to be lower than in the CE2 group. Histological examinations revealed a marked degeneration in hepatic tissue integrity in the Lar and CE2 groups but not in the DHA/Soy group. Hepatic levels of metal-related genes, transferrin and ceruloplasmin, as well as those related to bile acid synthesis were up-regulated, and an inflammation-related gene (cyclooxygenase [COX]-2) was down-regulated in the DHA/Soy group. Some proliferation-related genes were also affected by the dietary fatty acids. These results indicate that polyunsaturated fatty acids suppress the development of acute hepatitis and prolong survival in females, regardless of whether they are of the n-6 or n-3 type, which are associated with altered gene expressions.

- 2) Tokudome S., Ichikawa Y., Okuyama H., Tokudome Y., Goto C., Imaeda N., Kuriki K., Suzuki S., Shibata K., Jiang J., Wang J. and Takeda E. : **The Mediterranean vs the Japanese diet. Eur. J. Clin. Nutr., 58(9), 1323, 2004.**

Abstract: Both the Mediterranean and Japanese diets are known to be healthy (Tokudome et al, 2000; Trichopoulou & Vasilopoulou, 2000; Ferro-Luzzi et al, 2002; Serra-Majem et al, 2003). People of the Mediterranean countries enjoy a low risk of cardiovascular disease, while Japanese are famous for their longevity/health life expectancy (UN, 1998). However, there are both similarities and discrepancies in intake of foods and beverages between the two cases. The Mediterranean diet is characterized by high consumption of cereals (wheat), vegetables and fruit, fish and olive oil (Trichopoulou & Vasilopoulou, 2000; Ferro-Luzzi et al, 2002; Serra-Majem et al, 2003). Japanese also consume large amounts of cereals (rice), vegetables and fruit, and fish, but there is much lower intake of energy and oils/fats (Tokudome et al, 2000; Health Promotion and Nutrition Division, 2003).

In a recent issue of EJC, Dr Serra-Majem et al (2003) reported an interesting ecological finding that typical Mediterranean individuals consume high amounts of total lipids (approximately 100g/day in males and 80 g in females) and also polyunsaturated fatty acids (PUFAs) in males, and lipids (more than 40% energy) and PUFAs in both genders along with high concentrations and proportions of mono-unsaturated fatty acids (MUFAs), largely from olive oil.

In contrast, the traditional Japanese diet has been characterized by low intake of total lipids, including saturated fatty acids, MUFAs and PUFAs, particularly of n-6 PUFAs, not only absolute concentrations as well as proportions (Okuyama et al, 1997; Tokudome et al, 2000). However, the recent past has seen a change from 20% energy from lipids to 30%, whereas the ratio of n-6 PUFAs/n-3 PUFAs has shifted from 2-3 to 4-5. We assume that these changes will enhance the risk of fat-related cancers, cardiovascular disease and cerebrovascular embolisms.

Therefore, we wonder if Dr Serra-Majem et al could provide information that the risk of cardiovascular disease is explained with reference to concentrations and-or percentage energy from total lipids, n-6 PUFAs and n-3 PUFAs together with its ratio. Furthermore, comments on whether the risk is modulated when the intake of vegetables and

fruit is adjusted would be welcomed because they contain antioxidant nutrients, including α -tocopherol, carotenoids, vitamin C and folic acid.

There is evidence that not only absolute concentrations of total lipids but also the balance of fatty acids of n-6 PUFAs/n-3 PUFAs, in particular, are crucial to our health (Lands, 1995; Okuyama et al, 1997; Rose & Connolly, 1999). We propose that, even if olive oil comprises antioxidant nutrients, intake at high levels may be unhealthy. According to values for macronutrients set for the Japanese diet (Health Promotion and Nutrition Division, 2003), intake of 20-25% energy from lipids on average, with more than 50% from carbohydrates and 15-20% from proteins may be recommended for adults.

3) Tatematsu K., Hirose N., Ichikawa Y., Fujii Y., Takami A. and Okuyama H. : Nutritional evaluation of an inter-esterified perilla oil and lard in comparison with butter and margarine based on the survival of stroke-prone spontaneously hypertensive (SHRSP) rats. *J. Health Sci.*, 50(1), 108-111, 2004.

Abstract: Some kinds of vegetable oil and a partially-hydrogenated oil shorten the survival of the stroke-prone spontaneously hypertensive (SHRSP) rats compared with perilla seed oil, soybean oil and lard. The n-3/n-6 ratio of constituent fatty acids, phytosterol content and other factors in these oils have been proposed to affect the survival of this strain. Here, we examined the safety of a fat produced by the inter-esterification of perilla oil and lard (Perilla-Lard) on the bases of the survival of SHRSP rats. The mean survival time decreased in the order of the butter, the Perilla-Lard, the lard, the margarine and the partially-hydrogenated soybean oil (Hyd.Soy) group. The correlations between survival time and cholesterol content or phytosterol content in the diet were analyzed, and the probable health benefits of the new margarine-type fats made of animal fats and oils with high n-3/n-6 ratios were discussed.

4) Tatematsu K., Fuma S., Satoh J., Ichikawa Y., Fujii Y. and Okuyama H. : Dietary canola oil and soybean oil fed to SHRSP rat dams differently affect the growth and survival of their male pups. *J. Nutr.*, 134, 1347-1352, 2004.

Abstract: Canola oil (Can), as well as some other oils, shortens the survival of SHRSP rats compared with soybean oil (Soy). Although detrimental factors other than phytosterols have not been identified, they are likely to be hydrophobic and transmissible to pups. To test this possibility, female SHRSP rats (F0) were fed a diet supplemented with Can or Soy and mated at 11 wk of age. The growth of suckling pups (F1) from the Can-fed dams was significantly retarded compared with that of pups from the Soy-fed dams. Half of the male pups (F1) were weaned to the same diet as their dams (Can-->Can and Soy-->Soy groups) and the rest were weaned to the other diet (Can-->Soy and Soy-->Can groups). The survival rate of the male pups (F1) was significantly lower in the Can-->Can group than in the Soy-->Can group, and in the Can-->Soy group than in the Soy-->Soy group, indicating that the oils fed to dams differently affected the growth and survival of pups. There were fewer pups per dam in the Can-fed dams (F0) than in the Soy-fed dams, and in the dams (F1) of the Can-->Can and Soy-->Can groups than in those of the Can-->Soy and Soy-->Soy groups. Although Can is nutritionally detrimental to SHRSP rats compared with Soy, no direct evidence has been obtained thus far relating these observations to human nutrition.

5) Tatematsu K., Fuma S., Nagase T., Ichikawa Y., Fujii Y. and Okuyama H.: Factors other than phytosterols in some vegetable oils affect the survival of SHRSP rats. *Food Chem. Toxicol.*, 42, 1443-1451, 2004.

Abstract: Unusual survival-shortening activities of some vegetable oils were detected in stroke-prone spontaneously hypertensive (SHRSP) rats, and phytosterol (PS) in the oils and the tissue tocopherol status have been suggested to be the factors for the activities. Here, we re-evaluated the contribution of PS to the survival-shortening, and examined the hepatic tocopherol status. A basal diet for rodents and a test oil were mixed at a 9:1 ratio, and the diet was

given to male SHRSP rats upon weaning. The total and major PS contents of the diets and tissue lipids did not correlate with relative survival time. The free fatty acid fractions obtained by lipase and alkaline hydrolyses of canola oil (Can) and the original Can contained PS in comparable amounts but the free fatty acid fractions did not exhibit survival-shortening activities compared with the soybean oil (Soy) group. The activity was not detected in the ethyl acetate extracts of the aqueous phase after the hydrolysis. When a commercially available PS preparation was added to the Soy diet at an amount 2.8-fold higher than that in the Can diet, the mean survival time was shortened but was still significantly longer than that of the Can group. The hepatic tocopherol level was significantly higher in the Can group than in the hydrogenated Soy group and Soy group, but the former two groups exhibited a survival-shortening activity. These results indicate that factors other than PS, tocopherol status and fatty acid composition in some vegetable oils are critical for the survival-shortening activity observed in SHRSP rats.

- 6) **Fujii Y., Murase Y., Otake K., Yokota Y., Omoto S., Hayashi H., Okada H., Okada N., Kawai M. Okuyama H., and Imakawa K.: A potential live vector, foamy virus, directed intra-cellular expression of ovine interferon- τ exhibited the resistance to HIV infection. *J. Vet. Med. Sci.*, **66(2)**, 115-121, 2004.**

Abstract: Interferon-tau (IFN-tau), produced by the embryonic trophoblast, is a member of type I IFNs required for the establishment of pregnancy in the ruminant ungulates. Although this IFN possesses antiviral activity similar to other type I IFNs, the effectiveness of IFN-tau as an antiviral agent has not been well characterized. To investigate possible antiviral effects of ovine IFN-tau (oIFN-tau), oIFN-tau-GST fusion protein was expressed in *E. coli* BL21, from which the purified protein isolated possessed anti-viral activity. An apathogenic human foamy virus (hFV) was then used to establish a potential recombinant live vector consisting of oIFN-tau cDNA sense (+) or antisense (-) sequence, oIFN-tau(+)/hFV or oIFN-tau(-)/hFV, respectively. Human hematopoietic and other mammalian cell lines that had been transduced with hFV vector consisting of no oIFN-tau, oIFN-tau(+)/hFV or oIFN-tau(-)/hFV construct were cultured initially for 12 days, and three of cell lines were then maintained for up to 90 days. These cells with oIFN-tau expression directed by hFV exhibited the in vitro cytopathic effect minimally. Transduced cell lines that had been cultured for 90 days were subjected to studies on human immunodeficiency virus type-1 (HIV-1) infection, which was measured with infectivity of viral particles resulted from the GFP inserted T-cell tropic HIV SF2 or macrophage tropic HIV SF162: the number of HIV-1 positive cells was reduced by the hFV driven-intra-cellular oIFN-tau expression. Since oIFN-tau/hFV transduced cells exhibited the resistance to HIV-1 infection and/or replication, oIFN-tau could be considered as one of effective antiviral agents against HIV-1. These results suggest that the hFV genome could be an effective recombinant live vector for the expression of a targeted gene in various cell types.

- 7) **Otake K., Omoto S., Yamamoto T., Okuyama H., Okada H., Okada N., Kawai M., Saksena N.K., and Fujii, .R.: HIV-1 Nef protein in the nucleus influences adipogenesis as well as viral transcription through the peroxisome proliferator-activated receptors. *AIDS*, **18**, 189-198, 2004.**

Abstract: BACKGROUND : Although the HIV-1 Nef protein (27 kDa) localizes primarily in cytoplasm, there is considerable evidence suggesting its occasional localization in the nucleus. Nef is known to play an important role in transcriptional events and viral replication, but the actual target of Nef in the nucleus remains to be identified. OBJECTIVE: To examine the functional roles of Nef in the nucleus and its possible interactions with other unknown factors in the nucleus. METHODS: High-density microarray analysis was used to screen directly the unique functions of Nef on host gene transcription. The nuclear localization of Nef and its effects on the expression of peroxisome proliferator-activated receptors (PPAR) was examined using PPAR promoter/reporter assay and immunoblotting. A long terminal repeat/reporter assay was used to investigate the effects of Nef and PPAR on viral

transcription. RESULTS: Nef in the nucleus suppressed PPAR gamma expression and reduced fatty acid levels in human T and macrophage cell lines. Expression of Nef or PPAR suppressed viral replication; the effect of PPAR gamma or retinoid X receptor-alpha on viral replication were reduced by coexpression of Nef in MT(-)4 T cells. CONCLUSION: Nef may be involved in both viral replication and the wasting syndrome associated with AIDS.

8) Omoto S., Brisibe E.A., Okuyama H., and Fujii Y.R. : Feline foamy virus tas protein is a DNA-binding transactivator. *J. Gen. Virol.*, **85**, 2931-2935, 2004.

Abstract: Foamy viruses (FVs) harbour a transcriptional transactivator (Tas) and two Tas-responsive promoter regions, one in the 5' long terminal repeat (LTR) and the other an internal promoter (IP) in the envelope gene. To analyse the mechanism of transactivation of the FVs, the specificity of feline FV (FFV) Tas protein, which is more distantly related to the respective proteins of non-human primate origin, were investigated. FFV Tas has been shown specifically to activate gene expression from the cognate promoters. No cross-transactivation was noted of the prototype foamy virus and human immunodeficiency virus type 1 LTR. The putative transactivation response element of FFV Tas was mapped to the 5' LTR U3 region (approximately nt -228 to -195). FFV Tas binds to this element in addition to a previously described sequence (position -66 to -51). It is therefore concluded that FFV Tas is a DNA-binding transactivator that interacts with at least two regions in the virus LTR.

◇総説 Review papers

- 1) 大本真也, 伊藤真文, 奥山治美, 藤井陽一 : RNAi を用いた AIDS 治療の展望 RNA ワクチンの可能性. *Molecular Medicine*, 41 : pp44~49, 中山書店, 日本, 2004.

◇学会報告 Scientific presentation

- 1) 奥山治美 : 血清コレステロール値と脂質栄養. 日本脂質栄養学会第13回大会, 2004, 9, 山形.
- 2) 夫馬慎弥, 久井周子, 立松憲次郎, 市川裕子, 奥山治美 : 脳卒中易発症性 (SHRSP) ラットに対する寿命短縮作用を軽減したキャノーラ油の調製. 日本脂質栄養学会第13回大会, 2004, 9, 山形.
- 3) 野々垣知行, 立松憲次郎, 瀧井猛将, 林秀敏, 市川裕子, 藤井陽一, 奥山治美, 小野寄菊夫 : 脳卒中易発症性 (SHRSP) ラット脳のプリオン (PrP) 分子発現に及ぼす油糧種子の影響. 日本脂質栄養学会第13回大会, 2004, 9, 山形.
- 4) 池本敦, 堀田典子, 奥山治美 : 安静及び運動時のマウスの基礎代謝に及ぼす食事多価不飽和脂肪酸の影響. 日本脂質栄養学会第13回大会, 2004, 9, 山形.
- 5) 立松憲次郎, 野々垣知行, 夫馬慎弥, 大平真悠子, 市川裕子, 伊藤真文, 藤井陽一, 森幸雄, 奥山治美 : 脳卒中易発症性 (SHRSP) ラットにおける食用油の内分泌攪乱作用. 日本脂質栄養学会第13回大会, 2004, 9, 山形.

◇その他 Others

- 1) 奥山治美 : 特別講演「油脂 (あぶら) 選びは間違っていますか? ~あぶらえ油は新方向への切り札~古川町保健センター健康セミナー, 2004, 1, 岐阜.
- 2) 奥山治美 : 特別講演「脂質栄養の新方向と商品開発」. 岡山県食品新技術応用研究会第201回研修会, 2004, 3, 岡山.
- 3) 奥山治美 : 特別講演「がん, 心臓病と EPA/アラキドン酸バランス」. 尾西市医師会講演会, 2004, 3, 愛知.
- 4) 奥山治美 : 特別講演「健康長寿の油脂栄養-新方向」. NILS SEMINAR, 2004, 7, 愛知.
- 5) 奥山治美 : 特別講演「がん・炎症性疾患予防の油脂栄養 (ω バランス)」. 国際ネットワーク大学コンソーシアム, 2004, 7, 岐阜.
- 6) 奥山治美 : 特別講演「がん, 心臓病と EPA/アラキドン酸バランス」. 枝幸医師会学術講演会, 2004,

9, 北海道.

- 7) 奥山治美: 講演「油脂(あぶら)の選び方ーコレステロール説から脱却して新方向へー」, 名古屋市立大学2004市民公開講座, 2004, 9, 名古屋.
- 8) 奥山治美: 特別講演「心疾患予防ーコレステロール仮説から脂肪酸の $\omega 6/\omega 3$ バランスへー」. 豊橋内科医会学術講演会, 2004, 11, 愛知.
- 9) Okuyama H.: 招待講演 High Bolld Cholesterol as a Predictor for Low Cancer Mortality and Long-evity, 3rd International Congress on the Columbus Concept, 2004, 10, Brussels.
- 10) 奥山治美: 脳を襲う種子 (Seeds Invading Brain). 名市大薬学祭講演会, 2004, 11, 名古屋.
- 11) 奥山治美: アレルギー体質改善の油脂食品. 抗アレルギー食品開発シンポジウム2004, 2004, 12, 東京.