Dietary poly-unsaturated fatty acids and bioactive lipids

Harald S. Hansen

Abstract

The poly-unsaturated fatty acids of the omega-6- and omega-3-fatty acid families are essential fatty acids that serve a number of important functions in the multi-cellular mammalian organism. Thus, lack of linoleic acid in structural *O*-acylated ceramides of the epidermis is the reason for usual essential fatty acid-deficiency symptoms observed in young experimental animals, i.e. growth retardation, scaly skin, and increased trans-epidermal water loss. However, arachidonic acid also serves essential functions particular in cellular signaling *via* its precursor role for numerous oxygenated derivatives like prostaglandins, leukotrienes, hepoxilins and other eicosanoids. Furthermore, arachidonic acid is also a structural part of the molecules called endocannabinoids (anandamide and 2-arachidonoylglycerol) that have signaling functions in relation to modulation of neurotransmitter release, which may involve physiological and patho-physiological phenomena as regulation of appetite, energy metabolism, pain perception, memory and learning. Omega-3 fatty acids in form of docosahexaenoic acid serve structural functions in phospholipid membranes of neuronal cells, and to a minor degree docosahexaenoic acid is also be precursor for signaling molecules like neuroprotectin D1. As all these fatty acids are ultimately derived from the diet, one can ask what the effect is of different dietary fats on the degree of formation of these bioactive signaling molecules. Generally, *in vivo* eicosanoid production from arachidonic acid can be increased and decreased by prolonged feeding with pharmacological levels of arachidonic acid and long-chain omega-3 fatty acids, respectively. However, changes in the level of these two fatty acids within the usual human diet do hardly affect this eicosanoid production. The beneficial effect of 0.85 mg/day of long-chain omega-3 fatty acids on death from coronary heart disease seems not to be mediated by changes in eicosanoid production. Preliminary data suggests that endocannabinoid formation is equally difficult to affect by the dietary intake of these two fatty acids.

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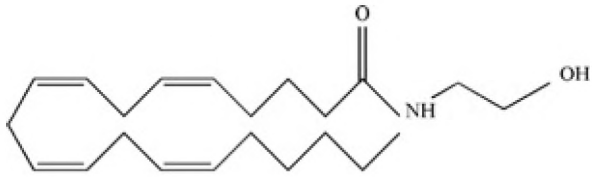
Introduction

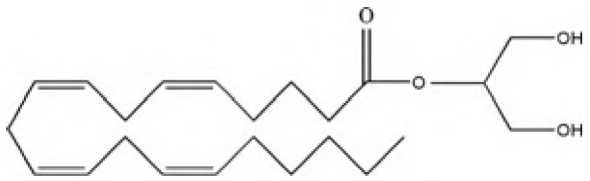
Poly-unsaturated fatty acids belonging to the omega-6- and omega-3-fatty acid families are considered as essential fatty acids, i.e. if deficient in the diet of young growing animals, a number of pathological deficiency symptoms will appear. Thus, the deficiency symptoms of the omega-6 fatty acids involve initially scaly skin, decreased growth, increased trans-epidermal water loss, and increased urinary excretion of vasopressin, and all these symptoms seem to be attributable to an essential structural function of linoleic acid in the epidermal water permeability barrier (Hansen *et al.,* 1986; Hansen, 1986; Hansen, 1989; Phinney *et al.,* 1993). A dietary intake of around 1 energy% should be enough to prevent these symptoms. Early symptoms of deficiency of omega-3 fatty acids involve delayed visual development probably caused by a defective structural function of docosahexaenoic acid in the retina (Lauritzen *et al.,* 2001; Lauritzen and Hansen, 2003; Wheeler *et al.,* 1975; Muskiet *et al.,* 2004). Docosahexaenoic acid is also essential for proper brain function. Different cell lines maintained in culture can exist without incorporation of any poly-unsaturated fatty acids (Stubbs *et al.,* 1992; Urade *et al.,* 1985) indicating that these fatty acids are not absolutely necessary for the basal function of cellular membrane structures of non-communicating cells in an optimal environment.

Bioactive lipids

Poly-unsaturated fatty acids acylated into membrane lipids like diacylglycerol have signaling roles (Wang, 2006) that may be influenced by the type of poly-unsaturated fatty acid present, i.e. arachidnonic acid versus docosahexaenoic acid (Madani *et al.,* 2004). The type of poly-unsaturated fatty acids in phospholipids of cellular membranes may also affect the function of selected integral enzymes in the membranes (Ruf *et al.,* 2006; Turner *et al.,* 2006). However, relatively little is known on this subject.



Anandamide
(AEA)



2-Arachidonoylglycerol
(2-AG)

Fig. 1. Structure of two endocannabinoids that can activate cannabinoid receptors.

Poly-unsaturated fatty acids as free acids, especially arachidonic acid, are also precursors for a vast number of enzymatically formed oxygenated derivatives, e.g. prostaglandins, leukotrienes, hydroxyeicosatetraenoic acids, epoxy-derivatives, neuroprotectins, resolvins, and hepoxilins (Funk, 2001; Montuschi *et al.,* 2004; Pace-Asciak, 2005; Spector and Norris, 2007; Bazan, 2007). Generally, those oxygenated compounds that are derived from arachidonic acid are all together called eicosanoids while those derived from docosahexaenoic acid are called docosanoids. Besides these enzymatically formed eicosanoids and docosanoids, non-enzymatically oxidation of phospholipid-bound arachidonic acid and other long-chain poly-unsaturated fatty acids can lead to formation of isoprostanoids and neuroprostanes that after hydrolysis from the phospholipids may have biological functions during oxidative stress (Montuschi *et al.,* 2004).

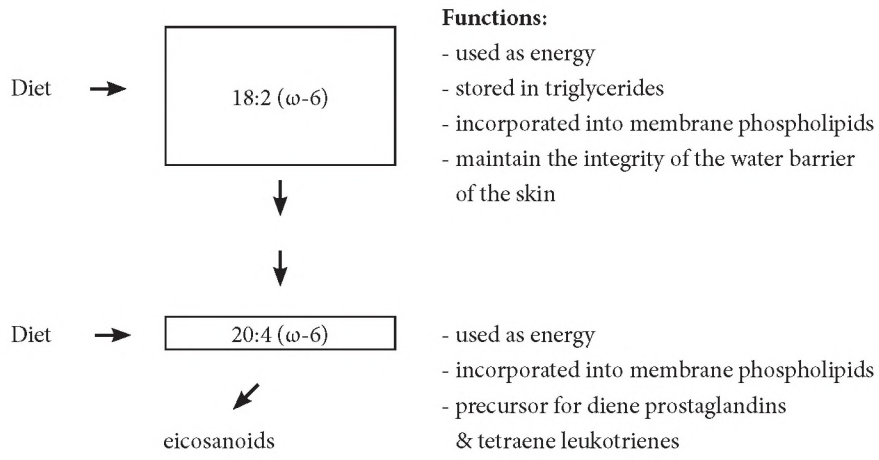


Fig. 2. Turnover and functions of omega-6 fatty acids. The figure illustrates the large dietary intake of linoleic acid (18, 2(ω-6)) and the corresponding large pool of linoleic acid in the body as typical for humans in western societies. A very minor amount of this linoleic acid can be elongated and desaturated to arachidonic acid (20, 4(ω-6)) that are found as a much smaller pool in the body. Omnivorous humans have a small dietary intake of arachidonic acid from animal products. The figure also illustrates the very small daily production of eicosanoids that is far smaller than the endogenous formation plus the dietary intake. Besides the eicosanoids, arachidonic acid is also precursor for endocannabinoids that probably also is formed in minute daily amounts.

Within the last decade, arachidonoylethanolamide (AEA, also called anandamide) and 2-arachidonoylglycerol (2-AG) (Fig. 1) have been demonstrated to be agonists for the cannabinoid receptors (Devane *et al.,* 1992; Mechoulam *et al.,* 1995; Sugiura *et al.,* 1995) that are involved in such diverse functions as regulation of appetite, neurotransmitter release, bone formation and pain (Hansen *et al.,* 2006; Pacher *et al.,* 2006; Sugiura *et al.,* 2006). Especially 2-AG appears to function as a retrograde messenger in regulating neurotransmitter release in the synapse (Hashimotodani *et al.,* 2005; Katona *et al.,* 2006). AEA is always formed *in vivo* together with other acylethanolamides, e.g. oleoylethanolamide (OEA) and palmitoylethanolamide, especially during tissue injury, and they have a variety of biological actions including modulation of food intake and neuroprotection (Hansen *et al.,* 2002; Hansen *et al.,* 2000; Sun *et al.,* 2007; Lo Verme *et al.*, 2005; Degn *et al.,* 2007; Petersen *et al.,* 2006).

It is astonishing that poly-unsaturated fatty acids, especially arachidonic acid, can serve as precursor for so many different signaling compounds.

Effects of diet on bioactive lipids

One can then ask whether the formation of all these different poly-unsaturated fatty acid-derived bioactive lipids are influenced by variation in the dietary intake of poly-unsaturated fatty acids, especially the dietary intake of the direct precursors, arachidonic acid and docosahexaenoic acid?

Generally, humans in the western world may have a rather large intake of linoleic acid, 18, 2ω6. i.e. 8-25 g/day, and thus also large stores of this fatty acid in both phospholipids, cholesteryl esters and triacylglycerol as shown in Fig 2.

Omnivorous humans also have an intake of

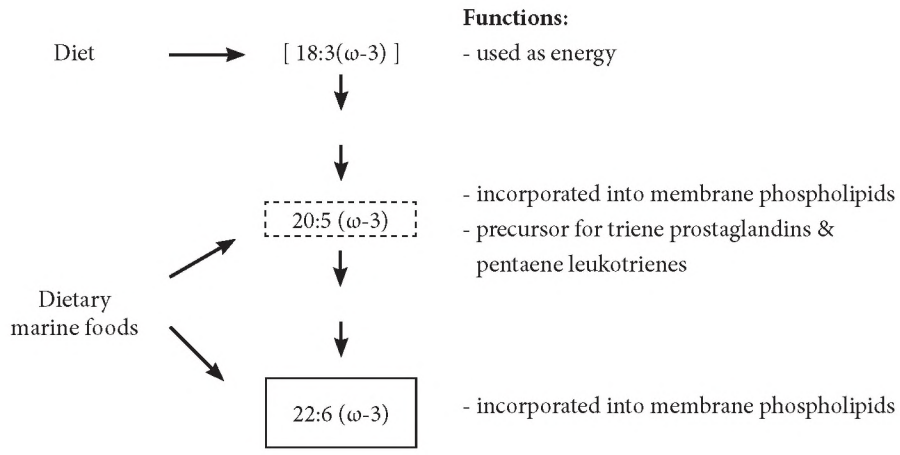


Fig. 3. Turnover and functions of omega-3 fatty acids. The figure illustrates the small dietary intake of alpha-linoleic acid (18, 3(ω-3)) and the nearly absent pool of alpha-linolenic acid in the body as typical of humans in western societies. Most of the ingested alpha-linolenic acid is oxidized and used for energy production while only an extremely small fraction is elongated and desaturated to omega-6 docosahexaenoic acid (22, 6(ω-6)) that is found specifically in relatively high amounts in neurons of the central nervous system. Humans ingesting seafood and fish oil can have a relatively large intake of omega-3 eicosapentaenoic acid (20, 5(ω-3)) and docosahexaenoic acid. Docosahexaenoic acid can also be oxidized and used for energy production. Only extremely small amounts of omega-3 fatty acids are converted to eicosanoids and docosanoids.

arachidonic acid from animal products amounting to 100-300 mg/day (Zhou and Nilsson, 2001). Arachidonic acid is not found in higher plants. Calculation of the endogenous production of arachidonic acid from linoleic acid indicates that this may be a bit higher, i.e. 180-800 mg/d depending among other things on the dietary intake of docosahexaenoic acid (Emken *et al.*, 1999). The daily endogenous prostaglandin formation seems to be quite low as estimated from urinary excretion of prostaglandin metabolites, i.e. in the order of 2-3 mg/day (Hansen, 1983; Zhou and Nilsson, 2001), and adding this up with all the other enzymatically produced eicosanoids, the total production of eicosanoids may probably not exceed 10 mg/day, thus being far lower than the daily arachidonic acid intake and endogenous arachidonic acid production. Humans in the western world ingest far less omega-3 fatty acids (around 1-3 g/day), and the dietary intake of eicosapentaenoic acid and docosahexaenoic acid varies a lot between individuals and populations being mainly related to the dietary intake of seafood (Fig 3).

In the USA, the average intake is around 130 mg/day while it in Denmark is around 500 mg/day (Gebauer *et al.*, 2006; Marckmann *et al.*, 1995; Tjonneland *et al.*, 1993) . In Japan it is around 800 mg/day (Hino *et al.*, 2004). It is clear that eicosapentaenoic and docosahexaenoic acids can inhibit the *in vitro* eicosanoid production from arachidonic acid (Hansen *et al.*, 1983; Rees *et al.*, 2006), but the *in vivo* eicosanoid formation seems to be much less influenced by dietary intake of eicosapentaenoic and docosahexaenoic acids (Murphy *et al.*, 2007; Ferretti *et al.*, 1998) probably because *in vitro* stimuli for prostaglandin formation often are stronger than those occurring *in vivo*. Generally, an intake of several grams per day of eicosapen-

taenoic and docosahexaenoic acid for many weeks is necessary for seeing a moderate decrease in the *in vivo* production of eicosanoids from arachidonic acid. Thus, the beneficial effect of 0.85 mg/day of long-chain omega-3 fatty acids on death frequency from coronary heart disease seems not to be mediated by changes in eicosanoid production (Marchioli *et al.,* 2002). Eicosapentaenoic acid is generally a rather poor substrate for prostaglandin-producing enzymes resulting in only very small levels of eicosapentaenoic acid-derived eicosanoids. Attempts to increase *in vivo* prostaglandin production by dietary supplements of pure arachidonic acid (e.g. 6 g/day for 2-3 weeks) have shown that a slight increase can be seen (Seyberth *et al.*, 1975) but the general impression is that eicosanoid production hardly is influenced by the variations in arachidonic acid content of usual human diets (Ferretti *et al.,* 1997; Pantaleo *et al.,* 2004). Studies of dietary influence on endogenous levels of endocannabinoids and acylethanolamides have been few. One study have shown that feeding of suckling piglets with a milk formula deficient in arachidonic acid decreased the brain levels of AEA and 2-AG and the levels can be increased by adding arachidonic acid to the formula (Berger *et al.,* 2001). Another study found that feeding young mice 10 wt% fish oil for 6 weeks, a diet that is not relevant for humans, resulted in decreased levels of 2-AG in the brain (Watanabe *et al.*, 2003). We have found that a one-week feeding of adult rats with diets containing 36 energy% of different fats (palm oil, safflower oil and olive oil) resulted in changes within both brain and intestine of levels of AEA, 2-AG and oleoylethanolamide. Thus, both type of dietary unsaturated fats increased levels of 2-AG and OEA in the brain and olive oil increased levels of AEA. These changes were seen without changes in the fatty acid composition of total brain phospholipids. All dietary fats decreased levels of acylethanolamides in the intestine (unpublished data).

Conclusion

The overall conclusion is that eicosanoid formation is not easily affected by variation in the dietary intake of arachidonic acid within the range of usual human diets. Prolonged intake of very high amounts of fish oil providing several grams of long-chain omega-3 fatty acids per day to humans will eventually decrease prostaglandin formation and thereby have weak anti-inflammatory and analgesic effects. Endogenous tissue levels of endocannabinoids and acylethanolamides seem in certain cases to be influenced in a complicated way by the type of dietary fat, a mechanism that perhaps is mediated through changes in expression of enzymes involved in the turnover of endocannabinoids and acylethanolamides.

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References

Bazan, N. G. Omega-3 fatty acids, pro-inflammatory signaling and neuroprotection. *Curr. Opin. Clin. Nutr. Metab. Care* **10**, 136-141 (2007).

Berger, A., G. Crozier, T. Bisogno, P. Cavaliere, S. Innis, and V. Di Marzo, Anandamide and diet, Inclusion of dietary arachidonate and docosahexaenoate leads to increased brain levels of the corresponding *N*-acylethanolamines in piglets. *Proc. Natl. Acad. Sci. USA* **98**, 6402-6406 (2001)

Degn, M., K. L. Lambertsen, G. Petersen, M. Meldgaard, A. Artmann, B. H. Clausen, S. H. Hansen, B. Finsen, H. S. Hansen, and T. M. Lund, Changes in brain levels of *N*-acylethanolamines and 2-arachidonoylglycerol in focal cerebral ischemia in mice. *J. Neurochem.* in press (2007).

Devane, W. A., L. Hanus, A. Breuer, R. G. Pertwee, L. A. Stevenson, G. Griffin, D. Gibson, A. Mandelbaum, A.

Etinger, and R. Mechoulam, Isolation and structure of a brain constituent that binds to the cannabinoid receptor. *Science* **258**, 1946-1949 (1992).

Emken, E. A., R. O. Adlof, S. M. Duval, and G. J. Nelson, Effect of dietary docosahexaenoic acid on desaturation and uptake *in vivo* of isotope-labeled oleic, linoleic, and linolenic acids by male subjects. *Lipids* **34**, 785-791 (1999).

Ferretti, A., G. J. Nelson, P. C. Schmidt, G. Bartolini, D. S. Kelley, and V. P. Flanagan, Dietary docosahexaenoic acid reduces the thromboxane/prostacyclin synthetic ratio in humans. *J. Nutr. Biochem.* **9**, 88-92 (1998).

Ferretti, A., G. J. Nelson, P. C. Schmidt, D. S. Kelley, G. Bartolini, and V. P. Flanagan, Increased dietary arachidonic acid enhances the synthesis of vasoactive eicosanoids in humans. *Lipids* **32**, 435-439 (1997).

Funk, C. D. Prostaglandins and leukotrienes. Advances in eicosanoid biology. *Science* **294**, 1871-1875 (2001).

Gebauer, S. K., T. L. Psota, W. S. Harris, and P. M. Kris-Etherton, n-3 fatty acid dietary recommendations and food sources to achieve essentiality and cardiovascular benefits. *Am. J. Clin. Nutr.* **83**, 1526S-1535S (2006).

Hansen, H. S. Dietary essential fatty acids and in vivo prostaglandin production in mammals. *World Rev. Nutr. Diet.* **42**, 102-134 (1983).

Hansen, H. S. The essential nature of linoleic acid in mammals. *Trends Biochem. Sci.* **11**, 263-265 (1986).

Hansen, H. S. Linoleic acid and epidermal water barrier. In *Dietary ω3 and ω6 Fatty Acids, Biological Effects and Nutritional Essentiality*. C.Galli and A.P.Simopoulos (eds.) Plenum Press, London, pp. 333-342 (1989).

Hansen, H. S., B. Fjalland, and B. Jensen, Extremely decreased release of prostaglandin E2-like activity from chopped lung of ethyl linolenoate supplemented rats. *Lipids* **18**, 691-695 (1983).

Hansen, H. S., B. Jensen, and P. von Wettstein-Knowles, Apparent in vivo retroconversion of dietary arachidonic to linoleic acid in essential fatty acid-deficient rats. *Biochim. Biophys. Acta* **878**, 284-287 (1986).

Hansen, H. S., B. Moesgaard, H. H. Hansen, and G. Petersen, *N*-Acylethanolamines and precursor phospholipids - relation to cell injury. *Chem. Phys. Lipids* **108**, 135-150 (2000).

Hansen, H. S., B. Moesgaard, G. Petersen, and H. H. Hansen, Putative neuroprotective actions of *N*-acyl-ethanolamines. *Pharmacol. Ther.* **95**, 119-126 (2002).

Hansen, H. S., G. Petersen, A. Artmann, and A. N. Madsen, Endocannabinoids. *Eur. J. Lipid Sci. Technol.* **108**, 877-889 (2006).

Hashimotodani, Y., T. Ohno-Shosaku, H. Tsubokawa, H. Ogata, K. Emoto, T. Maejima, K. Araishi, H. S. Shin, and M. Kano, Phospholipase Cb serves as a coincidence detector through its Ca^{2+} dependency for triggering retrograde endocannabinoid signal. *Neuron* **45**, 257-268 (2005).

Hino, A., H. Adachi, K. Toyomasu, N. Yoshida, M. Enomoto, A. Hiratsuka, Y. Hirai, A. Satoh, and T. Imaizumi, Very long chain n-3 fatty acids intake and carotid atherosclerosis - An epidemiological study evaluated by ultrasonography. *Atherosclerosis* **176**, 145-149 (2004).

Katona, I., G. M. Urban, M. Wallace, C. Ledent, K. M. Jung, D. Piomelli, K. Mackie, and T. F. Freund, Molecular composition of the endocannabinoid system at glutamatergic synapses. *J. Neurosci.* **26**, 5628-5637 (2006).

Lauritzen, L. and H. S. Hansen, Which of the n-3 FA should be called essential? *Lipids* **38**, 889-891 (2003).

Lauritzen, L., H. S. Hansen, M. H. Jorgensen, and K. F. Michaelsen, The essentiality of long chain *n*-3 fatty acids in relation to development and function of the brain and retina. *Prog. Lipid Res.* **40**, 1-94 (2001).

Lo Verme, J., S. Gaetani, J. Fu, F. Oveisi, K. Burton, and D. Piomelli, Regulation of food intake by oleoylethanolamide. *Cell. Mol. Life Sci.* **62**, 708-716 (2005).

Madani, S., A. Hichami, M. Charkaoui-Malki, and N. A. Khan, Diacylglycerols containing omega 3 and omega 6 fatty acids bind to RasGRP and modulate MAP kinase activation. *J. Biol. Chem.* **279**, 1176-1183 (2004).

Marchioli, R., F. Barzi, E. Bomba, C. Chieffo, D. Di Gregorio, R. Di Mascio, M. G. Franzosi, E. Geraci, G. Levantesi, A. P. Maggioni, L. Mantini, R. M. Marfisi, G. Mastrogiuseppe, N. Mininni, G. L. Nicolosi, M. Santini, C. Schweiger, L. Tavazzi, G. Tognoni, C. Tucci, F. Valagussa, and I. GISSI-Prevenzione., Early protection against sudden death by n-3 poly-unsaturated fatty acids after myocardial infarction - Time-course analysis of the results of the Gruppo Italiano per lo Studio della Sopravvivenza nell'Infarto Miocardico (GISSI)-Prevenzione. *Circulation* **105**, 1897-

1903 (2002).

Marckmann, P., A. Lassen, H. Haraldsdottir, and B. Sandström, Biomarkers of habitual fish intake in adipose tissue. *Am. J. Clin. Nutr.* **62**, 956-959 (1995).

Mechoulam, R., S. Ben-Shabat, L. Hanus, M. Ligumsky, N. E. Kaminski, A. R. Schatz, A. Gopher, S. Almog, B. R. Martin, D. R. Compton, R. G. Pertwee, G. Griffin, M. Bayewitch, J. Barg, and Z. Vogel, Identification of an endogenous 2-monoglyceride, present in canine gut, that binds to cannabinoid receptors. *Biochem. Pharmacol.* **50**, 83-90 (1995).

Montuschi, P., P. J. Barnes, and L. J. Roberts, II, Isoprostanes, markers and mediators of oxidative stress. *FASEB J.* **18**, 1791-1800 (2004).

Murphy, K. J., B. J. Meyer, T. A. Mori, V. Burke, J. Mansour, C. S. Patch, L. C. Tapsell, M. Noakes, P. A. Clifton, A. Barden, I. B. Puddey, L. J. Beilin, and P. R. Howe, Impact of foods enriched with n-3 long-chain poly-unsaturated fatty acids on erythrocyte n-3 levels and cardiovascular risk factors. *Br. J. Nutr.* **97**, 749-757 (2007).

Muskiet, F. A. J., M. R. Fokkema, A. Schaafsma, E. R. Boersma, and M. A. Crawford, Is docosahexaenoic acid (DHA) essential? Lessons from DHA status regulation, our ancient diet, epidemiology and randomized controlled trials. *J. Nutr.* **134**, 183-186 (2004).

Pace-Asciak, C. R. Novel eicosanoid pathways - The discovery of prostacyclin/6-keto prostaglandin F_{1a} and the hepoxilins. *Mol. Neurobiol.* **32**, 19-26 (2005).

Pacher, P., S. Batkai, and G. Kunos, The endocannabinoid system as an emerging target of phamacotherapy. *Pharmacol. Rev.* **58**, 389-462 (2006).

Pantaleo, P., F. Marra, F. Vizzutti, S. Spadoni, G. Ciabattoni, C. Galli, G. La Villa, P. Gentilini, and G. Laffi, Effects of dietary supplementation with arachidonic acid on platelet and renal function in patients with cirrhosis. *Clin. Sci.* **106**, 27-34 (2004).

Petersen, G., C. Sorensen, P. C. Schmid, A. Artmann, M. Tang-Christensen, S. H. Hansen, P. J. Larsen, H. H. O. Schmid, and H. S. Hansen, Intestinal levels of anandamide and oleoylethanolamide in food-deprived rats are regulated through their precursors. *Biochim. Biophys. Acta Mol. Cell Biol. Lipids* **1761**, 143-150 (2006).

Phinney, S. D., S. D. Clarke, R. S. Odin, L. L. Moldawer, G. L. Blackburn, and B. R. Bistrian, Thermogenesis secondary to transdermal water loss causes growth retardation in essential fatty acid-deficient rats. *Metabolism* **42**, 1022-1026 (1993).

Rees, D., E. A. Miles, T. Bannerjee, S. J. Wells, C. E. Roynette, K. W. J. Wahle, and P. C. Calder, Dose-related effects of eicosapentaenoic acid on innate immune function in healthy humans, a comparison of young and older men. *Am. J. Clin. Nutr.* **83**, 331-342 (2006).

Ruf, T., T. Valencak, F. Tataruch, and W. Arnold, Running Speed in Mammals Increases with Muscle n-6 Poly-unsaturated Fatty Acid Content. *PLoS. ONE.* **1**, e65 (2006).

Seyberth, H. W., O. Oelz, T. Kennedy, B. J. Sweetman, A. Danon, J. C. Frölich, M. Heimberg, and J. A. Oates, Increased arachidonate in lipids after administration to man, Effects on prostaglandin biosynthesis. *Clin. Pharmacol. Ther.* **18**, 521-529 (1975).

Spector, A. A. and A. W. Norris, Action of epoxyeicosatrienoic acids on cellular function. *Am. J. Physiol. Cell Physiol.* **292**, C996-C1012 (2007).

Stubbs, E. B., Jr., R. O. Carlson, C. Lee, S. K. Fisher, A. K. Hajra, and B. W. Agranoff, Essential fatty acid deficiency in cultured SK-N-SH human neuroblastoma cells. *Adv. Exp. Med. Biol.* **318**, 171-182 (1992).

Sugiura, T., S. Kishimoto, S. Oka, and M. Gokoh, Biochemistry, pharmacology and physiology of 2-arachidonoylglycerol, an endogenous cannabinoid receptor ligand. *Prog. Lipid Res.* **45**, 405-446 (2006).

Sugiura, T., S. Kondo, A. Sukagawa, S. Nakane, A. Shinoda, K. Itoh, A. Yamashita, and K. Waku, 2-Arachidonoylglycerol, a possible endogenous cannabinoid receptor ligand in brain. *Biochem. Biophys. Res. Commun.* **215**, 89-97 (1995).

Sun, Y., S. P. Alexander, M. J. Garle, C. L. Gibson, K. Hewitt, S. P. Murphy, D. A. Kendall, and A. J. Bennett, Cannabinoid activation of PPARalpha; a novel neuroprotective mechanism. *Br. J. Pharmacol.* (in press, 2007).

Tjonneland, A., K. Overvad, E. Thorling, and M. Ewertz, Adipose tissue fatty acids as biomarkers of dietary exposure in Danish men and women. *Am. J. Clin. Nutr.* **57**, 629-633 (1993).

Turner, N., K. L. Haga, P. L. Else, and A. J. Hulbert, Scaling

of Na^+,K^+-ATPase molecular activity and membrane fatty acid composition in mammalian and avian hearts. *Physiol. Biochem. Zool.* **79**, 522-533 (2006).

Urade, R., Y. Hayashi, and M. Kito, Monoenoic fatty acid requirement in V79 cells. *J. Biochem.* **97**, 391-398 (1985).

Wang, Q. M. J. PKD at the crossroads of DAG and PKC signaling. *Trends Pharmacol. Sci.* **27**, 317-323 (2006).

Watanabe, S., M. Doshi, and T. Hamazaki, n-3 poly-unsaturated fatty acid (PUFA) deficiency elevates and n-3 PUFA enrichment reduces brain 2-arachidonoylglycerol level in mice. *Prostaglandins Leukot. Essent. Fatty Acids* **69**, 51-59 (2003).

Wheeler, T. G., R. M. Benolken, and R. E. Anderson, Visual membranes, Specificity of fatty acid precursors for the electrical response to illumination. *Science* **188**, 1313-1314 (1975).

Zhou, L. and Å. Nilsson, Sources of eicosanoid precursor fatty acid pools in tissues. *J. Lipid Res.* **42**, 1521-1542 (2001).