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Role of PUFA, the precursors of endocannabinoids, in human obesity and type 2 diabetes

Alejandro Dain[1], Gaston Repossi[1, 2], Undurti Das[3], Aldo Renato Eynard[1, 2]

[1]Instituto de Biologia Celular y I Catedra de Biologia Celular, Histologia y Embriologia, Facultad de Ciencias Medicas, Universidad Nacional de Cordoba, Ciudad Universitaria, Barros esquina Gordillo, Cordoba (5000), Argentina, [2]CONICET, Argentina, [3]Jawaharlal Nehru Technological University, Kakinada-533 003, India

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1. ABSTRACT

Polyunsaturated fatty acids (PUFAs) serve as precursors of the endocannabinoids (ECs) that are bioactive lipids molecules. Recent studies revealed that ECs participate in several physiological and pathological processes including obesity and type 2 diabetes mellitus. Here we review the experimental and clinical aspects of the role of endocannabinoids in obesity and type 2 diabetes mellitus and the modification of the endocannabinoids by exogenously administered PUFAs. Based on these evidences, we propose that the endocannabinoid system (ECS) can be modulated by exogenous manipulation of PUFAs that could help in the prevention and management of human diseases such as obesity, metabolic syndrome and type 2 diabetes mellitus.

2. INTRODUCTION: THE ENDOCANNABINOID SYSTEM, AN OVERVIEW

The endogenous cannabinoid system (ECS) is a complex intercellular signaling system comprising of cannabinoid receptors (CBRs), their endogenous ligands, the endocannabinoids (ECs), and enzymes for their biosynthesis and degradation. The ECS is a ubiquitous lipid signalling system that appeared early in evolution and which has important regulatory functions throughout the body in vertebrales and is broadly distributed in human tissues.

The ECS have been studied using genetic and pharmacological methods but less in a dietary framework. Studies revealed a broad role for endocannabinoid

signaling in a variety of physiological and phatological processes, including neuromodulator release (1-3), motor learning (4), synaptic plasticity (5), appetite (6), pain sensation (7), reproduction and fertility (8), modulation of energy metabolism (9), mental disorders (10), neurodegenerative diseases (11), autonomic and neuroendocrine responses (12). In addition, ECS modulate immune responses (13), inflammation (14), may induce hypotension and bradycardia (15), inhibit cancer cell growth (16), among many other functions (17).

The main endocannabinoids (endogenous cannabis-like substances), and other lipid molecules derived from polyunsaturated fatty acid (PUFA) arachidonic acid (AA), such as anandamide (arachidonoylethanolamide, AEA) and 2-arachidonoylglycerol (2-AG) have been well studied. There are several other molecules, some of which are derived from PUFAs while some others are non-lipid in nature that are known to serve as endogenous agonists of CBRs (cannabinoid receptors) have also been described.

The ECs bind to a family of transmembrane G-protein-coupled receptors (called CB1 and CB2 receptors). CB1 is densely distributed in areas of the brain related to motor control, cognition, emotional responses, motivated behaviour and homeostasis. ECs also bind to CB1 in some peripheral tissues including pituitary gland, immune cells, reproductive tissues, gastrointestinal tissues, sympathetic ganglia, heart, lung, urinary bladder and adrenal gland (18, 19). On the other hand, CB2 receptors are located principally outside of central nervous system (CNS) in the autonomic nervous system. CB2 one of the mayor modulators of immune system, including leucocytes, lymphocytes from spleen and tonsils tissues and microcirculation (17, 19-21). Recent studies revealed that CB2 receptors are present in both cultured neuronal cells and many areas of CNS as well (22).

Endocannabinoids are released upon demand from AA, or other 20 carbons PUFAs in a receptor-dependent manner and serve as retrograde signalling messengers in GABAergic and glutamatergic synapses, as well as modulators of postsynaptic transmission, interacting with other neurotransmitters, including dopamine. ECs are transported into cells by a specific uptake system and degraded by two well-characterized enzymes, the fatty acid amide hydrolase (FAAH) and the monoacylglycerol lipase (MAGL) (22).

Currently progress is being made in the discovery of novel agonists and antagonists with receptor subtype selectivity which should help in providing a greater understanding of the physiological role of the ECS and perhaps also in a broad number of pathologies. This could lead to important advances in developing modulators of activity of ECS drugs that could have therapeutic potential (18).

3. THE ENDOCANNABINOIDS AND ENERGY BALANCE

The most notorious and recognized cannabinoid derivatives have been the marijuana products (23, 24), not only for its social role but also for its medical apply, like in the wasting syndrome, AIDS and cancer patients (25). The marijuana products have analgesic and orexigenic propiertes which in turn induce weight gain and produced some euphoria that allowed the patients to feel better and improved their quality of life (26-28).

3.1. The endocannabinoid system and central control of energy balance

The role of ECS in the brain is complex. CB1 receptors are expressed in all hypothalamic nuclei except, perhaps, in the suprachiasmatic and lateral mammillary nuclei, and they are mostly found at the presynaptic level (29, 30). AEA and 2-AG are also present in the hypothalamus (31, 32). Animal studies revealed that chronic blockade of CB1 receptor leads to sustained and significant weight loss, especially in obese rodents, suggesting that ECs in the brain control food intake and are elevated following food deprivation and during obesity. EC antagonists/inverse agonist like Rimonabant induced weight loss by reducing food intake (18, 26, 33-35).

Similar effects were noted with both AEA and 2-AG in the limbic forebrain level. In the brainstem nodose ganglion, which controls satiety, an increase in the expression of CB1 receptor during fasting leads to an enhancement in the action of ghrelin, a circulating hunger factor that increases food intake and fat mass, the levels of which rise under these conditions. In contrast, CB1 expression decreased following food intake, probably because CB1 is under the control of cholecystokinin (CCK), autacoid whose levels increase following food consumption (36, 37).

Leptin, a satiety hormone that plays a key role in regulating appetite and metabolism and acts as a long-term internal measure of energy state, could be responsible for the higher hypothalamic levels of ECs observed in rats following brief food deprivation, and specifically of AEA. This suggest that in obese humans, who rapidly develop leptin resistance, the hypothalamic levels of ECs might be increased, and possibly, contribute to hyperphagia. Despite these actions of ECs on leptin and ghrelin, intracerebral or intra-hypothalamic infusions of alfa-melanin stimulating hormone and insulin, which causes anorexia, do not appear to affect the levels of AEA and 2-AG. Conversely, indirect evidence exists suggesting a positive regulation on ECS by orexin A, a neuropeptide hormone wich increases the craving for food, stimulates wakefulness and energy expenditure. This is supported by the observation that CB1 antagonist Rimonabant attenuated the effects of orexin A (29, 37-39).

The activation of hypothalamic presynaptic CB1 receptors located on GABA terminals, decrease GABA release onto “orexigenic melanin concentrating hormone (MCH)-releasing neurons” of the lateral hypothalamus leads to increased feeding behavior (40-43). Leptin deficient obese mice (ob/ob) are characterized by an increase (6-fold times than controls animals) in activity of voltage-gated calcium currents and CB1 receptors in lateral hypothalamic neurons and this is supported by the

observation that elevated EC levels were found in the hypothalamus of these mice (40-43).

Recent studies suggested that the activity of anorectic proopiomelanocortin (POMC) expressing neurons in the arcuate nucleus are either stimulated or inhibited by presynaptic CB1 receptors, via inhibition GABAergic or glutamatergic inputs on these neurons, respectively (44-46). Activation of ghrelin receptors in postsynaptic paraventricular nucleus (PVN) neurons produces release of ECs that, in turn, inhibit glutamatergic signaling, and activity of CB1-expressing presynaptic parvocellular anorectic neurons (44-46).

A long-term/postsynaptic effects of ECs on the regulation of food intake is supported by the presence of CB1 mRNA in neurons that produce neuropeptides that regulate food intake such as the orexigenic agouti-related protein (AgRP), orexins and MCH, or the anorectic POMC and the cocaine- and amphetamine- related transcript (CART). For instance, the ability of Rimonabant to change the feeding behavior is well known (18, 38). Furthermore, CB1 receptors tonically down regulate CRH expression (39). Elevation of AEA levels, obtained by knocking out the FAAH enzyme, is accompanied by reduced CART release in several hypothalamic regions. This effect is antagonized by CB1 antagonist. CB1 activation increases the levels of neuropeptide Y (NPY), and the effect of the antagonism on this level could explain the anorectic effects of the inhibition of the CB1 receptors (6). Additionally, CB1 blockade can attenuate the phenotypic hyperphagia in genetic models of obesity (33, 47, 48).

In addition to their actions on the hypothalamus, ECs have other actions as well. ECs have distinct motivational actions on appetite, modulate the incentive and reward aspects of obtaining food and engage in eating, and influence the subjective palatability of food that maintains consumption within a meal. Observational and meal pattern analysis revealed that endogenous and exogenous ECs directly increase the relevance of food, independent of need or energetic status. ECS provoke feeding through adjustments to natural feeding processes and thus, they are likely to have a physiological role in appetite control. The shell subregion of the accumbens nucleus (AcbSh) has strong association with incentive-reward processes. The AcbSh contains a relatively high density of CB1 receptors, and is particularly sensitive to EC induced feeding. Exogenous instillation of 2-AG or AEA into this region induces substantial short term hyperphagia. Fasting that increases food incentive value and reduces eating latency, significantly increases AEA and 2-AG levels within the forebrain regions containing the AcbSh. This response may underlie the enhanced anorectic potency of Rimonabant in fasted animal and reduced hyperphagic response to fasting in CB1-/- animals(20, 49-52).

There are others interactions between ECS and central hormones or peptides, like dopamine and opioids. Dopamine (DA) is central to incentive processes in feeding, organism orientation toward motivationally significant stimuli and the elicitation of appropriate behavioral responses. Food stimuli cause DA release in the accumbens nucleus under conditions were food incentive is elevated, such as after fasting, or with the presentation of novel or palatable food; Rimonabant, reduces this effect. AEA increased extra-cellular DA levels in the AcbSh. ECs also contribute to the pleasure (reward) derived from eating. It may be mentioned here that CB1 agonist and antagonist effects are studied with highly palatable test foods. Suppression of feed behavioral responses by CB1 antagonist is also evident with low palatability foods (53). Rimonabant is equi-anorectic when tested with foods of differing macronutrient content, and agonists will increase intake of the less stimulating diets. It is possible, therefore, that ECs modulate appetitive processes *per se* to provide a general gain in the incentive and reward value of food. ECs may have important functional relationships with the endogenous opioid systems that have an established role mediating food palatability. There is ultrastructural evidence that ECS-opioid interactions are mediated by activation of CB1 and μ-opioid receptors within the same, or synaptically linked, reward-relevant neurons in the AcbSH (25, 50, 54-57).

There is evidence to suggest that ECs are critical to processes that underlie the normal control of appetite and eating motivation. The ECs are intimately associated with the brain mechanisms mainly via AcbSh, that mediate food craving and the pleasure that is derived from eating. ECs, acting in part by modulation of mesolimbic DA system, may be pivotal to the wanting of food and to the anticipation of eating (58). A deeper understanding of the involvement of ECs may help in the development of suitable intervention targets to manage the complex mechanisms involved in the pathobiology of obesity.

3.2. The hypothalamic-pituitary-adrenal (HPA) axis

The neuroendocrine cascade begins in the CNS with the release of corticotrophin-release hormone (CRH) from the PVN of the hypothalamus. CRH induces the release of adrenocorticotrophin hormone (ACTH) from the anterior pituitary, which regulates glucocorticoid production from the adrenal gland. Then, the glucocorticoids complete the negative feedback loop necessary for the regulation of CRH and ACTH release (58). The hierarchical regulation of the axis also includes extra-hypothalamic brain structures, such as the pre-frontal cortex, the hippocampus, and the amygdala, whose function integrates the HPA hormonal response. If theses mechanisms are inadequate or excessive, it might lead to metabolic and behavioral alterations, thus predisposing the organism to an increased risk of illness. Dysregulation of the HPA axis is often associated with affective disorders, depression, abdominal obesity, and cardiovascular disease. ECS modulate the HPA axis directly at hypothalamic level. Chronic treatment with the CB1 agonist CP-55,940 increases both CRH and POMC mRNAs in the PVN and pituitary, respectively. It is known that the action of ECs on the HPA axis is dose-dependent and relies on the activation of CB1 (29, 58-60). Administration of agonist CB1 doesn't increase ACTH levels, suggesting that ECs had no direct effect on the pituitary. Recent studies highlighted a role for

the ECS as a negative modulator of the HPA axis and suggest that alterations in the tone of ECS might be associated with the development of stress-related diseases. This may explain why some obese subjects treated with Rimonabant suspended the treatment due to the occurrence of anxiety and mood disorders (58-62).

3.3. Gut-brain interactions

Interactions between the gut and the brain are recognized to play important roles in the control of energy intake. These effects could be mediated by direct action on CNS neurons, but many others are mediated by afferents fibers of the vagal nerve. CCK was the first of the gut hormones to be shown to influence food intake. CCK acts via vagal neurons, which also function as gastric mechanoreceptors (63). In addition, a variety of peptide hormones from the gut have been shown to inhibit nutrient ingestion, including peptide YY (PYY) and glucagon-like peptide (GLP1). Ghrelin increases food intake by inhibiting the action of CCK by stimulating vagal afferent nerve discharge. Hence, CCK could be considered as the primary regulator in the response of vagal afferent neurons after meal and ECS may participate in this response (26, 33, 63-69).

The link between ECS and vagal afferent signaling is represented by the intestinal presence of CB1 receptors and its participation in the gastrointestinal motility and secretion. A 24-hour fasting increases the concentrations of AEA in the small intestine by 7-fold. However, it is likely that local changes in the levels of ECs may not modify have any direct effect on the brain but might modulate vagal afferent neurons to bring about their actions. Thus, it is possible that there is a central role for the regulation of feeding behavior by ECS and, in addition there could exist a peripheral action for the ECs in the maintenance of the energy balance at the gut level.

The expression of CB1 receptors by vagal afferent neurons are up regulated after 8 and 12-hours of fasting, and are rapidly down regulated after refeeding so that expression of the protein is virtually undetectable 5 hours after refeeding in a fasted rat. Down regulation of CB1 receptors is produced by CCK, and this effect could be responsible for the therapeutic actions seen by the CCK1 receptor antagonist, lorglumida (70).

The vagal afferent neurons express constitutively CCK1, ghrelin (GHS-1), orexin (Ox-R1), leptin (Ob-R), and MCH-1 receptors and do not change with feeding. On the other hand, the expression of both CB1 and MCH1 receptors are increased during energy withdrawal when plasma CCK concentrations are low, while the receptor levels (CB1 and MCH1) are depressed by refeeding in response to increasing plasma concentrations of CCK an action that seems to be mediated by protein kinase C and the transcription factor CREB. These findings suggest a response mechanism at the level of vagus nerve: withdrawal of food is associated with an increase in CB1 receptor expression allowing responses to orexigenic stimuli, such as AEA from the intestine (71). Administration of ghrelin inhibited the down regulation of CB1 receptor expression with refeeding, although it did not stimulate expression in rats fed *ad libitum,* implying that the interactions between ghrelin and ECS may be complex.

In summary, these evidences suggest a role for the gastrointestinal ECS in the stimulation of appetite via gut-brain signaling pathways. Food withdrawal induces both the synthesis in the gut of AEA and the expression of CB1 receptors modulated by vagal fibers. ECs inhibit gastrointestinal motility, gastric acid and intestinal secretion, suppress inflammation, cell proliferation and visceral pain (71-74), actions that are mediated by CB1 receptors while CB2 may have a role in gut inflammation. Energy restriction appears to stimulate both (CB1 and CB2) receptors and ligands and generate an appropriate increase in food intake. Further investigations are needed to understand the role of ECs and ECS in other gastrointestinal diseases such as gastrointestinal reflux and colon cancer (30, 40, 63).

3.4. Actions of ecs and ecs on adipose tissue, pancreas and liver

3.4.1. Role of the ECS in the adipose tissue

ECs and CB1 receptors are present in the gut, hepatocytes, the white adipose tissue, the skeletal muscle and the pancreas (68, 75). The ECS regulate fatty acid homeostasis and the activation of CB1 receptors increases de novo lipogenesis. The importance of the CB1 receptor in fat mass regulation had been pointed out by studies carried out with the use of antagonist Rimonabant in a diet-induced obesity mouse model (68). While its inhibitory effect on food intake was only transient, the CB1 antagonist induced a persistent fall in energy intake and a reduction in fat content following 5 weeks of treatment of diet-induced obese mice in comparison to the control (4, 8, 68). The effect of the ECS on lipogenesis may be indirectly mediated by its ability to decrease adiponectin levels. This is supported by the observation that Rimonabant, an antagonist of CB1 receptors, increased the expression of adiponectin in the adipose tissue. In addition, Rimonabant also regulates peroxisome proliferator activated receptor α (PPAR-alfa) in adipose tissue (76). CB1 receptors have a direct role in increasing lipid accumulation in adipocytes, and the stimulation of these receptors leads to the inhibition of adenylylcyclase and of cAMP formation, an intracellular event coupled to lipolysis and inhibition of lipogenesis in adipocytes. Furthermore, cAMP inhibition stimulates lipogenesis by inhibiting the AMP-activated protein kinase (AMPK) that serves as an energy sensor of the cell and plays a key role in the regulation of energy metabolism. ECS could exert an inhibition on AMPK that could lead to fatty acid synthesis in the adipose tissue leading to an increase in the mass of adipose tissue; and Rimonabant could restore the activity of AMPK and thus, enhance adiponectin synthesis. The inhibition of AMPK by CB1 agonist decreases the malonyl-CoA availability that contributes to the insulin resistance (68).

In addition, Rimonabant and other CB1 antagonists could regulate lipolysis by increasing the activity of the enzymes, such as CAT, CPT2, and crotonase and others involved in the tricarboxylic acid (TCA) cycle.

Recent studies showed that the effect of Rimonabant in the brown adipose tissue involved a change in energy storage and regulation of mitochondrial functions. It was shown that CB1 inhibits the thermogenic uncoupling protein 1 (UCP-1), which is a specific marker of the thermogenic recruitment process (26, 30, 68, 71, 73).

AEA concentrations are in direct relation with the differentiation process for preadipocyte to adipocytes and exert modifications in lipoprotein lipase and PPAR-γ levels. These data reinforce the concept that EC actively and directly participates in adipogenesis and fat accumulation (51).

ECS could block glycolysis in adipose tissue by decreasing of GAPDH and other glycolytic enzymes and by downregulation of the insulin-responsive glucose transporter, GLUT 4. These actions, which could be reversed by Rimonabant, leads to a decrease in glycolysis and lipogenesis, events that play a role in the pathogenesis of type 2 diabetes and obesity (59, 71, 73).

Furthermore, both leptin and PPAR-gamma seem to have the ability to regulate EC in the adipose tissue. Differentiation of human adipocytes with the PPAR-gamma agonist rosiglitazone was shown to downregulate CB1 expression and up regulates FAAH expression. These data suggest a feedback mechanism between PPAR-gamma and ECS at early stage of differentiation, while leptin would then decrease EC levels and inhibit PPAR-gamma expression at late stage of differentiation(23, 63, 65, 71, 74, 77) .

3.4.2. Role of the ECS in the endocrine pancreas

ECS is involved in the control of metabolism by regulating insulin levels as well as glucose uptake and utilization by tissues, with subsequent impact on glucose tolerance. The expression of CB1 in the endocrine pancreas depends of differentiation and/or metabolic state of beta cells and could promotes an impairment in the glucose tolerance by indirect mechanism. CB2 receptors are present in the pancreatic tissue, and improve glucose tolerance. Other data also show the opposite role of CB1 and CB2 receptors, and its coordination helps to maintain the glucose homeostasis (30, 68).

The levels of EC appeared to be under the regulation of glucose and insulin depending on the glucose concentration. High concentrations of glucose can elevate the levels of both AEA and 2-AG in preadipocyte cells.The contrary effects could be observed when glucose is low. Insulin has no affect on the levels of EC, but decrease in EC levels occurs in instances of reduced levels of glucose (78). Under both high and low glucose concentrations, leptin decreased 2-AG levels only after prolonged stimulation (30).

An overactive ECS in the pancreas in hyperglycaemic and obese states has been detected. An over activity of the enzymes N-acyl-phosphatidylethanolamine phospholipase D (NAPE-PLD) and diacylglycerol lipase alfa (DAGL-alfa) and a decrease in the expression of FAAH in these cells is likely. ECS have an impact on insulin levels and hence on glucose utilization and metabolism, contributing to hyperinsulinemia and hypoadiponectinemia (6, 30, 68).

In addition, ECS may modulate glucose and fatty acid metabolism at the level of the adipose tissue and pancreas. CB1 increase glucose levels by inhibition of insulin release and glucose utilization by peripheral tissue. Under normal conditions, CB1 receptor activation allows the optimal energy balance to compensate for the loss of energy that occurs during starvation and others stressful conditions and is a response mechanism to inflammatory situations. But the over activity, leads to high circulating and fat tissue EC levels, that could lead to obesity and in these states ECS system is overactive generating a vicious circle. Thus, antagonism of CB1 could be a therapeutic approach not only in type 2 diabetes but also obesity (6, 26, 30, 45, 68, 71, 73).

4. ENDOCANNABINOID SYSTEM IN OBESITY

Low-grade systemic inflammation occurs on obesity, type 2 diabetes mellitus and other related conditions (79). An increase of fatty acid amides (FAA) by alterations of oxidative pathways and the perturbation of the clearance in the liver, contribute directly to the insulin resistance and this is associated with decrease levels of adiponectin. These changes could result in the disturbances of the normal phosphorylation of the tyrosine substrates and result in hyperglycemia and insulin resistance (6, 26, 30, 45, 53, 76).

4.1. Activation of peripheral endocannabinoid system and obesity

Obesity is one of the main risk factors for the development of type 2 diabetes, and weight loss may be a successful means of ameliorating type 2 diabetes. Exogenous cannabinoids and EC increase food intake and promote weight gain in animals by activating central CBR receptors.

It has been reported that the CB-1 receptor and FAAH are markedly upregulated in mature human adipocytes compared with preadipocytes, suggesting a role for ECS in the maturity of adipocytes. Systemic EC levels are increased in postmenopausal women with uncomplicated obesity and are associated with decreased CB-1 and FAAH gene expression in adipose tissue. CB-1 is expressed in some peripheral human tissues relevant to the pathogenesis of obesity and obesity-associated metabolic disorders (6, 26, 68, 71). The marked downregulation of FAAH gene expression in adipose tissue of obese women suggests that increased EC levels may be secondary to altered activity of FAAH activity (28, 35, 37, 38, 80, 81).

Adipose tissue may be an important contributor to EC inactivation, given the great mass of adipose tissue present in it. But, it is not yet clear if a close interaction exists between leptin and FAAH activity in human adipose tissue. Visceral fat accumulation, typically referred to as abdominal obesity with a leptin relative

deficiency might represent a very important pathophysiologic mechanism for the clustering of metabolic abnormalities (50).

2-AG concentration is markedly increased in obese, especially in those with abdominal obesity compared with lean subjects and a significant correlation was found between circulating 2-AG and visceral fat mass(72, 82). The significant negative relationship between 2-AG levels and measures of insulin sensitivity was independent of the effects of body fat mass, suggesting additional peripheral effects of the ECS. Both 2-AG and AEA are also increased in blood of patients with type 2 diabetes (6, 34, 50, 63, 81, 82).

A selective upregulation of 2-AG, but not AEA, has recently been described in visceral adipose tissue of obese patients; and the reverse situation was described for EC in the liver of diet-induced obese mice. Both, hypothalamic and uterine levels of EC are increased in genetically obese animals presenting with leptin deficiency (*ob/ob* mice) or impaired leptin signaling (*db/db* mice and *fa/fa* rats) (73, 78, 83). These findings, however, appear to be related to interactions between leptin and EC, rather than reflecting obesity-associated changes. Thus, these findings suggest the complexities involved in the regulation of circulating and tissue concentrations of EC.

ECS seems to be involved with respect to adiponectin dysregulation in obesity in rodent and human adipocytes. This may explain weight loss in obese subjects who received Rimonabant that appears to be independent of its effect but possibly related to its action on circulating adiponectin levels at the adipocyte level (68, 71, 74, 84).

It is likely that increased supply of precursors for EC biosynthesis and/or increased activity of enzymes involved in EC synthesis may have a role in this action of Rimonabant (82). Decreased EC degradation must be considered as the other possibility that is supported by the observation that adipocytes could contribute to EC inactivation, which may be disturbed in subjects with abdominal obesity.

EC action is limited by intracellular enzymatic degradation and a marked downregulation of FAAH gene expression in adipose tissue of obese compared with lean subjects was recognized, with the lowest levels in visceral obese subjects. Downregulation of FAAH activity in the liver of mice with high-fat feeding was associated with increased AEA levels (68, 80). Another degrading enzyme, MAGL, has been found in human adipose tissue, but its expression is apparently not influenced by obesity (77).

A role of FAAH in human obesity has recently also been proposed due to the strong association of a FAAH missense gene mutation that leads to decreased enzymatic activity with human obesity (85). The mechanisms that contribute to changes in the regulation of the ECS in human abdominal obesity yet to be clarified.

4.2. Adipose tissue source of EC and related hormones

Adipocytes produce hormones, such as leptin and adiponectin, and peptides that can elicit insulin resistance, such as tumor necrosis factor α or resistin. CB1 activation enhance preadipocyte proliferation and increases expression of adipocyte-adiponectin, whereas its activation in isolated mouse adipocytes increases lipogenesis, confirming the participation of the ECS in adipocyte physiology. In animal studies, it was noted that adipocytes produce both 2-AG and AEA, suggesting that adipocytes could directly contribute to the peripheral dysregulation of EC levels during obesity (68, 86).

Human white subcutaneous adipocytes in primary culture produce AEA, palmitoylethanolamide (PEA), and oleoylethanolamide (OEA). Several studies revealed that human adipocytes possess all precursors and enzymes responsible for the biosynthesis of ECS ligands (87). The nature and content of such free fatty acids in adipocytes largely depend on the ingested fatty acids and de novo synthesis after lipolysis of stored triglycerides. This differential mobilization, which is positively correlated with the unsaturation and negatively with the chain length of fatty acids of arachidonic, oleic, and palmitic acids in adipocytes could explain why PEA is the most abundant EC circulating levels produced by human adipocytes (26, 30). However, it should be noted that levels of these metabolites usually depend on degrading enzyme activities, such as the FAAH for AEA and other *N*-acylethanolamines, and the MAGL for 2-AG (77).

Therefore, at the adipocyte level, some EC and related compounds may act as autocrine and paracrine signals affecting proliferation, differentiation, and metabolic functions of adipose cells in a CBR-independent and peroxisome proliferator-activated receptor gamma (PPAR-gamma) dependent manner It should be noted that PEA, detected as the most abundant EC, did not directly affect either leptin and adiponectin secretion or PPAR protein level in human adipocytes. However, PEA is known to exhibit immunosuppressive and anti-inflammatory effects (79) and might have other adipocyte targets and participate as a local mediator in the regulation of adipose tissue inflammation and role in immunity. Recent data demonstrated that PEA potentiates lipopolysaccharide-induced down-regulation of leptin secretion in human adipocytes (88). Moreover, the well-established pro-inflammatory and immunostimulatory effects of leptin might also provide a physiological significance to the down-regulation of PEA production in adipocytes exposed to leptin (6, 51, 53, 72) .

In summary, a physiological function is suggested for adipocytes as a source of EC and related derivatives. These molecules may participate in the efferent signaling from adipocytes and contribute to the wide range of biological roles attributed to cannabinoid ligands at both central and peripheral levels. Moreover, the degree of unsaturation and the length of the lipid chain are key factors for the clearance of free fatty acid

(FFA) from adipose tissue for the production of ECS and other compounds with important biological functions and play a central role in the human obesity.

4.3. The link between insulin resistance and ECS and free fat acids

Although the majority of circulating FFAs arise from outside the intra-abdominal region, the FFAs originated in the visceral fat, have a directly relation with cardiometabolic disease, promotion of insulin resistance, dyslipidaemia, and impaired beta-cell function. These adverse changes are consistent with increased risk of adverse cardiovascular outcomes and an increased risk of developing type 2 diabetes. The CBR activation enhances the development and metabolism on adipocytes. In addition, adipocytes can secrete EC and a range of bioactive substances ('adipokines') which have the potential to influence metabolism and cardiometabolic function directly. The extent of the contributions of individual adipokines is unclear, although increased secretion of pro-inflammatory mediators and decreased secretion of adiponectin from intra-abdominal adipocytes have been strongly associated with an increased risk of cardiovascular disease(89). Therapies that normalize FFAs secretion may improve cardio metabolic risk by mechanisms related to and/or independent of weight loss (68, 71, 72, 74, 83, 84).

4.4. Adiponectin, obesity and insulin resistance

An association between adipokines and insulin resistance has been noted in both diabetic and non diabetic states. Of particular is the recent demonstrations that adiponectin may play a direct role in determining insulin mediated glucose uptake (90). However, since adiponectin is the major adipokine secreted by fat cells and is linked to obesity physiology, it is unclear to what extent the association of adiponectin with insulin resistance is related to the presence of obesity (64, 90).

Studies have documented that adiponectin concentrations are significantly related to various measures of body fat and that significant weight loss leads to a rise in adiponectin levels (90). However, it is possible that the relationship between obesity and adiponectin is, in part, related to metabolic changes frequently associated with obesity. For instance, insulin resistance and hyperinsulinemia are frequently associated with obesity, and both decline with weight loss. Importantly, both in vitro and in vivo studies have demonstrated that insulin itself may lead to downregulation of adiponectin secretion from fat cells (91, 92); insulin in an indirect fashion can, decrease the levels of AEA and 2-AG (8). Several studies have reported that improving insulin resistance and reducing insulin levels with an insulin-sensitizing agent markedly increases adiponectin concentrations, even in the absence of or after adjustment of changes in weight (92).

It remains to be seen whether changes in adiponectin levels can be linked not only to the insulin concentrations but also to other factors such as body mass index, weight and fat tissue distribution and to EC levels in adipose tisssue (72).

4.5. Pro-inflammatory cytokines and adipose tissue

Data has shown that decreasing of pro-inflammatory cytokines levels when the CB1 has been blocked (e.g., TNF-alfa or IL6) leads to an increase in insulin sensitivity and facilitates differentiation of the preadipocyte to the mature one (8). TNF-alpha and its receptors also form part of a superfamily of related cytokines and receptors. The major TNF receptors that are known to be active in adipose tissue, TNF-R1 mediate apoptosis, whilst TNF-R2 induces one or more mitogen associated protein kinases. Pharmacological studies concluded that TNF-R2 is involved in the induction of insulin resistance, although studies with knockout animals suggested that the TNF-R1 is crucial for stimulating lipolysis (86, 93).

Insulin stimulates adipose tissue to produce more TNF-alpha, although the effect is less obvious in isolated adipocytes. Circulating TNF-alpha concentration tends to correlate with circulating insulinaemia, although this relationship is difficult to interpret in view of the close correlation between obesity and hyperinsulinemia. Thiazolidinediones have been reported to stimulate adipose tissue TNF-alpha production in lean human subjects, but suppresses it in the obese (24, 40, 45, 46, 77, 85, 86, 94). TNF-alpha is able to induce its own synthesis and may also regulate expression of its own receptors, and is a powerful regulator of other cytokines, e.g. increases the production of IL-6 (95, 96). These facts demonstrate the relationship between adipose tissue and the immune system. When the immune system is stressed, it needs to call on energy from the stores, hence cytokines such as TNF-alpha have anti-adipogenic actions. By contrary, when the adipose tissue stores are undesirably small, leptin and IL-6 signals would reduce immunological preparedness and reduce energy expenditure. It is possible that from an evolution point of view, leptin and IL-6 may have had a primary immunological message, but have eventually evolved to regulate adipose tissue metabolism both directly and indirectly by acting on the hypothalamus. This may explain the essential role of cytokines in adipose tissue.

4.6. Leptin-regulated EC and food intake

Leptin is the primary signal through which the hypothalamus senses nutritional state and modulates food intake and energy balance. Leptin reduces food intake by up regulating anorexigenic (appetite-reducing) neuropeptides, such as alpha-melanocyte-stimulating hormone and downregulating orexigenic (appetite stimulating) factors, primarily neuropeptide Y. Genetic defects in anorexigenic signal, such as mutations in the melanocortin-4 or leptin receptors, cause obesity. However, alternative orexigenic pathways maintain food intake in mice deficient in neuropeptide Y (NPY) (6). Leptin is down regulates the expression in the hypothalamus of orexigenic peptides such as NPY, orexins and melanin concentrating hormone. Di Marzo *et al* showed that administration of recombinant mouse leptin into normal Sprague-Dawley rat resulted in around 40-50% reductions in the hypothalamic levels of both AEA and 2-AG, compared to control. By contrast, defective leptin signal in obese Zucker rats was associated with elevated 2-AG levels in the hypothalamus

compared with non-obese controls. In young (6 at 8 week-old) obese db/db mice with defective leptin receptors, hypothalamic levels of both 2-AG and AEA were higher than the controls, whereas the levels of PEA were similar in the two groups (6). These findings suggest negative regulation of EC by leptin, which appears to be specific for EC since hypothalamic levels of PEA were similar and there was no difference in the cerebellar levels of anandamide. In the absence of a neuronal storage mechanism for EC, the effect of leptin on EC levels in the hypothalamus may reflect decreased synthesis, increased degradation or both. Hypothalamic activity of the enzyme FAAH was unaffected by leptin (74). On the other hand, the hypothalamic levels of the direct biosynthetic precursor of AEA, N-Ar-PE, increased significantly following treatment with leptin. This indicates that leptin may downregulate the activity of the phospholipase D (PLD) enzyme that catalyses the conversion of NAr- PE to AEA.

Leptin may trigger or inhibit the release of putative soluble inhibitors or activators, respectively, of the N-Ar-PE-specific PLD, and such soluble substances may be lost in the in vitro assay system used to measure enzyme activity (8, 10) . These results indicate that, the target of leptin may be at the level of a phosphatidylinositol- specific PLC, which are calcium sensitive. On the other hand, data indicate that ECs are involved in maintaining food intake in both the absence and the presence of NPY, and that the two systems operate essentially independently of one another. Simultaneous blockade of more than one orexigenic pathway may be necessary to achieve clinically relevant, long-term reductions in food intake in obese individuals (6, 30, 51, 53, 72, 89, 97). Hypothalamic EC appear to be under negative control by leptin and may be considered to belong to the growing family of orexigenic mediators, including NPY, the orexins and melanin concentrating hormone. However, the cell groups in which EC are synthesized and their neuronal connections with other hypothalamic nuclei that form the circuitry controlling food intake and satiety remain to be clarified.

5. THE ECS IN HYPERGLYCEMIA AND DIABETES

In adipocytes, EC and CB1 receptor levels increase during differentiation and CB1 stimulation leads to more rapid differentiation of pre-adipocytes, stimulation of lipoprotein lipase activity, up-regulation of glucose uptake, inhibition of AMP-activated protein kinase (AMPK) and stimulation of fatty acid synthase. These lipogenic actions of CB1 in adipocytes explain, in part, why CB1 knock-out mice fed with the same amount of food as wild-type mice still exhibit less fat mass. On the other hand, the role of the ECS in the endocrine pancreas is less well understood (30, 97).

In beta cells, both CB1 and CB2 stimulation has been reported to inhibit insulin release, whereas in insulinoma cells grown in a high glucose concentration, CB1, but not CB2, stimulation causes enhancement of glucose-induced insulin release. In human beta cells, despite the relatively low abundance of CB1 receptors, their stimulation also enhances insulin release. (30, 84, 97-100)

Metabolic dysfunctions such as dyslipidemia and dyslipoproteinaemia as well as insulin resistance and visceral adiposity and the associated inflammatory states, while being correlated with each other, clearly play a role in atherogenesis and the subsequent increased cardiovascular risk (3, 8). Therefore, the ECS is likely to play an important role in the cardiovascular consequences of these metabolic disorders, especially if these are caused in part by its dysfunction. However, the ECS also plays a direct role in the cardiovascular system by directly lowering blood pressure via both CB1 and non-CB1 receptors in both endothelial and smooth muscle cells of the vasculature by reducing the accumulation of atherosclerotic plaques via CB2 receptors and by exerting direct cardioprotective effects, again mostly via CB2 receptor. There is, however, evidence for the overactivity of the ECS (in terms of up-regulation of either CB1 receptor or EC (48, 77, 96)levels, or both) during conditions of unbalanced energy homeostasis (e.g. obesity and hyperglycemia). This overactivity occurs at the level of both the hypothalamus and peripheral tissues, including the liver, pancreas, and epididymal adipose tissue in animals fed with a high fat diet (HFD), and in the visceral fat and blood of obese patients.(26, 30-32, 40, 43-45, 81).

However, this is not without controversy. For instance, all tissues do not show EC increase during hyperglycemic and/or obesity following a high fat diet. In fact, a decrease has been observed in the subcutaneous fat and in the stomach of experimental animals (8)

The possible biochemical mechanisms underlying aberrant EC levels in obesity are not clear. Impairment of FAAH expression or activity seems to correlate with obesity and overweight in humans and with the elevated EC levels found in the liver of mice fed a HFD or in the blood of obese humans (31, 48). Changes in the levels and functional activity of metabolic hormones that seemingly control EC levels, such as leptin, insulin, glucocorticoids and possibly ghrelin, might underlie some of the effects of obesity and hyperglycemia on EC metabolic enzymes.

The skeletal muscle is the major tissue responsible for glucose utilization and oxidation, ATP production and energy expenditure, especially during exercise, which, among other things, stimulates AMPK and the subsequent oxidation of both glucose and fatty acids. This tissue will use glucose coming from the liver or the diet and taken up via the action of glucose transporters, whose translocation to the plasma membrane of myotubes is stimulated by insulin. Despite evidence suggesting that an overactive EC system participates in decreased energy expenditure, no data on the regulation of EC levels in the skeletal muscle during hyperglycemia and obesity have been studied.

The early elevation of the levels of 2-AG, either effect of diet obesity induce or the over activity of the system in relation with the insulin resistance ambience, which was the most abundant EC in all tissues analysed,

might cause a stronger inhibition of AMPK and, subsequently, of glucose uptake and oxidation from the skeletal muscle, whereCB1 receptors are present and functionally active with subsequent overall reduced energy expenditure. The increase of the levels of 2-AG could be produced of diets with high fat concentration, and even that with high levels of linoleic acid who is a precursor for arachidonic acid, and the higher availability of the latter fatty acid might cause elevation of 2-AG levels. These effects could bring an increase of glucose levels, with a alteration of the action and response of a second peak of insulin liberation(50, 55, 59, 61, 63, 68, 69, 77, 86).

Despite the regulation of glucose balance by ES, there is little information on its impact on other hormonal systems and impact on organs like heart and kidneys that are also involved when there are disturbances in the metabolism of glucose. Some of these action could include the effects of ES on visceral adipose tissue via its action on the PPAR-gamma expression, decrease of thermogenesis by altering signals in mithocondrial futil cycle in brown adipose tissue, hyperinsulinemia, hyper lipogenesis, increase of T3 and T4 hormones, decrease in adiponectin levels and others actions. In addition, the aberrant EC levels in endocrine and non-endocrine organs involved in energy homeostasis, and in organs that are the ultimate targets of metabolism-derived pathological conditions, are involved in these conditions via potential alterations in CB1, CB2 and TRPV1 activity. This concept opens the way to new therapeutic strategies for the treatment of cardiometabolic risk based on the ECS, including, but not limited to, the use of the CB1 receptor antagonists (30).

6. CLINICAL AND EXPERIMENTAL MANIPULATIONS OF THE ECS

Endocannabinoids are released “on demand” after a triggering signal, when it is necessary to maintain homeostasis and are quickly metabolized. An altered ECS appears to contribute to the etiology of several disease states that constitute significant global threats to human health (18). Natural compounds and *de novo* sythesized substances with an affinity to cannabinoid receptors act either as agonists simulating the activity of endocannabinoids, or as antagonists preventing the binding of endocannabinoids and thus inhibiting the activity of the ECS. CBRs agonists as well as agents that might modify cannabinoid transport or metabolism and that way increase the ECS activity are likely to be used as potential hypnotics, analgesics, antiemetics, antiasthmatics, antihypertensives, immunomodulatory drugs, anti-inflammatory and neuroprotective agents, antiepileptics, drugs for treatment of glaucoma, spasticity and other “movement disorders”, eating disorders, or alcohol withdrawal (17, 18, 21, 22, 101-103) CB2 receptor modulation has been implicated in processes as diverse as analgesia, hepatic fibrosis, bone growth, and atherosclerosis (104).

Experimental and clinical evidence supports the therapeutic use of CB1 receptor antagonists to treat overweight/obesity, obesity-related cardiometabolic disorders, and substance abuse (105). Laboratory data suggest that CB2 receptor antagonists might be effective immunomodulatory and, perhaps, anti-inflammatory drugs. One CB1 receptor antagonist/inverse agonist, rimonabant, has emerged as the first-in-class drug approved for weight control. Related drugs (taranabant, otenabant, surinabant, rosonabant, SLV-319, AVE1625, V24343) have also been studied in the clinic recently (105). The results of the RIO studies (the paradigm trials in the Rimonabant use) and others show effectiveness in weight loss; however, side effects such as nausea and psychiatric alterations, including depression and anxiety, have been reported. These side effects have led the FDA (Food and Drug Administration) to not approve this drug in the United States and suspension of rimonabant in the European Union. For a more complete evaluation on the safety of this drug, additional studies are in progress (87, 106).

Novel CB1 receptor ligands that are peripherally directed and/or exhibit neutral antagonism (the latter not affecting constitutive CB1 receptor signaling) may optimize the benefits of CB1 receptor antagonists while minimizing any risk. CB1 receptor-neutral antagonists appear from preclinical data to offer efficacy comparable to or better than that of prototype CB1 receptor antagonists/inverse agonists, with less propensity to induce nausea. Continued pharmacological profiling prior to human use of CB1 receptor antagonists with unique modes of targeting/pharmacological action, represents an important and critical path to CB receptor blockers therapeutical use (107). Some experimental data suggests that exogenous CBRs ligands modulates the ECS activity and in some pathological states such as multiple sclerosis, certain types of pain, cancer, schizophrenia, post-traumatic stress disorders, some intestinal and cardiovascular diseases, excitotoxicity and traumatic head injury, the up regulation of the ECS may cause a reduction in the severity of symptoms or a slowing of disease progression. However, there are other disorders as impaired fertility in women, obesity, cerebral injury in stroke, endotoxaemic shock, cystitis, ileitis and paralytic ileus, in which the unwanted effects appear to result from the up regulation of the ECS, suggesting that one has to be cautious about the unwanted effects of their use (17).

Selective inhibitors of EC degradative metabolism and transport prolong the actions of AEA and 2-AG. The compound URB597, inhibits FAAH activity, causes increments on brain AEA levels and produces marked anxiolytic-like and antidepressant-like effects in rats and mice (108). Selective inhibitors of MAGL, exhibited interesting *in vivo* analgesic and anti-inflammatory properties, suggesting that they may be valuable agents for the treatment of inflammatory pain(13, 93) . Many exhaustive revision of compounds active on ECS whit promisory therapeutical aplications have been published (13, 17, 21, 103, 104, 106, 109-111).

The intense pharmacological research, development and essay of new drugs targeting on ECS might lead in the near future to new classes of pharmaceuticals for several diseases.

7. MODULATION OF PUFAS AND THEIR EFFECTS ON THE ECS

Changes in dietary lipids modify the FA composition of membranes. This can significantly affect the fluidity of membranes, which in turn, influences the morphology and behaviour of membrane-associated enzymes, receptors and EC synthesis (47, 112-115). The arachidonic acid, a major precursor of EC, tissue concentration may affect EC levels and function (47, 65, 68, 88, 116-119). Inclusion of AA to the diet, in mouse and suckling pigs, significantly increased EC and N-acylethanolamines levels in several regions of the brain (117). Furthermore, it was reported that mice fed high levels of long-chain (n-3) fatty acids had significantly lower 2-AG concentrations in the brain, and diets deficient in docosahexaenoic acid (DHA) produced higher levels of 2-AG(116) . In viw of the competition between (n-3) and (n-6) FA, the concentration of AA in brain phospholipids was significantly lower in the mice fed high DHA (116). Mouse adipocytes incubated with DHA, significantly decreased the proportion of AA in membrane phospholipids as well as the cellular concentration of AEA and 2-AG, while incubation with AA significantly increased phospholipids AA and the concentration of 2-AG but not AEA (54). In (n-3) long chain PUFA-supplemented obese rats, liver and heart triglycerides and the peritoneal macrophage response to an inflammatory stimulus were significantly lower than in rats fed the control diet. These effects were associated with a lower concentration of the AEA and 2-AG in the visceral adipose tissue and of AEA in the liver and heart, and were associated with lower levels of AA in the membrane phospholipids, but not higher activity of endocannabinoid-degrading enzymes. Data suggests that the beneficial effects of a diet enriched with (n-3) PUFA are the result of changes in membrane FA composition. The reduction of substrates for inflammatory molecules (eicosanoids) and EC may account for the dampened inflammatory response and the physiological reequilibration of body fat deposition in obese rats (26).

The tissue phospholipids FA content also tended to reflect the major FA consumed in diet (120). A comparison of five experimental diets fatty acids enriched, informed that rats fed AA had higher concentrations of AEA and 2-AG in the small intestine and higher AEA in the brain and liver, whereas rats fed fish oil fatty acids had lower concentrations of AEA in the liver and small intestine, and conclude that short-term feeding of diets resembling human diets (Mediterranean diet high in monounsaturated fat, diet high in saturated fat, or diet high in polyunsaturated fat) can affect tissue levels of EC and N-acylethanolamines (120).

Data obtained from mice fed high fat diets (HFD) with different FA compositions and impact on fasting glucose levels provide evidence on the possible modification of endocannabinois levels in organs involved in energy expenditure (brown adipose tissue and skeletal muscle), endocrine function (adrenal glands and thyroid), or affected by the consequences of metabolic disorders (heart and kidney). In the skeletal muscle, heart and kidney were found elevations or of the levels of either 2-AG or AEA, or both, by contrast were found reductions in the thyroid (68) . In the adrenal gland, first a reduction and then an elevation of EC levels were observed, and a very early elevation of both AEA and 2-AG levels was observed with one of the diets in the brown fat, whereas delayed decreases were explained by an increase of the amount of fat tissue weight induced by the HFD (68). In another study, it observed that mice fed two different amounts (and types) of HFD increased AEA and 2-AG concentration in the pancreas but decreased their concentration in subcutaneous fat, which also showed that the direction and magnitude of the modification was dependent on duration of feeding and of specific tissue analyzed, and no correlation was noted between the amount of the determined FA and tissue endocannabinoids concentration (88). The extrapolation of these results to humans should be made with caution because the HFD used in this study contained up to 60% of energy from fat that is atypical in human diets (119).

In HFD, depending on the composition of diet, changes in ECS were preceded or accompanied by the development of overt obesity and/or hyperglycemia. Existent data from experimental animal studies indicate that the level of tissue EC can be significantly affected by modulation of dietary FA. Changes in the levels of dietary fat in terms of saturated, polyunsaturated and monounsaturated seems to affect tissue levels of endocannabinoids (121), and it can mediated mainly through the availability of levels of precursor arachidonate within membrane phospholipids.

8. PERSPECTIVES AND CONCLUSIONS

Dietary intake of PUFAs results in changes in the fatty acids of cell membrane composition. Considering that both, CB receptors, and the enzymes involved in synthesis and catabolism of EC are an integral part of cell membranes, their lipid domains are specifically and strongly modified by FA composition and in turns, for the dietary supply of PUFAs. These changes in FA membrane composition also could modify EC precursors substrates availability and thus modulate their levels.

Obesity could be linked to dietary pattern abnormalities and the dysregulation of the ECS via AEA and 2-AG and their respective receptors. The relationship of ECS with obesity and type 2 diabetes are very complex and further research is needed in order to integrate the biological process with therapeutics aims to achieve effective measures to prevent the world epidemic of obesity and diabetes.

More studies are needed to clarify the relationship between EC ligands, CB receptors in target tissues such as adipose tissue, SNC, liver and pancreas. Further research is needed to understand the complex interaction among EC and bioactive endogenous sustances like insulin, adiponectin, TNF or IL-6 in order to further understand of fine regulatory mechanisms involved in development of human obesity and type 2 diabetes.

Experimental and clinical evidences suggest the existence of an internal, complex and well tuned, "endocannabinoid tone" in the body. An increased knowledge of this signal system and their regulatory mechanisms could allow advances in the comprehension of many physiological and pathological processes. This knowledge would help in the development of drugs with potent inhibitory action on ECS related enzymes and synthetic agonists/antagonists for CBRs which could serve as therapeutic tools in the management of metabolic syndrome, cancer, neurodegenerative disorders, addictions and other pathologies. However, their safe clinical application in humans should be carefully evaluated in long-time trials. Based on the various evidences presented, it is clear that healthy dietary habits of consumption of PUFAs, the precursors of ECS, can offer an alternative approach that is natural, non-pharmacological, and safe, in the prevention, improvement or treatment of diverse diseases. In particular, the potential of dietary manipulations in order to modify the "up-stream" availability of endogenous PUFAs could be a valuable tool in the treatment of obesity and type 2 diabetes.

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Abbreviations: PUFA: Polyunsaturated fatty acid, EC: endocannabinoid, ECS: endocannabinoid system, CBR: cannabinoid receptor, AA: arachidonic acid, AEA: anandamide, 2-AG: 2-arachidonoylglycerol, CNS: central nervous system, FAAH: fatty acid amide, MAGL: monoacylglycerol lipase, CCK: cholecystokinin, POMC: proopiomelanocortin, PVN: paraventricular nucleus, AgRP: agouti-related protein, CART: cocaine-amphetamine related transcript, NPY: neuropeptide Y, MCH: melanin concentrating hormone, AcbSh: accumbens nucleus, DA: dopamine, CRH: corticotrophin-release hormone, ACTH: adrenocorticotrophin hormone, HPA: hypothalamic-pituitary-adrenal**,** PYY: including peptide YY, GLP1: glucagon-like peptide, AMPK: AMP-activated protein kinase, TCA: tricarboxylic acid**,** UCP-1: uncoupling protein 1, FAA: fatty acid amides, NAPE-PLD: N-acyl-phosphatidylethanolamine phospholipase D, DAGL-alfa: diacylglycerol lipase alfa, PEA: palmitoylethanolamide, OEA: oleoylethanolamide, PPAR-gamma: peroxisome proliferator-activated receptor gamma, FFA: free fatty acid, IL-6: interleukein 6, TNF-alpha: tumor necrosis factor alpha, HFD: high fat diet, FDA: Food and Drug Administration.

Key Words: Endocannabinoid, Polyunsaturated Fatty Acids, Human Obesity, Diabetes, Endocannabinoids Precursors, Energy Balance, CB1, CB2, Rimonabant, anandamide, 2-AG, Review

Send correspondence to: Aldo Renato Eynard, Instituto de Biologia Celular, Facultad de Ciencias Medicas, Universidad Nacional de Cordoba, Enrique Barros esq, Enfermera Gordillo Ciudad Universitaria, Cordoba 5014, Argentina., Tel: 54 3547 489518, Fax: 54 3547 489518, E-mail: aeynard@gmail.com

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