The endocannabinoids anandamide (AEA) and 2-arachidonoylglycerol (2-AG) are bioactive lipids derived from the n-6 family of polyunsaturated fatty acids that are essential fatty acids. Symptoms of essential fatty acid deficiency in rats – growth retardation, scaly skin, and increased transepidermal water loss – can mainly be attributed to lack of linoleic acid as a structural element of the epidermis. Arachidonic acid, however, also serve essential functions, particularly in cellular signalling via its precursor role for numerous oxygenated derivatives such as prostaglandins, leukotrienes, hepxoylinis and other eicosanoids. Furthermore, arachidonic acid is also a structural part of endocannabinoids that have signalling functions in relation to modulation of neurotransmitter release, which might involve physiological and pathophysiological phenomena such as regulation of appetite, energy metabolism, pain perception, memory and learning. Furthermore, along with AEA formation other acylethanlamides are always formed – e.g., oleoylethanalamide (OEA), that can inhibit food intake, and palmitoylethanalamide, that is anti-inflammatory – possibly through activation of peroxisome proliferator activated receptor α (PPAR α) and/or GPR119. As all these unsaturated fatty acids are ingested daily in smaller or larger amounts, one can ask whether different dietary fats can affect the levels of these fatty acids in the tissues and thereby the quantitative formation of these bioactive signalling molecules. Generally, in vivo arachidonic-acid-derived eicosanoid production can be increased and decreased by prolonged feeding with pharmacological levels of arachidonic acid and long-chain (n-3) fatty acids (fish oil), respectively. Changes in levels of these two fatty acids within the traditional human diet hardly affects the eicosanoid production, however. Moreover, preliminary data suggest that dietary intake of arachidonic acid and fish oil also doesn’t easily affect endocannabinoid formation; however, dietary fat in terms of saturated, polyunsaturated and mono-unsaturated seems to affect tissue levels of AEA, 2-AG and OEA.

Key words: diet, eicosanoid formation, endocannabinoid formation, anandamide, 2-arachidonic acid, essential fatty acids, oleoylethanalamide, levels.

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function of docosahexaenoic acid in the retina (5, 6). Docosahexaenoic acid is also essential for proper brain function, probably via its incorporation into specific cellular phospholipids (6).

Different cell lines maintained in culture can exist without any polyunsaturated fatty acids in the membranes (7, 8) indicating that these fatty acids are not absolutely necessary for the basal function of cellular membrane structures in an optimal environment. In such cell cultures the polyunsaturated fatty acids are substituted by endogenous production of oleic acid and its derivatives that are then incorporated into cellular lipids.

Precursor role of polyunsaturated fatty acids

Polyunsaturated fatty acids as free acids, especially arachidonic acid, are also precursors for a vast number of enzymatically formed oxygenated derivatives, e.g., prostaglandins, thromboxanes leukotrienes, hydroxyeicosatetraenoic acids, epoxy-derivatives and hepxilins (9–11). The oxygenated compounds that are derived from arachidonic acid are all together called ‘eicosanoids’. Prostaglandins are involved in a number of physiological and pathophysiological settings, such as stimulation of inflammation, regulation of blood platelet aggregation, stimulation of uterus during labour and closure of foetal ductus arteriosus after birth. Besides these enzymatically formed eicosanoids, non-enzymatically oxidation of phospholipid-bound arachidonic acid can lead to formation of isoprostanooids that, after hydrolysis from the phospholipids, might have pathophysiological functions during oxidative stress (12). Enzymatically produced eicosanoids are formed ‘on demand’ from arachidonic acid released from phospholipids by different phospholipases (13).

Endocannabinoids are derivatives of arachidonic acid

Within the past decade, arachidonoylethanolamide (AEA, also called anandamide) and 2-arachidonoylglycerol (2-AG) (Fig. 1) have been demonstrated to be agonists for the two known cannabinoid receptors, the CB1 receptor and the CB2 receptor (14–16), which are involved in such diverse functions as regulation of food intake, neurotransmitter release, bone formation and pain (17–20). Recently, it has been reported that AEA and 2-AG also can activate the orphan receptor GPR55 but the biological significance is at present unknown (21). 2-AG in particular appears to function as a retrograde messenger in regulating neurotransmitter release in the synapse via activation of pre-synaptic CB1 receptors (22, 23). In vivo, AEA is always formed together with other acylethanolamides, for example oleoylethanolamide (OEA) and palmitoylethanolamide (PEA), particularly during tissue injury (24–29). Endocannabinoids are not stored in intracellular vesicles prior to release but formed in the tissues ‘on demand’ from precursor phospholipids – for example, AEA from N-arachidonoyl-phosphatidylethanolamine and 2-AG from inositol phospholipids. The other acylethanolamines, such as OEA and PEA, appear to be formed by the same enzyme systems as does AEA, and they are usually formed in far greater amounts than AEA (30, 31).

Dietary polyunsaturated fatty acids and eicosanoid formation

How tight is the quantitative formation of all these different polyunsaturated fatty-acid-derived bioactive lipids controlled? Can it be influenced by variations in the tissue levels of arachidonic acid through variations in the dietary intake of polyunsaturated fatty acids – for example, intake of fish oil, which might decrease levels of arachidonic acid in phospholipids, and of meat, which has a certain content of the direct precursor, arachidonic acid? Generally the levels of arachidonic acid in tissue phospholipids are to some degree influenced by the dietary intake of different polyunsaturated fatty acids but the brain seems to be less influenced – especially the non-growing adult brain (32, 33).

Humans in the Western world generally have a rather large dietary intake of linoleic acid (18:2n-6) i.e. 8–25 g/day, and thus also have large stores of this fatty acid in the form of phospholipids, cholesteryl esters and especially triacylglycerol, as shown in Fig. 2. Omnivorous humans also have a dietary intake of arachidonic acid from animal products (meat, eggs and milk) amounting to
100–300 mg/day [13]. 300 mg/day corresponds to 0.1 energy% of an 11 MJ diet. Arachidonic acid is not found in higher plants, so vegans have no dietary intake of arachidonic acid. Calculation of the endogenous production in humans of arachidonic acid from linoleic acid indicates that this may be a bit higher than the dietary intake, i.e. 180–800 mg/day, depending on the dietary intake of long-chain polyunsaturated fatty acids [34]. The daily endogenous prostaglandin formation seems to be quite low, as estimated from urinary excretion of prostaglandin metabolites – in the order of 2–3 mg/day [13, 35] –and in addition to all of the other enzymatically produced eicosanoids, the total production of eicosanoids probably does not exceed 10 mg/day. This is far lower than the daily arachidonic acid intake and the endogenous arachidonic acid production. Presently, there are no data on the daily production of endocannabinoids.

Humans in the Western world ingest far less n-3 fatty acids (around 1–3 g/day) than n-6 fatty acids, and they mainly consume α-linolenic acid [18:3(n-3)] and, to a lesser extent, eicosapentaenoic acid [20:5(n-3)] and docosahexaenoic acid [22:6(n-3)]. The latter two are found in seafood. The dietary intake of long-chain n-3 polyunsaturated fatty acids (eicosapentaenoic and docosahexaenoic acids) varies greatly between individuals and between geographical populations and is mainly related to the dietary intake of seafood [36] (Fig. 3).

In the USA, the average dietary intake of eicosapentaenoic acid and docosahexaenoic acid is around 130 mg/day; in Denmark it is around 500 mg/day [37–39]; in Japan it is around 800 mg/day [40]. It is evident that eicosapentaenoic and docosahexaenoic acids can inhibit the in vitro production of arachidonic-acid-derived eicosanoid [41, 42], but the in vivo eicosanoid formation seems to be much less influenced by dietary intake of eicosapentaenoic and docosahexaenoic acids [43, 44]. The reason is probably that stimuli used to induce prostaglandin formation in vitro in experimental settings often are stronger and thereby involve different endogenous pools of arachidonic acid than the stimuli occurring in vivo. Generally, a dietary intake of several grams per day of eicosapentaenoic acid and docosahexaenoic acid for many weeks are necessary for observing a moderate decrease in the in vivo production of arachidonic-acid-derived eicosanoids. Thus, the beneficial effect of a dose of 0.85 mg/day of long-chain n-3 fatty acids on death from coronary heart disease in the large GISSI-study [45] seems not to be mediated by changes in eicosanoid production. Eicosapentaenoic acid is generally a rather poor substrate for prostaglandin-producing enzymes [46] resulting in only very small levels of eicosapentaenoic-acid-derived eicosanoids. Attempts to increase in vivo prostaglandin production by dietary supplements with pure arachidonic acid [e.g., 6 g/day for 2–3 weeks in humans] have shown that a slight increase can be observed [47] but the general impression is that eicosanoid production is hardly influenced by variations in arachidonic acid content of traditional human diets [48, 49].

**Dietary polyunsaturated fatty acids and endocannabinoid formation**

Only a few studies have been performed investigating the influence of dietary fatty acids on endogenous levels of endocannabinoids and acylethanolamides. A high-fat diet (60 energy% for 14 weeks) have been reported to increase levels of anandamide in mouse liver [50] and, interestingly, the authors suggested that this could contribute to diet-induced obesity via activation of CB1 receptors in the liver. Another study has shown that feeding suckling piglets with a milk formula deficient in arachidonic acid decreased the
Fig. 3. Turnover and functions of n-3 fatty acids. Humans in Western society typically have a low dietary intake of α-linolenic acid [18:3(n=3)] and an almost absent pool of α-linolenic acid in the body. Most of the ingested α-linolenic acid is oxidised and used for energy production, as are other fatty acids, while only an extremely small fraction is elongated and desaturated to docosahexaenoic acid [22:6(n-6)], which is found specifically in relatively high amounts in neuronal phospholipids of the central nervous system. Docosahexaenoic acid, through a poorly understood mechanism, optimises the function of neuronal tissues. It is unclear whether eicosapentaenoic acid has any essential functions. Humans ingesting seafood and fish oil can have a relatively large intake of eicosapentaenoic acid [20:5(n-3)] and docosahexaenoic acid, which also can be oxidised and used for energy production equivalent to other fatty acids and be incorporated into cellular lipids. Only extremely small amounts of n-3 fatty acids are converted to eicosanoids and other oxygenated metabolites.

brain levels of AEA and 2-AG and that the levels can be increased by adding arachidonic acid plus docosahexaenoic acid to the milk formula (51). In a study by Berger et al. (51), mice supplemented with 0.5 weight% of arachidonic acid for 58 days had dramatically increased brain levels of AEA. Dietary arachidonic acid (270 mg/day, corresponding to approximately 1 energy%/day) given for 7 days to mice did not affect the expression of CB1 receptor or endocannabinoid enzymes (52). Another study found that feeding young mice 10 wt% fish oil for 6 weeks, a diet that is not relevant for humans in terms of the very high fish oil content, resulted in decreased levels of 2-AG in the brain (53). Hanus et al. (54) observed that a fat-rich diet (46 energy%, mainly soybean oil) for 12 days induced a fall in mouse brain levels of 2-AG of 60%. No data on brain fatty acid composition were reported.

We have found that feeding adult rats for 1 week with diets comprising 36 energy% via different fats [palm oil (= mainly saturated fat), safflower oil (= mainly polyunsaturated fat) and olive oil (= mainly monounsaturated fat)] significantly changed brain and intestinal levels of AEA, 2-AG and OEA (55). Thus, both type of unsaturated dietary fats significantly increased levels of 2-AG and OEA in the brain relative to saturated fat, and olive oil increased brain levels of AEA. These changes were seen without changes in the fatty acid composition of total brain phospholipids (55). Conversely, when we fed rats the olive oil diet supplemented with arachidonic acid (equivalent to 4.9 energy%) for 1 week we found no changes in brain fatty acid composition or of 2-AG or AEA (55). This extreme diet corresponds to an intake of 12.5 g/day for a human having an energy intake of 12 MJ/day — and no human would ever have such a diet. An arachidonic-acid-enriched diet increased arachidonic acid in intestinal lipids (both phospholipids and triacylglycerol) and it doubled the level of both 2-AG and AEA in the jejunum (55).

All three high-fat diets (palm oil, safflower oil and olive oil enriched diets) decreased the total levels of acylethanolamides, including OEA, in the intestine relative to the rat chow diet, which had only 11 energy% of fat. The olive-oil-enriched diet resulted in enrichment of jejunal lipids with oleic acid; and despite this, OEA levels decreased. OEA is an anorectic lipid working through activation of PPARα and/or GPR119 (27, 56, 57) and a decrease in the level of OEA in the intestine might promote increased food consumption. Whether this could be a part of the mechanism behind the increased energy intake seen with high-fat diets must await further studies.

The overall conclusion is that eicosanoid formation is not easily affected by variations in the dietary intake of arachidonic acid within the range of traditional human diets. Prolonged intake of very high amounts of fish oil, providing several grams of long-chain n-3 fatty acids per day, in humans will eventually decrease prostaglandin formation and thereby having weak anti-inflammatory and analgesic effects. Endogenous tissue levels of endocannabinoids and acylethanolamides seems in certain cases to be influenced in a complicated way by the type of dietary fat in short-term studies, a mechanism that perhaps is mediated through changes in expression of enzymes involved in the turnover of endocannabinoids and acylethanolamides. It has recently been shown that a high-fat diet (45 energy%) for only 1 week can
influence feeding behaviour, locomotor activity rhythm, circadian clock genes and clock-controlled genes involved in fuel utilisation [58]. Whether this is related to changes in the tissue levels of endocannabinoids and acylethanolamides is at present unclear.

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Conflicts of interest

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