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REVIEW

Effect of dietary fat on endocannabinoids and related mediators: Consequences on energy homeostasis, inflammation and mood

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Among the several known fatty acid-derived chemical signals, the endogenous ligands of cannabinoid receptors type-1 and -2, two G-protein-coupled receptors involved in several aspects of mammalian physiology and pathology, are perhaps those the levels of which have proven to be most sensitive to the fatty acid composition of the diet. The two most studied such ligands, known as endocannabinoids, are *N*-arachidonoyl-ethanolamine and 2-archidonoylglycerol, and are found in tissues together with other *N*-acyl-ethanolamines and 2-acylglycerols, not all of which activate the cannabinoid receptors, although several of them do exhibit important pharmacological effects. In this review article, we describe literature data indicating that the tissue concentrations of the endocannabinoids and related signalling molecules, and hence the activity of the respective receptors, can be modulated by modifying the fatty acid composition of the diet, and particularly its content in long chain PUFAs or in long chain PUFA precursors. We also discuss the potential impact of these diet-induced changes of endocannabinoid tone on three of the major pathological conditions in which cannabinoid receptors have been involved, that is metabolic dysfunctions, inflammation and affective disorders.

Keywords:
2-arachidonoylglycerol / *N*-arachidonoyl-ethanolamine / Cannabinoid / Omega-3 fatty acids / PUFA

1 The endocannabinoid system: The main members of the ''family'' and their close ''relatives''

The endocannabinoid signalling system was originally defined as the system of G-protein-coupled receptors (GPCRs) and their endogenous ligands discovered from studies on the molecular mechanism of action of the major psychotropic principle of *Cannabis sativa*, Δ^9-tetrahydrocannabinol [1]. Two GPCRs with high affinity and specificity for Δ^9-tetrahydrocannabinol have been cloned to date, the cannabinoid receptor type 1 (CB_1) and type 2 (CB_2) [2, 3], which several endogenous lipids, known as endocannabinoids (Fig. 1), bind to and activate. The best studied

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Abbreviations: 2-AG, 2-archidonoylglycerol; **AA,** arachidonic acid; **ACTH,** adrenocorticotropic hormone; **anandamide,** *N*-arachidonoyl-ethanolamine; **$CB_{1\ and\ 2}$,** cannabinoid receptor type 1 and 2; **CLA,** conjugated linoleic acid; **CORT,** corticosterone; **DAG,** diacylglycerol; **DHA,** docosahexanoic acid; **EPA,** eicosapenatenoic acid; **FO,** fish oil; **GPCR,** G-protein-coupled receptor; **GPL,** glycerophospholipid; **HFD,** high-fat diet; **KD,** ketogenic diet; **KO,** krill oil; **LA,** linoleic acid; **LC-PUFA,** long chain PUFA; **MAGL,** monoacylglycerol lipase; **NAE,** *N*-acylethanolamine; **NAPE,** *N*-acyl-phosphatidylethanolamine; **NAPE-PLD,** NAPE-specific ''phospholipase D''; **NArPE,** *N*-arachidonoyl-phosphatidylethanolamine; **OA,** oleic acid; **OEA,** *N*-oleoylethanolamine; **PEA,** *N*-palmitoylethanolamine; **PPAR-α/γ,** peroxisome proliferator-activated receptor-α/γ; **TRPV1,** transient receptor potential of vanilloid type-1

endocannabinoids are all derivatives of an important n-6 PUFA, arachidonic acid (AA), which is also the biosynthetic precursor of a plethora of other chemical mediators, known with the general name of ''eicosanoids''. In particular, *N*-arachidonoyl-ethanolamine (anandamide) [4] and 2-AG [5, 6] (Fig. 1) have been thoroughly investigated in terms of their biosynthesis and inactivation, pharmacological actions and physiopathological role.

With the cloning of endocannabinoid metabolic enzymes, the identification of additional molecular targets for anandamide, and the revisitation of already known (and the isolation of novel) endocannabinoid-like molecules that do not necessarily interact with CB_1 and CB_2 directly, the number of small molecules and proteins belonging to the endocannabinoid system has considerably increased during the last 10 years. For example, we know that anandamide and 2-archidonoylglycerol (2-AG) are accompanied in tissues by congeners that are less active or inactive at CB_1 and CB_2. Long chain *N*-acylethanolamines (NAEs) are often more abundant than anandamide, and, except for homologues with 3–4 double bonds and 18–20 carbon atoms, which do activate cannabinoid receptors, they exert biological actions by interacting with other receptor types. *N*-oleoylethanolamine (OEA), an anorectic mediator that also affects lipid and glucose metabolism, acts by activating the nuclear peroxisome proliferator-activated receptor-α (PPAR-α) [7] or, in some cases, the transient receptor potential of vanilloid type-1 (TRPV1) cation channels. *N*-palmitoylethanolamine (PEA) exerts anti-inflammatory actions *via* several molecular mechanisms, including direct activation of PPAR-α [8] or enhancement of anandamide actions at CB_1, TRPV1 or PPAR-γ [9] receptors. In fact, apart from CB_1 and, less efficaciously, CB_2, anandamide interacts also with a variety of other GPCRs and ion channels, by either activating or, in most cases, inhibiting them [10]. However, the only such effects of anandamide that have been validated *in vivo*, also through the use of mice genetically invalidated for these potential targets, are those exerted by activating TRPV1 channels [11]. 2-AG congeners are, instead, mostly inactive at cannabinoid receptors [12], although some of them enhance some of the cannabinoid receptor-mediated actions of 2-AG [13, 14].

Anandamide, like other NAEs, is produced from the hydrolysis of the phosphoester bond of the corresponding *N*-acyl-phosphatidylethanolamine (NAPE) (that is, *N*-arachidonoyl-phosphatidylethanolamine (NArPE)), which in turn originates from the *trans*-acylase-catalyzed transfer of AA from the *sn*-1 position of phospholipids to the nitrogen atom of phosphatidylethanolamine [15] (Fig. 2). NAPEs and NArPE can be converted into NAEs and anandamide, respectively, in a one-step hydrolysis reaction, catalysed by the NAPE-specific ''phospholipase D'' (NAPE-PLD) [16]. Despite its name, this enzyme is not a phospholipase D-like enzyme, and belongs instead to the metallo-β-lactamase superfamily. It is composed of 393 amino acids, binds one or two zinc ions *per* subunit and is stimulated by divalent cations including Ca^{2+}. It catalyses the hydrolysis of all tested NAPEs regardless of their *sn*-1, 2- and *N*-acyl substituents, but not of other phospholipids, and produces the corresponding NAEs together with phosphatidic acid [17]. However, since NAPE-PLD ''knock-out'' mice do not exhibit reduced levels of AEA in most tissues [18], anandamide and other NAEs were suggested to be formed from NAPEs also *via* other pathways and enzymes, including (i) formation of the corresponding phospho-NAEs *via* phospholipase C and hydrolysis of phospho-NAEs to NAEs by protein tyrosine phosphatase N22 [19]; (ii) phosphodiesterase-mediated hydrolysis of glycerophospho-NAEs, which in turn are produced *via* sequential cleavage of the two *sn*-1 and 2- acyl groups of NAPEs, catalysed by α/β-hydrolase 4 (Abdh4) [20] and (iii) lyso-phospholipase D-mediated hydrolysis of 2-lyso-NAPEs, which in turn would be formed through the action on NAPEs of a soluble form of

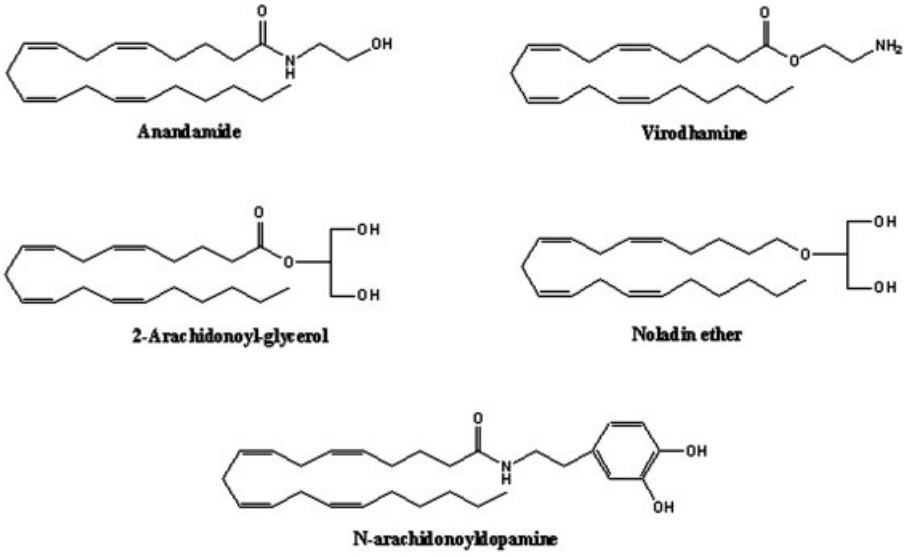

Figure 1. Chemical structures of some of the best studied endocannabinoids.

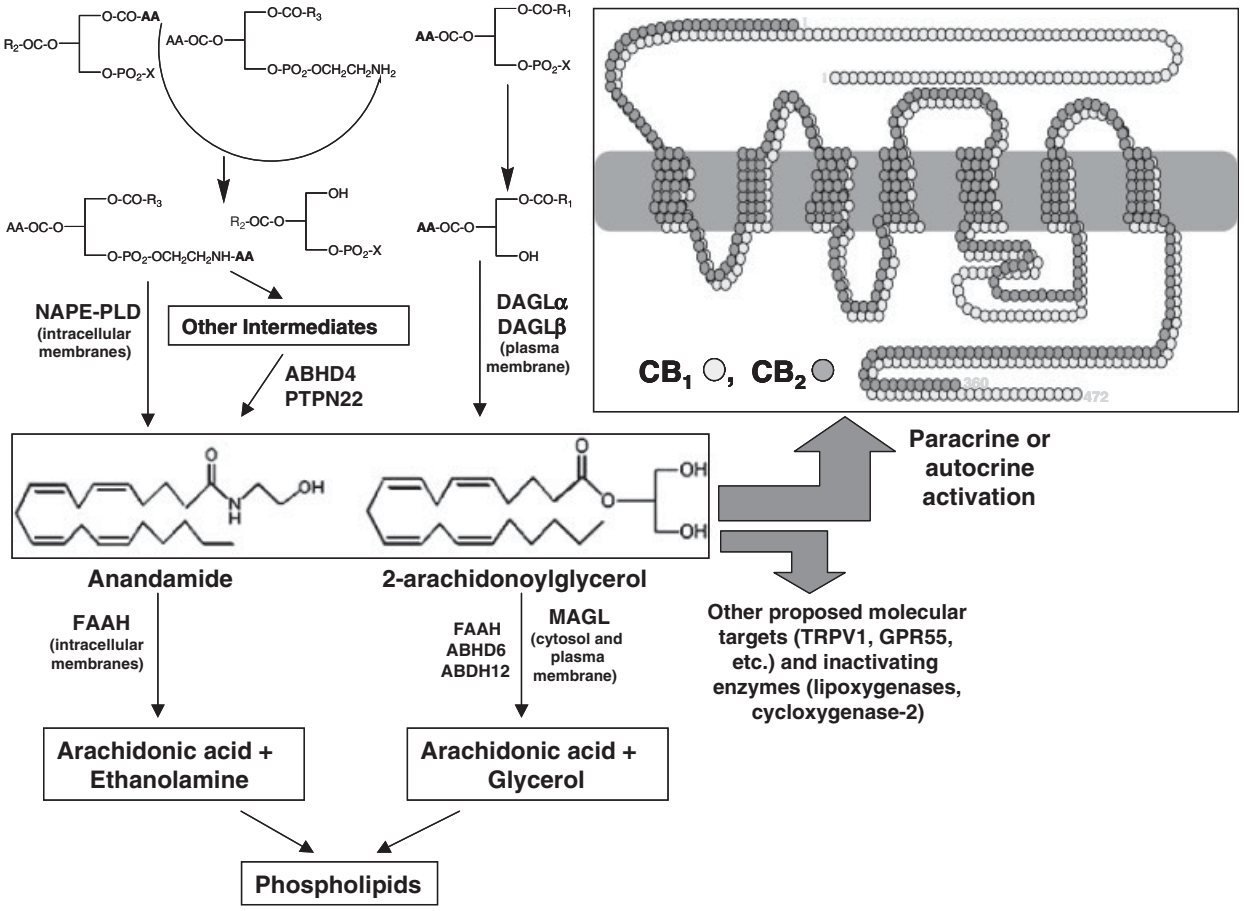

Figure 2. Biosynthesis, action and inactivation of anandamide and 2-AG. Note how the amounts of anandamide and 2-AG ultimately depend on the amounts of the arachidonoyl moiety (AA) on the *sn*-1 and *sn*-2 positions of phospholipids, respectively. Thus, dietary interventions that modify more or less specifically the relative amount of AA esterified to phospholipids might alter correspondingly anandamide and/or 2-AG precursor availability.

phospholipase A_2 [21]. These alternative routes for anandamide (and NAE) biosynthesis might occur in distinct tissues and under different physiopathological conditions.

The Ca^{2+}-dependent acyl-transferase responsible for the formation of NAPEs [22, 23] has not yet been cloned, but the corresponding enzymatic activity was suggested to recognise equally well as substrates most types of glycerophospholipids (GPLs), and to transfer almost equally well all types of fatty acids from the *sn*-1 position of GPLs to the nitrogen atom in phosphatidylethanolamine [24]. Recent data indicate that, in brain homogenates, the Ca^{2+}dependent acyl-transferase might cause the preferential formation of PUFA-containing NArPE species [25].

Whilst anandamide derives ultimately form AA esterified to the *sn*-1 position of phospholipids (Fig. 1), diacylglycerols (DAGs) with AA on the 2-position are instead the most frequent biosynthetic precursors for 2-AG. Two *sn*-1-selective DAG lipases, known as DAGL-α and DAGL-β, were cloned and identified as responsible for 2-AG biosynthesis in most cells and tissues [26]. The proteins possess a 4-*trans*-membrane domain that is probably responsible for their localisation to the cell plasma membrane and exhibit different sizes (1042 amino acids for DAGL-α, 672 for DAGL-β). DAGL-α and -β, are stimulated by 0.1–1.0 mM concentrations of Ca^{2+} and glutathione, and inhibited by sulphydryl reagents, and, like NAPE-PLD with GPLs, do not exhibit strong preference for any of the tested acyl groups in *sn*-1 and 2- position of DAGs. The latter can be obtained *via* various pathways, the most important of which are the hydrolysis of phosphatidylinositol by phospholipase C-β [27] or, possibly to a more limited extent, the hydrolysis of phosphatidic acid using a specific phosphatase [28].

The enzymatic inactivation of anandamide and 2-AG occurs through the hydrolysis of their amide and ester bonds to AA and ethanolamine or glycerol, respectively. Fatty acid amide hydrolase, the first of the ''endocannabinoid enzymes'' to be cloned, has 579 aminoacids and, as surmised by X-ray crystallography of its crystals in conjugation with a covalent inhibitor [29], is a dimer in its membrane associated quaternary structure, employing, unlike most serine hydrolases, a catalytic Ser-Ser-Lys triad for its catalytic action [30]. It can recognise as substrates

several types of long chain, preferably unsaturated, fatty acid amides, including ethanolamides like anandamide, OEA and PEA, taurinamides and primary amides. It also hydrolyses long chain fatty acid esters, including 2-AG and the less characterised endocannabinoid, virodhamine (Fig. 1). However, the enzyme that most specifically controls the levels of 2-AG is the monoacylglycerol lipase (MAGL) [31, 32], a cytosolic enzyme of about 303 aminoacids, which associates to the plasma membrane, and recognises 2- and 1(3)-monoacylglycerols, better if mono- or polyunsaturated. It is sensitive to sulfhydryl-specific reagents and comparison models constructed to get a view on the cysteines located near the binding site strongly suggest the role of these residues in the catalytic mechanism [33], which, however, also involves a catalytic triad of Ser122, Asp239 and His269 (in the human enzyme) [31]. Apart from MAGL, two other α/β-hydrolases, Abhd6 and 12, also recognise 2-AG as substrate [34]. Although in whole brain homogenates and isolated cells, MAGL is a major contributor to 2-AG inactivation, the situation *in vivo* might be different, depending on the physiopathological conditions and the tissue analysed. AA, and possibly also glycerol and ethanolamine, produced from the hydrolysis of 2-AG and anandamide, are rapidly incorporated into membrane phospholipids [35, 36].

From these observations, it is reasonable to hypothesise that the tissue levels of endocannabinoids might be regulated not only by the activity of the corresponding biosynthetic and catabolic enzymes, but also by the availability of their ultimate biosynthetic precursors, and hence by the amounts of AA in the *sn*-1 or *sn*-2 position of phospholipids, for anandamide and 2-AG, respectively. Indeed, whilst anandamide is a minor species among NAEs, 2-AG is the most abundant among its congeners, thus possibly reflecting the relatively little and high amounts of AA esterified to the *sn*-1 or *sn*-2 position of phospholipids, respectively. It has been shown that the amounts of fatty acids esterified on phospholipids reflect their dietary intake by the animal, and this is particularly true for essential fatty acids, such as α-linolenic acids and linoleic acids (LAs), which act as biosynthetic precursors for n-3 and n-6 long chain-PUFAs (LC-PUFAs), respectively (including AA). Therefore, it was reasonable to postulate that the tissue concentrations of the endocannabinoids might depend also on the diet and the presence therein of high or low amounts of LC-PUFAs or of their biosynthetic precursors. Hereafter, we review the available data that support this possibility.

2 Impact of dietary fatty acids on endocannabinoid and NAE tissue concentrations

In newborn piglets, supplementation of milk formulas with AA and docosahexanoic acid (C22:6n3, DHA) increased, in specific brain regions, the concentrations of the corresponding NAEs, anandamide and *N*-docosahexaenoyl-ethanolamine (22:6n3 NAE) [37], the latter of which binds to CB_1 with low affinity. Intriguingly, 2-AG levels were not modified. In the same study, mice fed with an AA-rich diet from post-natal day 1–58 exhibited increased anandamide concentrations in the whole brain [37]. The diet-induced changes were accompanied by changes in the corresponding fatty acids esterified to individual phospholipids, thus suggesting that the increased anandamide levels were due to increased incorporation of AA in the *sn*-1 position of phospholipids [38–40]. In a study (so far published only in the form of abstract) carried out in adult mice fed for 2 wk with either normal lab chow or chow formulated with high DHA fish oil (FO), most of the measured fatty acid levels were found to remain constant; however, DHA, 22:5n3, eicosapenatenoic acid (EPA) and their ethanolamide or glycerol derivatives did change. Although DHA and 22:6n3 NAE levels increased in plasma, they were not altered in the brain. However, when comparing 22:6n3 NAE levels with those of anandamide, there was an increase in both the brain and plasma. Furthermore, when comparing the levels of 2-docosahexaenoyl-glycerol with those of 2-AG, an increase was observed in plasma. Finally, the levels of both EPA and 2-eicosapentaenoyl-glycerol were also increased in brain and plasma [41]. The authors interpreted these data to support the idea that increases in dietary DHA affect fatty acid ethanolamide and glycerol synthesis in favour of the DHA derivatives over the AA derivatives [41]. Accordingly, in another study, Watanabe *et al.* [42] had previously found that mice fed with an n-3 PUFA-deficient diet exhibited higher brain 2-AG levels. On the other hand, in a second experiment, short-term supplementation of DHA-rich FO reduced brain 2-AG levels as compared with the diet supplemented with low n-3 PUFA. The authors observed a concomitant decrease in AA levels and increase in DHA levels in the major brain phospholipid species of mice fed the FO diet as compared with those fed the low n-3 PUFA diet. These results, taken together, indicate that n-3 PUFA deficiency elevates, while n-3 PUFA enrichment reduces, brain endocannabinoid levels in mice, and suggest that CNS physiological functions and pathological conditions involving the endocannabinoids could be correspondingly modified by the manipulation of dietary n-3 LC-PUFAs.

Also the presence in the diet of certain unusual fatty acids, such as conjugated linoleic acid (CLA), was recently found to be linked with changes in brain endocannabinoid levels. Thus, when 3-wk-old mice were fed diets containing 3% of either LA or CLA for 4 wk, the amounts of 2-AG, but not anandamide, OEA and PEA, in the cerebral cortex, but not the hypothalamus, were significantly decreased by CLA treatment as compared with LA [43]. It should be noted, however, that the biological effects of dietary CLA are usually already evident at levels of 0.5–1% in the diets [44]. Furthermore, the authors of this study used LA, the biosynthetic precursor of AA, as a control fatty acid, and, as a consequence, the CLA diet was 3% lower in LA than the control diet, thus probably influencing both brain AA and

2-AG levels, also independently from dietary CLA. In summary, the possible effects of CLA on endocannabinoids should be investigated using lower doses and a more suitable control diet.

Also in peripheral tissues the concentrations of endocannabinoids and their congeners could be modified by the dietary content of LC-PUFAs or of their essential biosynthetic precursors. In a feeding trial of only 1 wk in rats, Artmann *et al.* [45] administered rats five different dietary fats: palm oil (rich in palmitic acid), olive oil (rich in oleic acid, OA, as in a Mediterranean diet), safflower oil (rich in LA as in a North-American diet), FO (as in a Japanese diet) and an AA-containing diet. The authors then examined the effects of these diets on the concentrations of 2-AG, anandamide, OEA PEA, *N*-stearoylethanolamine, *N*-linoleoyl-ethanolamine, 22:5n3 NAE and 22:6n3 NAE in the brain, liver and duodenum. The results obtained can be summarised as follows: (i) the LA diet increased *N*-linoleoylethanolamine in the jejunum and liver; (ii) the OA diet increased OEA in the liver, a finding potentially relevant to the PPAR-α-mediated anti-lipogenic effects of this compound in hepatocytes [46] and to the beneficial effects of the Mediterranean diet; (iii) all five dietary fats decreased OEA in the jejunum without changing the levels of anandamide, thus suggesting that dietary fat may have an orexigenic action; (iv) the AA diet increased anandamide and 2-AG in the jejunum with no effect on the liver, thus suggesting that dietary AA might also cause orexigenic effects (endocannabinoids exert potent orexigenic actions at both the central and small intestine levels, see below); (v) the FO diet decreased liver levels of NAEs, except for 22:5n3 NAE and 22:6n3 NAE; (vi) the LA diet increased *N*-linoleoylethanolamine in the brain, whereas the OA diet increased the brain concentrations of anandamide and OEA (but not 2-AG); (vii) no effect on PEA and SEA levels were observed with any of the diets; (viii) unlike described previously [38, 41, 42] for longer feeling periods, the AA diet and FO diet had no effect on endocannabinoids in the brain after this 1 wk period and (ix) none of the observed effects seemed to be related with changes in the fatty acid composition of phospholipids in the various tissues, suggesting that, after such a short period of administration, the diet were acting possibly *via* mechanisms other than alterations in phospholipids. Nevertheless, this study was important in as much as it demonstrated that even relatively short-term exposures of mammals to diets enriched in certain fatty acids might cause profound tissue-specific effects on NAE levels, with subsequent modulation of the physiopathological function played by these mediators and their molecular targets.

To date, only one study has been carried out *in vitro* to determine whether incubation of cells with certain free fatty acids can affect locally produced NAE and 2-AG levels. Matias *et al.* [47] showed that incubation of 3T3F442A mouse adipocytes with AA strongly elevates 2-AG levels as well as the amounts of AA esterified in triacylglycerols and on the glycerol *sn*-2 carbon, but not on the *sn*-1, in phospholipids. Incubation with DHA, instead, decreased 2-AG and anandamide levels and the amounts of AA esterified on both *sn*-2 and *sn*-1 position of phospholipids, but not on triacylglycerols. The authors suggested that dietary LC-PUFA and/or their biosynthetic precursors might modulate fatty acid composition of adipocyte phospholipids that act as endocannabinoid precursors, and that this, in view of the CB_1-mediated lipogenic actions of endocannabinoids in adipocytes (see below) [48–50], might participate in the beneficial effects of n-3 PUFA, and in the worsening effect of n-6 PUFA, in abdominal obesity, dyslipidaemia and insulin resistance.

Possibly relevant to the above studies, at least to some extent, are the recent findings that ketogenic diets (KDs) or high-fat diets (HFDs) affect endocannabinoid and/or NAE levels in the mouse brain or gastrointestinal tract and adipose tissue, respectively, in some cases time-dependently [51–55]. A KD (*i.e.* high in fat, adequate in protein and low in carbohydrate) consisting of 78% of weight in fat, administered to 3-wk-old mice for 4 wk, was found to reduce OEA and total NAE levels in the hippocampus [51]. A non-statistically significant reduction was also observed for anandamide and PEA levels. Given the proposed neuroprotective activity of NAEs, these effects are unlikely to be involved in the antiepileptogenic action of KDs [51].

In mice, after both 8 or 14 wk on an HFD, both OEA and PEA levels increased in the stomach as compared with a normal chow diet over the same period, whereas anandamide, but not 2-AG, levels decreased, and only after 14 wk [52, 53]. OEA and PEA levels, instead, decreased in the small intestine, and only after 8 wk on the same HFD [52]. The potential importance of these alterations in the regulation of gastric emptying and small intestine motility have been discussed [52, 53]. An HFD for 14 wk is also accompanied by the decrease of all three NAEs in the mouse subcutaneous, but not mesenteric, adipose tissue [48, 54], with possible impact on the regulation of adipogenesis in the various fat depots (see Section 3).

3 Dietary control of endocannabinoid levels: effects on energy metabolism, metabolic disorders and inflammation

The endocannabinoids, by activating cannabinoid CB_1 receptors, can profoundly affect energy metabolism both by stimulating food intake and by affecting energy processing in the adipose tissue, liver, pancreas and skeletal muscle [48, 50, 55]. Indeed, it has been shown that persistent elevation of peripheral endocannabinoid levels in both fasted and postprandial obese and overweight individuals correlates with intra-abdominal obesity, glucose intolerance, dyslipidaemia and dylipoproteinaemia [55–59], and that mice with HFD-induced obesity are also characterised by higher endocannabinoid levels in the epididymal adipose tissue, liver, skeletal

muscle and pancreas [48–50]. That this ''overactivity'' of the endocannabinoid system does contribute to obesity and related metabolic disorders is strongly suggested by the observed orexigenic, pro-lipogenic and insulin desesensitising effects of CB_1 activation, and by the opposite effects observed following chronic blockade of these receptors or in transgenic mice with functionally inactive CB_1 [60]. Although the malfunctioning of metabolic hormones, namely leptin and insulin, seems to underlie most of the chronic dysregulation of central and peripheral endocannabinoid levels observed in obese, leptin- and/or insulin-resistant individuals, the possible role of HFDs in determining increased availability of endocannabinoid biosynthetic precursors has also been suggested [55, 59]. Indeed, as anticipated above, changes induced in endocannabinoid concentrations of both central and peripheral tissues by diets with varying amounts of LC-PUFAs might have a strong impact on metabolism. That the fatty acid composition of the diet might specifically affect endocannabinoid tissue concentrations with potential impact on metabolism was initially suggested by a comparative study carried out by Matias *et al.* [49]. These authors analysed organs with endocrine function (adrenal glands and thyroid), and/or involved in energy expenditure (brown adipose tissue and skeletal muscle), or affected by the consequences of metabolic disorders (heart and kidney), obtained from mice fed for 3, 8 and 14 wk with two different HFDs exhibiting different fatty acid compositions and impact on fasting glucose levels. Although the two diets used for experiments exhibited similar n-6/n-3 PUFA precursor ratios, the 2nd HFD was characterised by significantly higher amounts of monounsaturated fatty acids and PUFAs than the 1st HFD, and by the capability of inducing high fasting glycaemia after 14 wk of the dietary regimen, instead of the only 3 wk required by the 1st HFD. The authors observed elevations (in skeletal muscle, heart and kidney) or reductions (in thyroid) of the levels of either anandamide or 2-AG, or both. Depending on the diet, these changes preceded or accompanied the development of overt obesity and/or hyperglycaemia. Although the authors did not observe clear correlations between abundance of a certain family of dietary fatty acids and effects on endocannabinoid levels, in the adrenal gland, where anandamide was first decreased and then increased by diet, the 2nd HFD (higher in PUFAs) produced this effect earlier. By contrast, in the thyroid, where anandamide levels were decreased by the diet, this effect was observed earlier again with the 2nd HFD. Finally, in those organs where a significant increase of anandamide levels was observed with the diets (*i.e.* kidney and the heart), this effect was often extended to 2-AG only with the 1st HFD (lower in PUFAs). These differences might be due to the higher amounts, in the 2nd *versus* the 1st HFD, of precursors for both n-6 and n-3 LC-PUFAs, the latter of which, as mentioned above, can reduce both anandamide and 2-AG levels in tissues, and the effect of which might have predominated, in some tissues but not in others, over the stimulatory effect on endocannabinoid levels by n-6-PUFAs.

Two studies have been published so far that strongly support, either indirectly or directly, the hypothesis that dietary fatty acids can affect energy homeostasis *via* changes in endocannabinoid levels. First, it was shown that food-restriction of dams, during either gestation or lactation, or both, causes a decrease in hypothalamic anandamide levels in pups, which persists until weaning, but not in adult rats [61]. Interestingly, the observed dam dietary restriction-induced decrease in pup hypothalamic anandamide levels directly and strongly correlated with pup reduced body weight. The authors speculated that, as pups depend on the dams for their LC-PUFA precursor intake, their ability to synthesise AA from dietary precursors was limited by the food restriction imposed on the dams. This might, in turn, have affected the pup capability of synthesising the needed amount of hypothalamic anandamide necessary for optimal food intake and weight gain.

In a second, more recent study [62], the effects of dietary n-3 LC-PUFAs, in the form of either FO or krill oil (KO), balanced for EPA and DHA content, on liver and heart fat and inflammation in Zucker rats, a model of obesity and related metabolic dysfunctions, was compared with that of a control (C) diet containing no EPA and DHA and similar contents of oleic, linoleic, and α-linolenic acids. Concomitantly, the authors measured the amounts of anandamide and 2-AG in the abdominal and subcutaneous fat, liver and heart. Diets were fed for 4 wk. In n-3 LC-PUFA-supplemented rats, liver triglycerides and the peritoneal macrophage response to an inflammatory stimulus were significantly lower than in rats fed the control diet, and heart triglycerides were lower, although only in KO-fed rats. These effects were associated with a lower concentration of the endocannabinoids, anandamide and 2-AG, in the visceral, but not subcutaneous, adipose tissue, and of anandamide in the liver and heart. The decreased endocannabinoid levels were, in turn, associated with lower levels of AA in membrane phospholipids, but not with higher activity of fatty acid amide hydrolase and MAGL. The authors suggested that the beneficial effects of a diet enriched with n-3 LC-PUFAs are the result of changes in membrane fatty acid composition, with subsequent impairment of endocannabinoid, and particularly anandamide, biosynthesis and reduction of CB_1 overactivation, the latter of which was shown to occur in Zucker rats in a separate study [63]. Since also CB_1 antagonists can produce anti-inflammatory effects in macrophages [64], the observed n-3 LCPUFA-induced reduction in endocannabinoid levels in the visceral adipose tissue might also be responsible for the dampened inflammatory response caused by FO and KO, although in this case the reduction of substrates for other inflammatory eicosanoids might also partly underlie this further beneficial effect of the diets.

If dietary fat composition affects the peripheral tissue concentrations of endocannabinoids involved in adipose tissue remodelling *via* activation of CB_1, compounds that antagonise these receptors should exhibit different efficacies

in rodents fed with normal chow or different types of HFDs. In fact, one would expect that the same dose of a CB_1 antagonist is more effective in rodents fed with diets that increase peripheral endocannabinoid levels, whereas diets that reduce these levels (such as those rich in n-3 LCPUFAs [62]) should render the antagonist less efficacious. Nevertheless, a very recent study [65] showed that, whether mice were equicalorically fed a low-fat diet, an HFD or an HFD diet in which 10% of the saturated fatty acids were replaced by n-3 LCPUFAs (HF-FO diet), the chronic treatment with rimonabant always improved metabolic derangements and led to significantly lower body weight gain than that observed in the respective controls for these diets. In fact, some of the metabolic the effects of rimonabant appeared to be strongest in the HF-FO group, thus arguing against the hypothesis that the effect of dietary fat on endocannabinoid levels plays a role in metabolic control [65]. However, these findings could also be explained by hypothesising that the two strategies (*i.e.* n-3 LCPUFAs and CB_1 antagonism) might produce synergistic effects, possibly by reducing endocannabinoid tone in different organs. Indeed, another recent study carried out in rats showed that the hypophagic effect of another CB_1 antagonist, AM251, increased during high fat feeding, and was greater in animals fed an HFD diet than in animals with established obesity. This might suggest that the hyperphagia usually observed with HFDs is caused also by a centrally overactive endocannabinoid system, thus explaining the higher efficacy of CB_1 antagonism in this case [66]. Conversely, in obese Zucker rats, 1 month feeding with n-3 LCPUFAs reduced endocannabinoid levels more in the visceral fat than the brain [62, 67].

4 Dietary control of endocannabinoid levels: Effects on mood and stress

Since dietary changes in the intake of LC-PUFAs appears to alter endocannabinoid levels in the brain, and given the important function of CB_1 in the control of emotional responses to stress, anxiety reactions and extinction of aversive memories (see [68] for a recent review), it would not be surprising that prolonged diets enriched with either n-3 or n-6 LC-PUFAs produce effects on mood. In the aforementioned study in which mice were fed with an AA-rich diet from post-natal day 1–58, and in which increased anandamide levels in whole brain were observed [37, 38], the animals were also subjected to tests of depressive-like and anxiety-like behaviours. In the Porsolt forced swim test, in which mice are forced to swim in a situation from which they cannot escape, and anti-depressants decrease the duration of immobility, the supplementation of DHA or of AA+DHA did not affect immobility. The AA diet showed a trend to reduce immobility duration (anti-depressant-like activity) relative to the control diet ($p<0.1$) [69], which could be an endocannabinoid-CB_1-mediated effect, since the AA diet increased anandamide levels in the brain of similarly fed mice [37], and anandamide elevation and CB_1 activation is known to reduce immobility duration in this test [70]. In the elevated plus maze test, a test of anxiety-like behaviours in laboratory animals, none of the diets (AA, DHA and AA+DHA diets), relative to the control diet, affected entries or time spent in open arms ($p>0.1$), the primary end-point that indicates anxiolytic-like behaviour, and which was accordingly increased significantly by clobazam. However, also closed arm entries were increased by clobazam, thus possibly suggesting that also this end-point is indicative of anxiolysis. Closed arm entries also increased with the AA- ($p<0.05$), DHA- ($p<0.05$), and AA+DHA- ($p<0.01$) supplemented diets, indicating that these LC-PUFA-enriched diets may induce anxiolytic-like effects. Importantly, closed arm entries showed a statistical trend to decrease with AA+DHA-supplemented diet in the presence of the CB1 antagonist, AM251 ($p<0.1$; the *p*-value for this comparison is reduced to 0.08 after log10 transformation), suggesting that part of the anxiolytic-like effects induced by this diet might have been mediated by elevated endocannabinoid levels [69]. Closed arm entries showed a trend to increase when passing from control diet mice treated with AM251 to AA+DHA-supplemented diet mice treated with AM251 ($p<0.1$), indicating that CB_1 inhibition with AM251 could not completely block the dietary affects on closed arm entries. Importantly, none of the treatments or diets affected any parameter of locomotion, thus suggesting that the effects observed were not biased by actions on motor behaviour. Clearly, however, further studies are required in order to confirm that these findings do indicate that LC-PUFA-supplemented diets produce anxiolytic-like behaviours, and that they do so by elevating brain anandamide levels.

In a very recent study [71] pregnant rats were fed a 5% (C) or 30% fat diet rich in either n-6 (HF) or n-3 (HF-FO) PUFA during the last week of gestation and lactation. Post-natal day 10 offspring were then tested for metabolic hormones, brain phospholipid content in AA and endocannabinoid levels, and for the effects of CB_1 blockade (with AM251) on stress responsiveness. On post-natal day 4–5 of lactation, milk from dams belonging to the HF-FO group displayed a reduced n-6/n-3 fat ratio compared with C and HF milk, thus reflecting the composition of the maternal diet. Levels of phospholipid-esterified AA in the hypothalamus and hippocampus were diet-specific, and reflected the n-6/n-3 ratio of the maternal milk and the diets, in a way that HF-FO offspring exhibited a reduced AA content relative to C and HF offspring. AA phospholipid contents correlated with endocannabinoid concentrations, in a way that was diet- and brain region-specific, with positive correlations found in both the hippocampus and hypothalamus for 2-AG, and a negative correlation found for anandamide in the hypothalamus. Post-stress elevation of corticosterone (CORT) appeared to be stronger in pups from HF-FO-fed dams, which would fit with the potentially reduced levels of hypothalamic and hippocampal 2-AG observed in these mice and with the suggested tonic inhibition by CB_1 on

CORT and adrenocorticotropic hormone (ACTH) release. Indeed, also in pups pre-treated with the CB_1 antagonist AM251, stress-induced ACTH secretion was increased. Interestingly, however, the sensitivity to AM251 was reduced in HF and, particularly, HF-FO pups, again in agreement with the potentially reduced levels of brain 2-AG levels observed in these mice. The authors suggested that the nature of perinatal dietary fat can differentially influence neonatal AA levels in brain phospholipids, and hence brain endocannabinoid levels, with potential consequences on hypothalamic-pituitary-adrenal axis modulation during stress in developing pups [71].

These data, taken together, confirm that prolonged diets enriched in various n-3 or n-6 LC-PUFAs can affect, on the one hand, brain anandamide and/or 2-AG levels, and on the other hand, the stress response, anxiety and depression. However, they do not allow us yet to draw any definitive conclusion as to whether these two parallel biochemical and behavioural alterations are in fact always strictly related to each other. Further specific studies will be required to further investigate this possibility.

5 Concluding remarks

The literature data reviewed in this article clearly indicate that the tissue concentrations of anandamide and 2-AG, as well as those of their congeners, can be modified by feeding both perinatal and adult mammals with diets enriched in certain fatty acids, particularly LC-PUFAs and their essential biosynthetic precursors. Such alterations appear to play an important role in the actions exerted by these lipid mediators on mood and, particularly, energy homeostasis. Indeed, while excessive endocannabinoid levels in both the hypothalamus and visceral adipose tissue, liver, skeletal muscle and pancreas appear to be among the causes of hyperphagia leading to obesity, dyslipidaemia and insulin resistance, a defective endocannabinoid tone in certain brain areas, by reducing the capability of the animal to adapt to stressful conditions, might underlie the development of anxiety- and depressive-like behaviours. Therefore, it can be foreseen that diets supplemented with certain fatty acids and LC-PUFAs might be used in the future as an alternative to pharmacological ''reducers'' (*e.g.* CB_1 antagonists) or ''enhancers'' (*e.g.* inhibitors of endocannabinoid inactivating enzymes) of endocannabinoid activity, to treat metabolic or affective disorders, respectively. However, further studies are required to fully evaluate this possibility, particularly in view of the fact that endocannabinoids and related molecules often exert their effects not only in a time-restricted way, but also in a strictly site-specific manner [72]. Thus, any dietary manipulation of their levels needs to be tissue-specific in order to restore the time- and site-specificity of a correct endocannabinoid function, and, hence, to exert therapeutic effects with little adverse events. Furthermore, in view of the great metabolic flexibility of mammals, the possibility that long-term dietary manipulation of tissue endocannabinoid levels undergoes to adaptive mechanisms aiming at counterbalancing its impact, also needs to be evaluated, for example by analysing the effect of prolonged administration of certain diets on the expression of cannabinoid receptors and endocannabinoid biosynthetic and degrading enzymes. Finally, the relevance of the studies reviewed in this article to human nutrition *versus* human endocannabinoid activity still needs to be investigated. Therefore, the way to the use of dietary interventions to manipulate with therapeutic benefit the tone of cannabinoid receptors, or of other molecular targets of endocannabinoid-related fatty acid derivatives, such as the PPARs and the TRPV1 channels, is still long and unpaved, and will require a major multi-disciplinary effort from nutritionists, biochemists, pharmacologists and physicians.

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