Endocannabinoid System: An overview of its potential in current medical practice

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Abstract

The endocannabinoid system (ECS) is a lipid signalling system, comprising of the endogenous cannabis-like ligands (endocannabinoids) anandamide (AEA) and 2-arachidonoylglycerol (2-AG), which derive from arachidonic acid. These bind to a family of G-protein-coupled receptors, called CB_1 and CB_2. The cannabinoid receptor 1 (CB_1R) is distributed in brain areas associated with motor control, emotional responses, motivated behaviour and energy homeostasis. In the periphery, the same receptor is expressed in the adipose tissue, pancreas, liver, GI tract, skeletal muscles, heart and the reproduction system. The CB_2R is mainly expressed in the immune system regulating its functions. Endocannabinoids are synthesized and released upon demand in a receptor-dependent way. They act as retrograde signalling messengers in GABAergic and glutamatergic synapses and as modulators of postsynaptic transmission, interacting with other neurotransmitters. Endocannabinoids are transported into cells by a specific uptake system and degraded by the enzymes fatty acid amide hydrolase (FAAH) and monoacylglycerol lipase (MAGL).

The ECS is involved in various pathophysiological conditions in central and peripheral tissues. It is implicated in the hormonal regulation of food intake, cardiovascular, gastrointestinal, immune, behavioral, antiproliferative and mammalian reproduction functions. Recent advances have correlated the ECS with drug addiction and alcoholism. The growing number of preclinical and clinical data on ECS modulators is bound to result in novel therapeutic approaches for a number of diseases currently treated inadequately. The ECS dysregulation has been correlated to obesity and metabolic syndrome pathogenesis.

Rimonabant is the first CB_1 blocker launched to treat cardiometabolic risk factors in obese and overweight patients. Phase III clinical trials showed the drug's ability to regulate intra-abdominal fat tissue levels, lipidemic, glycemic and inflammatory parameters. However, safety conerns have led to its withdrawal.

The role of endocannabinoids in mammalian reproduction is an emerging research area given their implication in fertilization, preimplantation embryo and spermatogenesis. The relevant preclinical data on endocannabinoid signalling open up new perspectives as a target to improve infertility and reproductive health in humans.

REVIEW

Basic Pharmacology

Cannabinoid Pharmacology, Endogenous Cannabinoids and the Endocannabinoid Signaling System

Marijuana has been known for thousands years as a psychoactive substance altering sensory perception, relieving from anxiety and pain (Pertwee, 2008). However, it was only in 1964 when its main constituent, Δ^9-tetrahydrocannabinol (Δ^9-THC) *(figure 1)* was first isolated and stereochemically characterized among the other 60 phytocannabinoids present in *Cannabis sativa* (Pertwee, 2005). Δ^9-THC is the only one of these phytocannabinoids exhibiting anti-inflammatory, anti-convulsive and anti-emetic effects, which has been pharmacologically studied and used accordingly (Nixon, 2006). This research led to the conclusion that Δ^9-THC and its synthetic analogues elicit biological effects in a stereo-selective manner implying their binding in brain plasma membranes. These characteristics strongly suggested that cannabinoid pharmacology is receptor-mediated (Kofalvi, 2007). It was 25 years later, when the cellular target of Δ^9-THC was characterized, molecularly cloned and named cannabinoid receptor 1 (CB_1R) (Pacher, 2006) ***(table 1)***.

Further research led to cloning of one more G protein-coupled receptor (GPCR) for cannabinoids (CB), designated as CB_2 (Onaivi, 2006). In humans CB_1 and CB_2Rs receptors share ≈ 44% sequence homology. The CB_1R is mainly localized in CNS, among the cortex, cerebellum, hippocampus, basal ganglia and brain regions controlling motor, cognitive, emotional, and sensory functions. The CB_1R is also present in the brainstem, hypothalamus, and pituitary gland, regulating pain perception, hormonal activity, thermoregulation, cardiovascular, gastrointestinal and respiratory physiology. CB_1Rs at peripheral sites (e.g. adipocytes, liver, GI tract, pancreas, muscles, heart, uterus) regulate physiological processes such as energy balance and expenditure as well as lipid metabolism and reproduction. CB_2Rs are slightly expressed in the CNS and are primarily expressed by immune and hematopoietic cells, osteoclasts, and osteoblasts, mediating immune responses, inflammation, neuropathic pain, bone remodeling and metabolism (DiMarzo, 2004; Howlett, 2004; Onaivi, 2006; Pacher, 2006; Pertwee, 2006; Cota, 2007; Stern, 2007; Woelkart, 2008) ***(table 2)***.

Cannabinoid receptors, especially the CB_1R, have been uniquely preserved throughout evolution: e.g. human, rat and mouse CB_1Rs have 97–99% amino acid

Table 1: Historical discovery and exploitation of the endocannabinoid system

Cannabis research	Cannabinoid research	Endocannabinoid research
200bC: Chinese pharmacopoeia describes the therapeutic properties of cannabis **1840:** The medicinal properties of cannabis are described W.B. O' Shaughnessy **1899:** Cannabinol is isolated from cannabis resin **1932:** Partial characterization of cannabinol structure Cahn **1940:** Cannabinol synthesis Todd, Adams	**1964:** Δ^9-THC isolation and chemical characterization, the active constituent of *Cannabis sativa* Gaoni, Mechoualam **1988:** High-affinity THC-binding site is identified in rats' brain Howlett **1990:** ▪ Cloning of the rat G-protein-coupled CB_1R ▪ Cloning of the human G-protein-coupled CB_1R Matsuda **1992:** Discovery of the first endocannabinoid: AEA Mechoualam, Pertwee **1993:** Cloning of the peripheral CB_2R Munro	**1994:** Synthesis and characterization of the first CB_1R blocker, Rimonabant Rinaldi-Carmona **1995:** Discovery of the second endocannabinoid: 2-AG in brain Mechoualam, Waku **1996:** ▪ Activation of CB_1Rs found to suppress neurotransmitters release Shen ▪ Cloning of the first endocannabinoid degrading enzyme FAAH Cravatti **1998:** Evidence published on the interaction between cannabinoid and vanilloid receptors DiMarzo **1999:** AEA activates vanilloid receptors as well Zygmunt, Smart **2000:** CB_1Rs found in human vascular endothelial cells Liu **2002:** Rimonabant blocks nicotine effects on rats Cohen **2003:** ▪ Cloning of the first endocannabinoid biosynthesizing enzymes Bisogno ▪ Preclinical data that CB_1 knock out in mice leads to resistance to diet-induced obesity, increased leptin sensitivity and weight loss Bonz, Ravinet-Trillou **2004-2005:** : The RIO clinical program results are published. Rimonabant is launched in EU for the treatment of cardiometabolic risk factors in overweight and obese patients **2006- up today:** ▪ Characterization and cloning of novel non CB_1/ non CB_2 cannabinoid receptors ▪ Synthesis of novel CB_1R inhibitors, agonists and antagonists ▪ The second CB_1 selective CB_1R inverse agonist, Taranabant is synthesized and enters Phase III clinical trials Addy et al. ▪ The cannabinoid CB_1R is expressed in pancreatic delta-cells Tarp ▪ Results on Rimonabant's ability to reduce atherosclerotic process and treat fatty liver disease are published **October, 2008:** ▪ EMEA calls for marketing license suspension of Acomplia® (rimonabant)

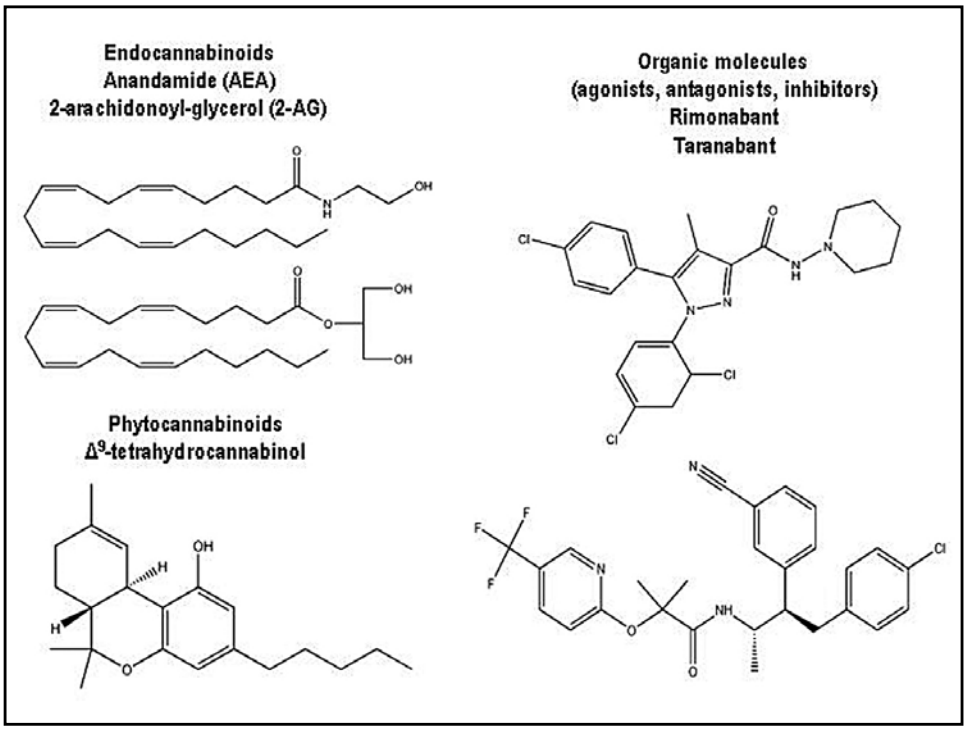

Figure 1: Chemical structures of endocannabinoids: anandamide (AEA) and -arachidononoylglycerol (2-AG); phytocannabinoid: Δ^9-THC and synthetic cannabinoids: rimonabant and Taranabant

Table 2: Major activities of the Endocannabinoid System

Central nervous system	Peripheral systems
Thalamus, hypothalamus, hippocampus Control of ▪ Pain initiation ▪ Wake/sleep cycles ▪ Thermogenesis ▪ Food intake Impairment of working memory and memory consolidation Inhibition of long-term potentiation and glutamatergic transmission	**Cardiovascular system** Decrease of blood pressure and heart rate Induction of hypotension during hemorrhagic shock or endotoxic shock Vasodilation Platelet aggregation
Basal ganglia, striatum, globus pallidus Psychomotor disorders control Interference with dopaminergic transmission Inhibition of γ-GABAergic transmission Potentiation of γ-GABA-mediated catalepsy Satiety control	**Immune system** Alteration of synthesis and secretion of ILs Stimulation of hematopoietic cell growth Inhibition of LIF release and neutrophil recruitment
Cortex, cerebellum, spinal cord Blockade of *N*-methyl-D-aspartate (NMDA) receptors Tremor and spasticity control	**Gastrointestinal tract** Inhibition of peristalsis and intestinal motility Food assimilation Satiety control Secretion of enterokines (ghrelin, PPY etc)
	Liver Control of lipogenesis and peripheral energy balance
	Adipose tissue Secretion of adipokines (adiponectin, leptin etc) and inflammatory markers (CRP, IL-6 etc) Lipid metabolism, lipogenesis, FFA oxidation Lipocytes proliferation
	Pancreas Hepatic lipogenesis Insulin sensitivity and secretion

sequence identity (Pertwee, 2008). This preservation depicts the important role kept for the ECS in human physiology. CB_1Rs are highly expressed in the brain, especially during brain development, controlling cell differentiation. The CB_1R is the most abundant G-protein-coupled receptor, with densities 10–50 fold higher than those of classical neurotransmitters (Reggio, 2005).

Both cannabinoid receptors CB_1 and CB_2 are coupled to similar transduction systems. Cannabinoid receptor activation has not been yet clarified. Initially, it was considered that this activation inhibits cAMP formation through its coupling to Gi proteins, resulting in a decrease of the protein kinase A-dependent phosphorylation processes (Poso, 2008). However, additional studies revealed that the CBRs were also coupled to ion channels through the Golf protein, resulting in the inhibition of Ca^{2+} influx. These actions are related to the ability of cannabinoids to modulate neurotransmitter release and short-term synaptic plasticity (Lopez-Moreno, 2008). Recently, it was shown CB_1Rs stimulate formation of cAMP by coupling to the Gs protein

A few years ago the existence of other endocannabinoid targets including the vanilloid receptor and at least two non-CB_1/non-CB_2 'CB-like' receptors were discovered (Brown, 2007; Hiley, 2007; Pertwee, 2007), one in the vascular bed and the other in glutamatergic axon terminals.

The discovery of the cannabinoid receptors led to the identification of a family of lipid transmitters mainly serving as natural ligands for the CB_1R: arachidonoylethanolamide (AEA), named AEA and 2-arachidonoylglycerol (2-AG) (Hillard, 2000; Basavarajappa, 2007). Soon afterwards, the complicated biochemical pathway responsible for the synthesis, release, transport and catabolism of endocannabinoids completed our knowledge of the new signalling system nowadays known as the 'endocannabinoid system'.

More than 5000 scientific reports have been published the last years, exploring the functions of the endocannabinoid system, which is currently considered as a major modulator of physiological functions in the central and autonomic nervous system, the endocrine network, the immune system, the gastrointestinal tract, the reproductive system and in microcirculation (total reference list).

Endocannabinoids are derivatives of arachidonic acid conjugated with ethanolamine or glycerol. So far, four of them have been characterized: AEA, 2-AG, virodhamine and noladin ether (Bisogno, 2008; Ho, 2005).

In the brain, AEA concentration is 200-fold lower than that of 2-AG. The monoglyceride 2-AG is a metabolic intermediate in lipid metabolism whereas AEA is the cleavage product of a membrane phospholipid (Smita, 2007).

In contrast to other classical neurotransmitters that remain stored within intracellular vesicles awaiting mobilization, endocannabinoids are only synthesized "on demand" in response to stimulus-induced intracellular Ca elevation (Vaughan, 2005).

AEA is formed by the cleavage of a phospholipid precursor called *N*-arachidonoyl-phosphatidylethanolamine (NAPE). This is synthesized by the enzyme *N*-acyltransferase (NAT), which catalyses the transfer of arachidonic acid from phosphatidylcholine to the head group of phosphatidylethanolamine (Paradisi, 2006).

This enzyme needs the presence of Ca^{2+} and is regulated by cAMP, which enhances the activity of NAT by phosphorylation mediated through the cAMP-dependent activity of protein kinase A. The derivation of AEA from NAPE is also catalysed by a specific phospholipase D, whose activity is regulated by several mechanisms like depolarization or by activation of the ionotropic glutamate *N*-methyl-*D*-Aspartate (NMDA) receptors or by nicotinic α7 neuronal receptors or via stimulation of the receptors of dopamine, glutamate or acetylcholine (Paradisi, 2006).

2-AG synthesis is mediated by the metabolism of triacylglycerol. The major synthetic pathway for 2-AG synthesis includes the 2-arachidonoyl-phosphatidylinositol hydrolysis by phospholipase C (PL-C) to diacylglycerol (DAG), which is further hydrolysed to 2-AG by DAG lipase or, in some tissues, by phospholipase A1 and subsequent lysophospholipase activity (Hermann, 2006).

A two-step process including transport into cells and hydrolysis completes endocannabinoid signalling. Endocannabinoid levels in tissues are controlled via their rapid degradation (Bari, 2006).

Endocannabinoids are removed from their sites of action by cellular uptake. In most tissues, AEA is metabolized by a membrane-bound amidase, belonging to the serine–hydrolase family: fatty acid amide hydrolase (FAAH) (Puffenbarger, 2005; Maccarone, 2006; Fowler, 2007; Labar, 2007;Saario, 2007; Vandevoorde, 2008), and 2-AG by a serine hydrolase, the soluble monoacylglycerol lipase (MAGL) (Jhaveri,2007; Saario,2007; Viso, 2008). FAAH degrades many fatty acid amides and although it can also deactivate 2-AG, the main enzyme responsible for the inactivation of this monoglyceride remains MAGL. A number of oxidative enzymes including lipoxygenases, cytochrome P450s, and cyclooxygenase-2 transform endocannabinoids into eicosanoid-related bioactive products. Within most brain areas, MAG lipase and CB_1Rs (and far fewer CB_2Rs) are localized presynaptically, whereas FAAH is predominantly postsynaptic in somata and dendrites of principal neurons. This distribution of the ECS components reveals the endocannabinoid synthesis in postsynaptic neurons, via the stimulation of intracellular Ca^{2+} increase and membrane phospholipid hydrolysis (Howlett, 2004; Reggio, 2005; Pacher, 2006; Pertwee, 2006; Brown, 2007).

Endocannabinoids are then released/transported into the synaptic cleft to act on pre-synaptic neurons. In this retrograde manner, endocannabinoids are bound

to regulate the synaptic transmission of excitatory and inhibitory neural circuits by modulating neurotransmitter release.

Endocannabinoids exhibit different binding properties towards CB_1 and CB_2 receptors. AEA acts as a partial agonist for both CB_1 and CB_2 receptors, but has higher affinity for the CB_1R. Its activity on CB_1Rs is 4–30 fold higher than on CB_2Rs. 2-AG is a complete agonist for both CB_1 and CB_2 receptors, exhibiting less affinity than AEA (Macki, 2008).

Metabolic functions

Endocannabinoid-mediated hypothalamic control of food-intake

The ECS holds a regulatory role in the neuroendocrine food intake process, since CB_1Rs are distributed in the hypothalamus in a way suggesting their effect on orexigenic or anorexigenic signals (Howlett, 2004; Onaivi, 2006; Pacher, 2006; Pertwee, 2006; Matias, 2007). In particular, CB_1Rs are found in:

- neurons of the ARC expressing cocaine- and amphetamine-regulated transcript (CART, an anorexigenic mediator). Osei-Gyiaman (2006) provided evidence that AEA elevation obtained by knocking-out FAAH, inhibits CART release in several hypothalamic regions via CB_1Rs.
- lateral hypothalamus (LHA) neurons containing the orexigenic melanin-concentrating hormone (MCH) and orexins
- the PVN, in neurons expressing the anorexigenic corticotropin-releasing hormone (CRH), whose levels are higher in CB_1-deficient mice, indicating that CB_1Rs downregulate CRH expression.

Endocannabinoids acting at CB_1Rs control homeostatic regulation of energy imbalance by stimulating the central, hypothalamic, orexigenic system and enhance food consumption by mediating motivational processes of the nucleus accumbens (DiMarzo, 2004; Howlett, 2004; Bellocchio, 2006; Onaivi, 2006; Pacher, 2006; Pertwee,2006; Cota, 2007; Stern, 2007; Woelkart, 2008).

A number of preclinical studies have been published on the mechanisms through which the ECS centrally regulates food intake (Bellocchio, 2006; Pacher, 2006; Cota, 2007; Despres, 2007).

Kirkham and Williams (2004) were the first to show the endocannabinoid presence in the hypothalamus as well as their fluctuation during feeding phases. Endocannabinoids levels are higher in rodents deprived of food for several hours compared with *ad libitum* fed animals. On the contrary, palatable foods consumption is blocked by CB_1 antagonists also in rodents fed *ad libitum*. Cota (2003) showed that endocannabinoids induce food-intake in satiated animals, when directly injected into the hypothalamus or the nucleus accumbens shell. Gomez (2002) provided evidence that the injection of endocannabinoid inactivation inhibitors in the nucleus accumbens, causes food intake increase in parallel to *Fos* expression in the arcuate and periventricular nuclei and in the dorsomedial and lateral hypothalamus.

Di Marzo (2001) correlated the variation in endocanabinoid levels with the orexigenic signals of leptin. Hypothalamic endocannabinoid levels decrease after systemic leptin administration in rats, and increase in rodent models of congenital hyperphagia and obesity, such as db/db, ob/ob mice and Zucker rats.

Moreover, activation of presynaptic CB_1Rs located on GABA terminals (Foldy, 2006), decreases GABA release onto MCH-releasing neurones of the lateral hypothalamus in cases of hyperfagia. The leptin receptors activation on these neurones inhibits endocannabinoid biosynthesis. Perifornical lateral hypothalamic neurones in ob/ob mice (leptin-deficient) exert larger Ca currents, consistent with upregulated endocannabinoid signalling, enhanced excitability and consequent hyperphagia.

Given that leptin stimulates POMC/CART-expressing neurones, which mediate melanocortins, a potential effect of the latter on endocannabinoid levels has been declared (Matias, 2008). Verty (2004) showed that Δ^9-THC-induced food intake was not blocked by α-MSH, whereas rimonabant enhanced the stimulatory effect on feeding by an experimental MCR-4 antagonist (JKC-363). However, Hentges (2005) contradicts the above assumptions suggesting that POMC-expressing neurones are modulated by CB_1Rs via the inhibition of GABAergic or glutamatergic inputs.

Valassi (2008) has associated some more neuropeptides regulating food-intake, e.g. AGRP, or POMC with CB_1Rs ***(table 3)***. Gamber (2005) correlated NPY levels with the stimulation or blockade of hypothalamic CB_1Rs. Although CB_1 and NPY receptors do not co-exist, CB_1 blockade reduces food intake in NPY knockout mice as efficaciously as in wild-type mice suggesting that NPY signalling is not necessary for CB_1-mediated food-intake.

Peripheral endocannabinoid control of energy metabolism

In 2003, Cota *et al.* demonstrated the presence and role of CB_1Rs in mouse adipocytes and adipose tissue and Spoto *et al* reconfirmed. Ever since, research on the periperal actions of the ECS have been profound, adding on to the regulatory role of the system on major endocrine organs like the liver, the pancreas and even the skeletal muscles ***(figure 2)***. The ECS presence has been verified in both human and rodent white adipocytes and adipose tissue (Bensaid, 2003; Onaivi, 2006; Pacher, 2006; Roche, 2006; Despres, 2007; Gonthier, 2007; Kovalfi, 2007; Matias, 2007; Matias, 2008;Valassi, 2008). So far, it has been demonstrated that the ECS is involved in FFA synthesis and therefore in cholesterol metabolism, in adipokines biosynthesis as well as in their signalling pathways where applicable, in glu-

Table 3: Hormones, neuropeptides and neurotransmitters known to affect both metabolic and reproductive functions

Name	Site of derivation	Metabolic effect	Reproductive effect
AgRP	**Hypothalamus**	Decrease of insulin sensitivity and energy expenditure	Decrease of GnRH and LH
α-MSH		Increase of insulin sensitivity and energy expenditure	Increase of steroidogenesis Decrease of LH and ovulation
β-endorphin		Increase of energy expenditure Insulin sensitivity and secretion Glucose availability	Decrease of ovulation, GnRH, LH, steroidogenesis
CART		Decrease of insulin secretion Increase of lipolysis and GH	Increase of GnRH Decrease of estradiol levels
CRH		Increase of energy expenditure Decrease of GH	Decrease of GnRH and LH
NPY		Decrease of energy expenditure, insulin sensitivity and secretion	Increase of steroidogenesis Decrease of GnRH and LH
Orexins		Increase of insulin secretion and energy expenditure	Increase of GnRH and LH
Catecholamines	**Adrenals**	Decrease of insulin sensitivity Increase of glucose availability and energy expenditure	Increase of GnRH and steroidogenesis
Cortisol		Decrease of insulin sensitivity Increase of glucose availability, lipolysis, proteolysis, energy expenditure	Decrease of GnRH, LH/FSH, ovulation and steroidogenesis
Adiponectin	**Adipose tissue**	Increase of insulin sensitivity Energy expenditure Lipolysis Decrease of GH	Decrease of LH and GnRH-stimulated LH secretion
Leptin		Increase of insulin sensitivity and energy expenditure, regulation of food intake	Increase of GnRH, LH/FSH, ovulation, implantation Cycle-dependent effect on steroids levels
Resistin		Decrease of insulin sensitivity in obese	Prevents ovulation and implantation
IL-6		Increase of energy expenditure Decrease of insulin sensitivity Inflammation inducer	Decrease of LH, ovulation and oestrogen levels
PAI-1		Decrease of insulin sensitivity and energy expenditure	Increase of ovulation rates
TNF-α		Increase of lipolysis and insulin sensitivity	Decrease of GnRH, LH, steroidogenesis
Ghrelin	**GI tract- stomach**	Increase of glucose oxidation, lipogenesis, energy expenditure and GH Food intake regulator	Decrease of GnRH, LH/FSH and implantation rates
CCK		Increase of insulin secretion and energy expenditure	Increase of GnRH, LH/FSH
PYY-36		Increase of insulin secretion and sensitivity, lipolysis and glucose availability Food intake regulator	Decrease of GnRH, LH
GLP-1		Decrease of insulin sensitivity Increase of insulin secretion and energy expenditure	Increase of GnRH, LH
GIP		Increase of insulin secretion and sensitivity	Decrease of FSH
Insulin	**Pancreas**	Increase of glucose storage and uptake and protein synthesis Decrease of lipolysis	Increase of GnRH and steroidogenesis Hyperinsulinemia induces hyperandrogenemia and PCOS

Abbreviations: AgPR= Agouti Related Peptide; MSH= Melanocyte Stimulating Hormone; CART= Cocaine and Amphetamine Regulated Transcript; CRH= Corticotropin Releasing Hormone; NPY=Neuropeptide Y; IL-6= Interleukin –6;TNF-α= Tumor Necrosis Factor- alpha; PAI-1= Plasminogen Activator Inhibitor 1; CCK= Cholecystokinin; PYY-36= Pancreatic Peptide YY_{3-36}; GLP-1= Glucagon-like Peptide-1; GIP= Gastric Inhibitory Polypeptide; GH= Growth Hormone; LH= Lutenizing Hormone; GnRH= Gonadotropin Releasing Hormone; FSH= Follicle Stimulating Hormone

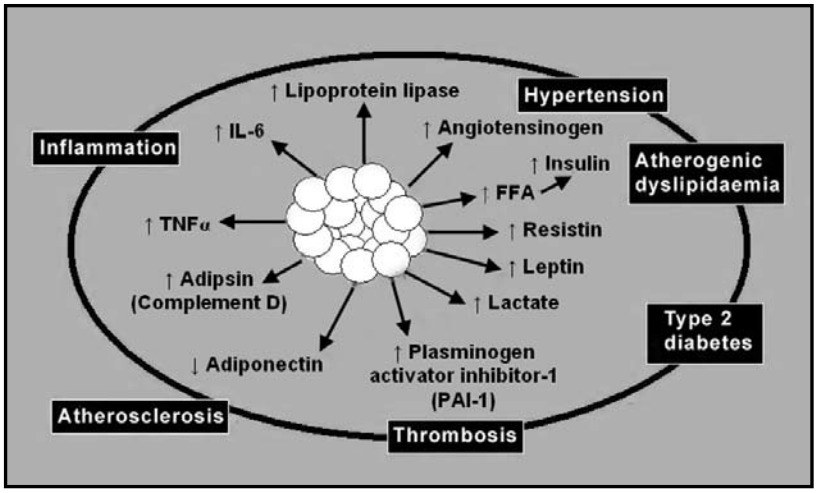

Figure 2: Cardiometabolic risk factors promoted by intra-abdominal adiposity

cose metabolism, insulin sensitivity and inflammation processes.

In his experiment, Cota (2003) showed that wild-type mice exhibit much higher amounts of fat mass in comparison with $CB_1^{-/-}$ mice as well as a tedency for lower energy even when they are equally fed with transgenic mice. A relationship between CB_1Rs presence and stimulation of lipoprotein lipase activity was also established (Bari, 2006), substantiating the ECS involvement in fat accumulation, regardless of the intaking food quantities. In addition, several other evidence (Cota, 2003; Jbilo, 2005) link the ECS functions with adipose tissue regulation. For example:

- CB_1Rs blockade arrests adipocyte proliferation
- endocannabinoids synthesis precedes pre-adipocyte differentiation
- CB_1Rs chronic stimulation during adipocyte differentiation enhances PPAR-γ activity.

The ECS takes part in adipogenesis and fat accumulation. Yan (2007) discovered that prolonged high-fat diet is associated with CB_1Rs expression in adipose tissue and contributes to adipocyte size. What is even more important, Osei-Hyiaman (2005) showed that CB_1Rs stimulate the expression of the steroid regulatory element-binding protein-1c (SREBP-1c), an important transcription factor and its targets, acetyl-CoA carboxylase-1 (ACC1) and fatty acid synthase (FAS).

Bensaid (2003), Roche (2006), Gonthier (2007) and Matias (2008) have also supported the ECS direct effect on lipogenesis by showing that the CB_1R blockade leads to an increase of adiponectin expression in the adipose tissue of obese Zucker (fa/fa) rats. Adiponectin is a 30-kDa protein exclusively produced by adipocytes (Antuna-Puente, 2008; Beltowski, 2008). Its levels are decreased in obesity. Its main functions include enhancement of insulin sensitivity, increase of fatty acid oxidation and glucose uptake and finally the suppressing of hepatic glucose production. In all, adiponectin enhances insulin sensitivity (Kong, 2006) ***(table 4)***. Rimonabant (the first CB_1R antagonist) induces adiponectin overexpression in the mouse adipocyte cell line but not in adipocytes from CB_1R knockout mice. Since adiponectin stimulates AMPK activity, the CB_1-mediated inhibition of its expression in adipocytes might also be implicated in lipogenesis regulation (Mathieu, 2008).

Endocannabinoids are able to enhance glucose uptake in adipocytes via the stimulation of basal and insulin-induced translocation to the plasma membrane of the GLUT-4 (Bari, 2006; Gasperi, 2007; Pagano, 2007) and this may be another pathway through which they trigger lipogenesis. The subsquent glycolysis in combination with FAS activity assist the adipocyte with biosynthetic precursors for *de novo* fatty acid biosynthesis and partially account for the pro-lipogenetic effect of CB_1R agonists in these cells.

On the other hand, endocannabinoids might also negatively affect fatty acid oxidation. It has been demonstrated that CB_1Rs chronic blockade by rimonabant enhances fatty acid oxidation and energy expenditure in diet-induced obese, mice possibly by up-regulating the expression of the enzymes involved in fatty acid oxidation such as carnitine acetyltransferase (CAT), carnitine palmitoyltransferase-2 (CPT2), and crotonase, as well as enzymes involved in the TCA cycle, such as fumarase, aconitase, and oxoglutarate dehydrogenase (Bensaid, 2003; Jbilo, 2005; Osei-Hyiaman, 2005; Roche, 2006; Gonthier, 2007; Yan, 2007Matias, 2008).

The ECS is thought to have a stimulatory effect on adipocyte differentiation as established by the progressive increase of CB_1R expression in adipocytes through their differentiative process. Gasperi (2007) has provided some interesting data on this issue while working with 3T3-L1 adipocyte cell line. AEA increased the PPAR-γ, a well known marker of adipogenesis, as well as *in vitro* differentiation of adipocytes, transport rate, hydrolysis and binding efficiency of AEA to CB_1Rs, suggesting a positive role of the ECS in adipocyte differentiation. Bellocchio (2006) has confirmed the above by

stimulating pre-adipocytes with a CB_1 agonist and one antagonist. The former up-regulated whereas the latter (rimonabant) downregulated CB_1R mRNA expression.

The interaction of endocannabinoids with PPARs

PPARs are a family of nuclear receptors acting as transcription factors and comprise of different isoforms (PPARα, PPARγ and PPARδ) that interact with the retinoid X receptor and a number of nuclear regulatory proteins. PPARs bind to retinoid X receptor (RXR) to form heterodimers that bind to DNA response elements. The regulation of PPAR target genes is involved in energy homeostasis, fat cell differentiation and inflammation, while PPARα and PPARγ isoforms are mainly involved in lipid metabolism regulation by controlling fatty acids catabolism, inducing fat cell differentiation and lipid accumulation and improving insulin sensitivity (Ferre, 2004; Kuusisto, 2007; Kawada, 2008).

Natural ligands for PPARs include fatty acids and eicosanoids and it is therefore possible that endocannabinoids, whose chemical structure derives from arachidonic acid, might also act through PPARs. O'Sullivan (2007) has actually proposed an interaction between endocannabinoids and PPARs that may be mediating feeding behaviour and lipid metabolism. Several authors (Guzman, 2004; Bouaboula, 2005; Burstein, 2005; Lenman, 2007; Sun, 2007) have shown that AEA and 2-AG bind to both PPARα and PPARγ at physiological concentrations.

Endocannabinoids (Sun, 2007) do not only act as ligands of PPARs. Chronic stimulation of cannabinoid receptors in 3T3-F442A pre-adipocytes stimulates PPARγ, while rimonabant reverses the effect. Furthermore, stimulation of human pre-adipocytes with CBR agonists increases PPARγ mRNA expression occuring in early stages of differentiation, whereas rimonabant inhibits the induced upregulation of PPARγ, indicating that this effect is mediated by CB_1Rs (Lenman, 2007).

In contrast to the above, it has been shown (Bouaboula, 2005) that stimulation of human adipocytes during differentiation with rosiglitazone, reduces the mRNA expression of CB_1R and upregulates FAAH, assuming that endogenous cannabinoids cooperate with PPARγ to enhance early stages of fat cell differentiation. A negative feedback between PPARγ and endogenous cannabinoids would control lipid deposition, whereas leptin at late stages of differentiation would inhibit both endocannabinoids and PPARγ.

PPARδ is another potential potential regulator of metabolic function and there may be a link between ECS and PPARδ(Guzman, 2004; Sun, 2007). High-fat feeding increases CB_1R expression and reduces PPARδ, whereas exercise exerts opposite effects on both CB_1R and PPARδ. In addition, silencing PPARδ by RNA interference increased CB_1R expression while overexpression of PPARδ significantly reduced CB_1R expression.

ECS in the pancreas

Juan-Pico (2006) first reported on the existence of cannabinoid in freshly dissociated pancreatic islets from mice. It was observed that CB_1Rs are mainly expressed in non-β-cells whereas CB_2Rs occur in both β-cells and non-β-cells. Later, the same group reported that in rats, both CB_1Rs and CB_2Rs are expressed in both β- and non-β-cells(Bermundez-Silva, 2007). Nakata and Yada (2008) showed that CB_1Rs are expressed in almost the entire area of the islet, including the central portion, possibly corresponding to β-cells. A strong staining was also observed in the periphery of the islet, suggesting that CB_1Rs are present in both α- and β-cells. Matias (2006) investigated the cannabinoid receptor localisation in the mouse pancreas and observed CB_2 staining in both insulin-producing β-cells and glucagon-producing α-cells, whilst CB_1 staining was almost exclusively present in α-cells. Additionally Matias (2008) observed CB_1 staining in several β-cells in rats. Starowicz (2008) showed that β-cells selectively express MAGL and FAAH, while DAG and NAPE-PLD are mostly abundant on α-cells.

Although no studies on human pancreas have been reported, Matias (2006, 2007) did prove that the occurrence of hyperglycaemia and obesity is accompanied by the over-expression of biosynthetic endocannabinoid enzymes and by a decrease of FAAH expression in β-cells. However, the high levels of CB_1Rs in rat insulinoma cells imply that their expression in β-cells depends on the animal species and cell differentiation and/or metabolic state.

Regarding the ECS effect on insulin release, opposing studies have been realeased. Juan-Pico (2006) and Bermundez-Silva (2008) showed that stimulation of CB_2Rs reduces insulin release via calcium transients inhibition in Langerhans' islets from lean, normoglycaemic mice, whereas CB_1Rs, exert the same effect to a much lesser extent. Nevertheless, other authors (DePetrocellis, 2007; Matias, 2008; Nakada, 2008) suggest that CB_1, but not CB_2, receptors are coupled to inhibition of both insulin release and Ca^{2+} transients in mouse pancreatic islets, since the stimulation of CB_1Rs by selective CB_1 agonists were shown to inhibit the glucose-induced insulin release and to reduce the $[Ca^{2+}]_i$ pattern.

Under conditions mimicking hyperglycemia, insulin does not inhibit glucose-induced endocannabinoid upregulation but increases endocannabinoid levels *per se*. In agreement with the above, increased levels of both AEA and 2-AG have been found in the pancreas of hyperglycaemic DIO mice in comparison to mice fed a normal diet (Jesudason, 2008; Matias, 2007). The ECS overactivity in the pancreas probably affects insulin (and also glucagon and GLP-1 levels) and glucose distribution and metabolism (Jellinger, 2007). Furthermore, it might affect adipoinsular interactions, thus contributing to insulin resistance.

The ECS effect on glucose homeostasis *in vivo* has been studied by Bermudez-Silva (2008) and Lafontan

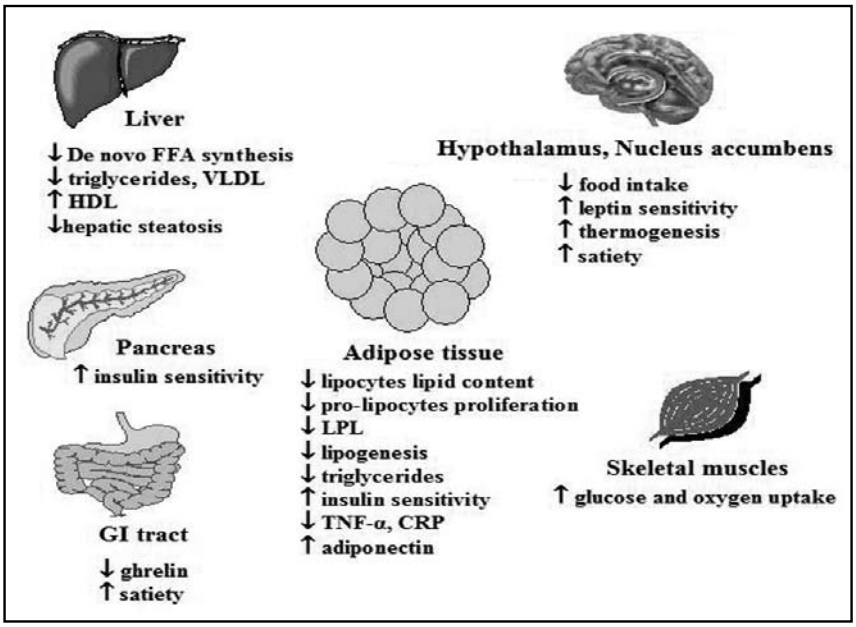

Figure 3: Sites of CB_1Rs and potential effects of CB_1R blockade.
Patients with excess visceral fat exert increased plasma level endocannabinoids (ECs), which increase food intake through a central hypothalamic-mediated mechanism. In addition, through CB_1R expression in adipocyte, ECs are decreasing adiponectin production. In the liver, high expression of CB_1R increases FFA synthesis thus enhancing the production VLDL and LDL particles while lowering HDL particles along with triglycerides increase. The ECS regulation prevents cardiometabolic risk factors accumulation, thus disabling the progression of type 2 diabetes and coronary artery disease.

(2007) who reported that systemic CB_1R stimulation in lean normoglycaemic rats, causes slower blood glucose clearance *in vivo* and that activation of CB_2Rs improves glucose clearance. The simultaneous activation of CB_1 and CB_2Rs results in a null net effect on glucose levels, thus leading the authors to propose that CB_1 and CB_2 have opposite roles in glucose homeostasis, suggesting a coordinated action of both receptors in the regulation of glycaemia.

Ravinet Trillou (2004) studied the effect of CB_1R blockade in a mouse model of diet-induced obesity. During a 5-week treatment period, rimonabant managed to reduce food intake in the first week, producing remarkable weight loss. CB_1R antagonist treatment also decreased fasting glycaemia in high fat diet (HFD) mice. The same author has reported on rimonabant's effect to enhance lipolysis on treated mice (Jbilo, 2005). These data imply that CB_1R blockade modulates insulin sensitivity and glucose homeostasis ***(figure 3)***.

Correlation of ECS overactivity and obesity pathogenesis

Hypothalamic levels of AEA and 2-AG are increased in Zucker rats and ob/ob and db/db mice (DiMarzo, 2001; Howlett, 2004; DiMarzo, 2004; Pacher, 2006; Despres, 2007; Lafontan, 2007; Matias, 2007). Respectively, blood endocannabinoid levels are high in patients with type 2 diabetes (T2D) (DiMarzo, 2008). These findings suggest that the malfunctioning of leptin and insulin signalling might be related to the ECS overactivity in obesity.

In addition, the phenotypic missense mutation of FAAH resulting to its rapid degradation in people with BMI >25, substantiates the need for an overactive ECS in order to maintain a stable level of the catabolizing endocannabinoids (Bari, 2006; Basasavarajappa, 2007; DiMarzo, 2008). Continuous ECS overactivity though, is positively signalling the intake of palatable food, therefore enhances obesity. Obese postmenopausal women bear AEA increased blood levels which are inversely correlated with reduced FAAH mRNA in adipose tissue, on the contrary, elevated AEA levels in the liver of DIO mice are followed by decreased FAAH expression (Puffenbarger, 2005; Maccarrone, 2006; Labar, 2007; Engeli, 2008).

A correlation between endocannabinoid levels and type of food intake has also been suggested. Diets rich in ω6-polyunsaturated fatty acids (PUFAs) and poor in ω3-PUFAs have shown to increase AEA and 2-AG levels in postnatal and adult brain, respectively (Matias, 2007; Watanabe, 2003). The explanation for that lies within the ability of certain free fatty acids to alter the levels of phospholipid precursors of AEA and 2-AG. The endocannabinoid metabolism is therefore affected causing the system to remain continuously active. Recent evidence showed that the remodeling of the amide-linked fatty acids of N-acyl-phosphati- dyletha-nolamines (i.e. AEA precursors) is responsible for the opposite effects of food deprivation and refeed-ing in the small intestine (Matias, 2007; Petersen, 2006).

Several studies involving humans link ECS overactivation with obesity. Monteleone (2005) was the first to determine that young obese women with binge eating disorder (BED), exert high levels of circulating AEA. In the same study, AEA levels were also found increased in patients with anorexia nervosa. A few months later, Engeli (2005, 2008) found increased AEA and 2-AG concentrations in the plasma of menopausal obese women in comparison to lean women of the same age. AEA levels were correlated to decreased FAAH mRNA expression in the subcutaneous adipose tissue depot. Finally, Matias (2007, 2008) have found increased AEA

and 2-AG concentrations in the plasma of obese diabetic subjects.

Bluher (2006) and Cota (2006)in their own studies, measured both adipose tissue distribution and insulin sensitivity or glucose tolerance on obese patients. In the first study, 2-AG plasma concentrations were correlated with visceral adipose tissue mass, whereas obese subjects with subcutaneous adipose tissue accumulation were not different from the lean control group with regard to 2-AG plasma concentration. AEA on the other hand, was higher in women than in men, but no correlation with obesity or body fat distribution was assigned. The second study established a relationship between 2-AG levels and increased visceral adipose tissue mass regardless of the BMI of the subjects. AEA was negatively correlated with visceral fat mass.

The above studies implicate increased 2-AG plasma levels with fatty acids and glucose metabolism dysregulation.

Plentiful *in vitro* data also support the link between ECS overactivation and abdominal obesity (DiMarzo, 2004; Engelli, 2005; Bari, 2006; Bluher, 2006; Kyrou, 2006; Pacher, 2006; Despres, 2007; DiMarzo, 2008; Engeli, 2008). For example, increased concentrations of 2-AG in visceral adipose tissue have been associated with morbid obesity (Matias, 2007, 2008) while the gene expression of the enzymes participating in the metabolism of 2-AG, does not change in obese subjects.

Many authors (Engeli, 2005; Monteleone, 2005; Bluher, 2006; Pacher, 2006; Despres, 2007; Matias, 2007, 2008; DiMarzo, 2008; Engeli, 2008) report on the decreased mRNA levels for both CB_1 and FAAH genes in subcutaneous and visceral adipose tissue depots of obese subjects independent of fat-distribution phenotype, thus suggesting that EC metabolism interefers with obesity pathogenesis

Regarding the potential genetic association of the ECS with obesity, the C385A missense mutation of the FAAH gene, which is associated with decreased FAAH stability and activity, has been linked to obesity prevalence (Sipe, 2005). This missense AA genotype has been found more frequent in obese Caucasians (4.8%) than in lean controls (2.1%). However, contradicting observations were made for African–American subjects (Jensen, 2007). The gene encoding CB_1 (*CNR1*) has been studied but so far, its known polymorphisms have not been linked to obese phenotypes (Russo, 2007).

Cardiovascular functions

Both endogenous and synthetic cannabinoids exert noticeable cardiovascular effects. CB_1Rs have been detected in the human, rat, and mouse myocardium mediating negative inotropy, which leads to vasodilation and eventually to a hypotensive effect of AEA in anaesthetized rodents (Sarzani, 2008). Upon injection of AEA, there is a vagally mediated period of bradycardia and hypotension, which is followed by a brief, pressor reaction, which then gives way to a prolonged hypotensive effect. This third phase is prevented by selective CB_1 blockade. AEA-induced cardiovascular effects appear to be absent in CB_1 knockout mice (Stein, 1996).

CB_1Rs stimulation in sympathetic nerve terminals inhibits norepinephrine release, thus contributing to the bradycardic effects of AEA *in vivo* (Ashton, 2007).

A vasorelaxant effect of endocannabinoids and their synthetic analogues *in vitro* has been reported and associated with CB_1 and $TRPV_1$ receptor- and NO-mediated or NO-independent mechanisms (Randall, 2007; Sarzani, 2008)

During shock states, platelets and macrophages exert elevated levels of endocannabinoids and their injection into normal rodents resulted in CB_1 mediated hypotension. CB_1R blockade prevented or reversed the hypotension associated with shock states; however, mortality was increased in the setting of cardiogenic shock, suggesting endocannabinoid-mediated vasodilation may be protective by improving tissue oxygenation and countering vasoconstriciton mediated by increased sympathetic tone (Grassi, 2008).

In a recent experiment, Nieswandt (2005) showed that the injection of platelets and macrophages into normal rodents during shock states, resulted in CB_1 mediated hypotension, while CB_1R blockade prevented or reversed the effect. However, mortality was increased in the setting of cardiogenic shock.

Batkai and Patcher (2005) showed that spontaneously hypertensive rats exert an upregulation of cardiac and vascular CB_1Rs compared to normotensive controls, while AEA administration was found to induce a larger and longer lasting hypotensive effect in these rats compared to controls. Furthermore, Patcher (2005) showed that AEA and FAAH transport inhibitors do not lower blood pressure in normotensive animals and FAAH deficient mice.

The above suggest that the ECS is mainly inactive under normal hemodynamic conditions and gets activated when the body finds itself in a stressful situation.

CB_2Rs have been assigned with a role in the atherosclerotic progression given their ability to modulate immune functions. (2005) used the apolipoprotein E knockout mouse model of atherosclerosis, to show that orally administered THC inhibits disease progression. CB_2 receptors expressing immune cells were found in human and mouse atherosclerotic plaques. Lymphoid cells isolated from THC-treated mice exhibited no proliferation capacity and decreased interferon-γ production. A selective CB_2R antagonist blocked these effects, suggesting that targeting CB_2Rs may offer a new approach in the treatment of atherosclerosis. The influence of the ECS on adiponectin ***(table 4)*** may also constitute a mechanism linking cannabinoids to atherogenesis (Kong, 2006; Antuna-Puente, 2008; Beltowski, 2008). CB_1R activation in isolated adipocytes is known to suppress adiponectin expression, whereas CB_1R

blockade increases adiponectin secretion by stimulating adiponectin mRNA expression.

Further data also link atherosclerosis progression and ECS functions. For expample, Batkai (2004) investigated the age-associated decline of cardiac function and changes in inflammatory gene expression, nitrative stress and apoptosis in $FAAH^{-/-}$ mice compared with wild-type mice. Enhanced AEA levels in the $FAAH^{-/-}$ animals were protective, which further supports the protective role of endocannabinoids in inflammatory disorders such as atherosclerosis. Moreover, AEA dose-dependently attenuated TNFα-induced intercellular adhesion molecule-1 (ICAM-1) and vascular cellular adhesion molecule-1 (VCAM-1) expression in human coronary artery endothelial cells (HCAECs), and the adhesion of THP-1 monocytes to HCAECs in a CB_1 and CB_2 dependent manner.

Regarding direct cardiac effects, AEA dose dependently decreases contractile performance in isolated, electrically paced human atrial muscles, an effect inhibited by CB_1 antagonists. Ford (2002) using isolated, perfused, rat Langendorff heart preparations, studied AEA effect on coronary perfusion pressure and left ventricular developed pressure, suggesting the involvement of a cardiac site of action distinct from CB_1 and CB_2 receptors.

Finally, in recent study, Fajardo (2007) provided evidence that AEA is overproduced in a well-established model of DOX-induced acute heart failure, and that CB_1 antagonists administration improve compromised contractile function. In addition, CB_1 antagonists were found to exert powerful cytoprotective effect in cardiomyocytes against DOX-induced cardiotoxicity both *in vivo* and *in vitro* by reducing apoptosis. The protective effect was not observed with CB_1 and CB_2 agonists and CB_2 antagonists *in vitro*. The authors conclude that CB_1 antagonists may protect against DOX-induced cardiotoxicity by exerting potent cytoprotective effects and by antagonizing AEA-mediated cardiodepression.

Table 4: Adiponectin main properties and interventions to increase its levels

Antiantherogenic properties of adiponectin
Stimulates NO production
Suppresses: ▪ Human umbilical vein endothelial cells apoptosis ▪ Macrophage-to-foam cell transformation ▪ Proliferation and migration of smooth muscle cells ▪ Expression of growth factor in endothelial cells
Reduces expression of adhesion molecules in endothelial cells and cytokine production from macrophages
Improves endothelium-dependent vasodilation
Induces production of IL-10
Inhibits: ▪ Expression of scavenger receptor class A-1 of macrophages ▪ Proliferation of myelomonocytes and function of mature macrophages
Increases tissue inhibitor of metalloproteinase-1 expression in human monocyte-derived macrophages
Anti-diabetic properties of adiponectin
Improves insulin resistance and increases FFA oxidation
Decreases hepatic glucose production by inhibiting gluconeogenesis enzymes
Interventions to increase adiponectin levels
Lifestyle modifications Weight loss (waist circumference reduction) Smoking cessation Decrease of the sympathetic nervous system activity
Pharmaceutical treatments CB_1 blockers: Rimonabant Antihypertensives: Temocapril, Ramipril, Losartan, Candesartan, Nebivolol PPARα Agonists: Fenofibrate PPARγ Agonists: Thiazolinediones Antidiabetic drugs: Glimepiride, Metformin

Gastrointestinal functions

Cannabis has been widely used as an appetizer stimulant and in order to decrease emesis and diarrhoea.

Recent evidence has correlated ECS dysregulation with a number of liver diseases, including hepatitis, non-alcoholic fatty lever disease (NAFLD), progression of fibrosis to cirrhosis, hepatic ischemia perfusion ((I/R) injury as well as their cardiovascular-related side effects (like cirrhotic cardiomyopathy and hyperdynamic circulatory syndrome) (Caraceni, 2008). Additionally, the EC has been found to regulate the mechanisms mediating cell injury and inflammatory responses during acute liver damage(Izzo, 2008).

CB_1Rs are found in cholinergic neurons across the enteric nervous system, including sensory and interneuronal as well as motoneural cell bodies of the myenteric plexus, in mice, rats, guinea pigs, and pigs. They are also colocalized with neuropeptide Y and the intestinal peptide in the submucous plexus neurons. Both CB_1 and CB_2 receptors have been found on plasma cells in the lamina propria, whereas only CB_2R has been detected on macrophages. CB_1 mRNA is expressed in liver or in various liver cells (e.g. hepatocytes, stellate cells and vascular endothelial cells). CB_2R mRNA has been found in cirrhotic liver tissues (Howlett, 2004; Onaivi, 2006; Pacher, 2006; Despres, 2007; DiMarzo, 2008).

Endocannabinoids are also present in the gastrointestinal tact. 2-AG was originally isolated from gut tissue, while it is known that AEA levels are regulated by feeding status. In a recent study, AEA was found to mediate development of diet-induced obesity and fatty liver in mice via the increase of *de novo* fatty acid synthesis through the induction of lipogenic transcription

factor and its target enzymes (Capasso, 2008). CB_1R antagonist rimonabant arrested this effect in pretreated mice and in $CB_1^{-/-}$ knockout mice fed with a high-fat diet. Accordingly, treatment with rimonabant to obese Zucker (fa/fa) rats improved dyslipidemic parameters, reversed fatty liver and attenuated serum markers of liver injury, possibly through the reduction of TNF-α helpatic levels and an increase in plasma adiponectin levels.

Furthermore, AEA and some CB_1 agonists but not CB_2-selective agonists inhibit gastrointestinal motility in rodents *in vivo* and in isolated ileum and colon from both experimental animals and humans (Massa, 2006).

Endogenous substrates of FAAH (e.g. N-arachidonoylserotonin, palmitoyliso-propylamide, palmitoylethanolamide, oleamide, and oleoylethanolamide) also inhibit intestinal motility in wild-type but not in FAAH knockout mice.

It should be noted that in clinical trials with rimonabant, patients receiving the drug, encountered diarrhoea 2,5 times more frequently than placebo ones, suggesting accelerated transit and/or enhanced secretion caused by CB_1 blockade.

The mechanism through which CB_1Rs mediate enteric contractility and peristalsis reduction remains unclear. The effect is possibly achieved through (Caraceni, 2008):

- Reduction of acetylcholine release from enteric nerves
- Inhibition of nonadrenergic / noncholinergic excitatory transmission
- Activation of apamin-sensitive K^+ channels
- Modulation of adenosine release

The ECS has also been implicated in the regulation of gastric acid and intestinal secretions. At high doses, THC decreases histamine-induced gastric acid secretion in isolated stomach preparations and in pylorus-ligated rats. AEA, the AEA transport inhibitor VDM11 and the CB_1 agonist ACEA all inhibit intestinal secretion and fluid accumulation in mice treated with cholera toxin, whereas rimonabant exerts opposite effects (Tyler, 2000).

Several findings also support the protective role of the ECS against inflammatory bowel disease (IBD). In a mouse model of colitis induced by 2,4-dinitrobenzene sulfonic acid and dextrane sulfate, Massa (2008) confirmed the up-regulation of CB_1Rs in experimental colitis. Furthermore, it was shown that the inflammation was more severe in $CB_1^{-/-}$ knockout mice than in wild-type ones, whereas genetic ablation of FAAH resulted in protection against the chemically induced colitis.

However, Croci's (2003) study revealed contradictory results. This group reported on a CB_1R independent protective effect of rimonabant against indomethacin-induced inflammation and ulcer formation in the small intestine of rats. AEA elevated levels and desensitization of the presynaptic neural CB_1R suggests that the ECS is involved in the pathophysiology of this frequent complication of colitis and/or colon cancer.

Endocannabinoids and CB_1Rs have been implicated in the systemic and portal vasodilation and hypotension associated with chronic liver cirrhosis (Mallat, 2006, Caraceni, 2008). CB_1R blockade with rimonabant reverses hypotension and low peripheral resistance and decreases elevated mesenteric blood flow and portal pressure in rats with biliary and carbon tetrachloride-induced cirrhosis. The hemodynamic parameters remain unaffected in noncirrhotic control subjects. This implies that CB_1Rs are upregulated in hepatic vascular endothelial cells and that circulating monocytes overproduce AEA. There is recent experimental evidence implicating increased signalling through myocardial CB_1Rs in the pathogenesis of cirrhotic cardiomyopathy.

The ECS may also be involved in the pathogenesis of liver fibrosis (Caraceni, 2008; Siegmund, 2008). It was recently reported that AEA exerts antifibrogenic effects *in vitro* by inhibiting activated hepatic stellate cells at low micromolar concentrations and by inducing their necrosis at higher concentrations, via CB1/2 and $TRPV_1$-independent mechanism(s).

In another study, liver fibrosis induced by carbon tetrachloride was more severe in CB_2-/- knockout mice compared with their wild-type littermates. The expression of CB_2 receptors was strongly induced in liver biopsy specimens from patients with active cirrhosis of various etiologies. Furthermore, CB_2 receptor activation triggered growth inhibition and apoptosis in myofibroblasts and in activated hepatic stellate cells, highlighting the antifibrogenic role of CB_2 receptors during chronic liver injury (Wright, 2008).

In all, and despite the lack of knowledge regarding exact mechanisms, it is obvious, that the ECS holds an important role on the modulation of inflammatory response in a variety of liver disorders

It seems that CB_1 agonists and FAAH antagonists may afford a beneficial effect on increased gastrointestinal motility, bowel inflammation, and associated diarrhoea, whereas CB_1 antagonists may be further used in the treatment of constipation (Capasso, 2005).

In chronic liver cirrhosis, CB_1 antagonists not only improve the hemodynamic side effects, offering an improvement in the quality of life of these patients, but might also reverse fibrosis progression and neurological decline associated with hepatic encephalopathy.

A protective role has also been demonstrated for CB_1 antagonists in the treatment of obesity-associated liver diseases and related features of metabolic syndrome through the amelioration of dyslipidaemia parameters and reversion of systemic and liver inflammation (Pacher, 2006; Cota, 2007; Despres, 2007; Cota, 2003; DiMarzo, 2008) ***(figures 2,3)***.

The potential of selective CB_2R agonists lies within the protection against liver fibrosis progression and perhaps against the chronic inflammation associated with IBD, mainly through attenuation of the endothelial cell

activation/inflammatory response. In this case, lack of psychiatric side effects may constitute a further advantage (Pacher, 2006; Caraceni, 2008; Massa, 2006; Mallat, 2006). Whether the above effects are exclusively attributed to CB_1 and CB_2 receptors, or non- CB_1/non CB_2 receptors are engaged in these processes, remains to be clarified.

Immune and Behavioral functions

Depression and suicidality are mediated by the monoamine neurotransmitter pathways in the prefrontal cortex and in particularly with 5-hydroxytryptamine (5-HT) and noradrenaline (NA) levels (Wolf, 2008). Given the side-effects and pharmacological profile of currently used drug treatments, the existence of new potential therapeutic targets like the ECS have earned a growing interest for the treatment of depression, suicidality, forms of addictions and schizophrenia, given its crucial role in mood regulation, cognition, motivation and emotional behaviour (Laviolette, 2006; Vinoid, 2006; Hashimorodani, 2007; Martinez-Orgado, 2007; Mackie, 2008; Cabral, 2008).

Though the available data are limited, there is a number of preclinical and clinical data assigning the ECS with several functions in the above conditions.

The pathophysiology of depression has been linked to CB_1Rs over-expression from post-mortem studies, which demonstrated higher levels of both CB_1R and CB_1-receptor-mediated G-protein activation in the dorsolateral prefrontal cortex (DLPFC) of depressed suicide victims compared with normal controls. Additionally, the polymorphisms of the *CNR1* gene have been associated with a genetic risk predisposition for depression in Parkinson's disease (PD). PD patients with long alleles in the *CNR1* gene were found susceptible to depression, indicating a potential role of the gene in the depressive disorder (Pacher, 2006; Onaivi, 2006; Vinoid, 2006; Kovalfi, 2007).

Other studies, correlate cannabis abuse, mood alteration and the involvement of the ECS in the aetiology of schizophrenia, since long-term cannabis abuse alters cognition and attention and might provoke symptoms of anhedonia, which resemble negative symptoms of schizophrenia (Muller-Vahl, 2008). To support this, post-mortem studies have shown an over-expression of the CB_1R in the prefrontal cortex, striatum and anterior cingulate cortex of schizophrenics while enhanced CB_1-receptor signalling in the specific brain regions are associated with symptoms of psychotic and affective disorders.

Furthermore, there is evidence linking sensitization of cortical CB_1-receptor-mediated G-protein activation to suicide. Higher AEA and 2-AG levels have been observed in the DLPFC of alcoholic suicide victims (Hashimotodani, 2007; Wolf, 2008).

Vinod (2005) reported an increase in CB_1R mRNA in the dorsolateral prefrontal cortex of subjects with a lifetime diagnosis of major depression that committed suicide, compared to normal controls that died by accident or natural causes. Hill (2008) reported on reduced 2-AG serum levels in drug-free females diagnosed with major depression compared to demographically matched controls. 2-AG levels were negatively correlated to the duration of depressive episode, while serum AEA was not associated with major depression, but was negatively correlated with anxiety parameters.

Pharmacological studies have revealed the importance of the ECS in depressive-like responses in rodents (Laviolette, 2006; Vinod, 2006; Hashimotodani, 2007; Martinez-Orgando, 2007; Cabral, 2008; Mackie, 2008). For example, rimonabant bears antidepressant-like effects in animal models. In addition, the same agent yields antidepressant activity similar to that of fluoxetine in various animal models of depression. CB_1 knockout mice display increased anxiety-like behaviour compared to wild-type controls under stressful conditions. Additionally, CB_1R knockout mice have increased sensitivity to develop anhedonia in the CUS model of depression, and display several other behavioral responses similar to the symptoms of melancholic depression.

It is not yet clear how this effect is yielded but according to some theories, rimonabant increases 5-HT, NA and dopamine levels in the prefrontal cortex by enhancing the AEA–CB_1R signaling pathway, therefore regulating mood behaviour. However, it should be noted, that in clinical trials of rimonabant for the treatment of obesity, anxiety and depression are among the most frequent adverse events (Xie, 2008).

Stress-induced alterations in the central ECS might also be associated with depression mood changes. Hill (2005) demonstrated downregulation of the ECS in the rat hippocampus by chronic unpredictable stress. Acute stress, however, induces elevated levels of prefrontal cortical AEA, and midbrain AEA and 2-AG in rodents.

Finally, abnormalities in the cAMP–PKA–CREB pathway have also been reported in the post-mortem prefrontal cortex of depressed suicide victims. Because of the abundance of the CB_1R, its levels alterations have a greater impact on the cAMP pathway. Upregulation of the CB_1R might therefore enhance the ability of the G_i protein to inhibit AC activity, accounting for the decreased activity of the cAMP–PKA–CREB pathway, which may be implicated in the pathophysiology of depression and suicide (Vinod, 2005; Laviolette, 2006; Vinod, 2006; Hashimotodani, 2007; Martinez-Orgando, 2007; Cabral, 2008; Mackie, 2008)

Finally, an interaction of the ECS with the hypothalamic–pituitary–adrenal (HPA) axis has been established (Cota, 2008). HPA axis has a crucial role in mood regulation via the control of glucocorticoid hormones – cortisol in humans and corticosterone in rodents – circulating levels, which are elevated in depression and in response to stress. CB_1Rs have been found to acti-

vate the HPA axis following stimulation of the neurons containing corticotrophin-releasing factor. Furthermore, rimonabant attenuates a CB_1-receptor-stimulated increase in corticotrophin and corticosterone levels. The levels of adrenocorticotrophin are lower in $CB_1^{-/-}$ mice. ECS signaling inhibits the HPA axis through the CB_1R as well. Basal and stress-induced plasma levels of adrenocorticotrophin and corticosterone are higher in $CB_1^{-/-}$ mice, indicating a context-dependent alteration in the function of the HPA axis. Given the importance of the HPA axis in the pathophysiology of depression and suicidality, the ECS might have an important role in the regulation of mood and emotional response, which are impaired in patients with suicidal behaviour (Laviolette, 2006; Vinoid, 2006; Mackie, 2008; Zhao, 2008)

It is evident, that current research is bound to reveal the exact mechanisms through which the ECS is implicated in human behavioural disorders and answer the questions rising by the contradicting preclinical and clinical data.

Bone metabolism

The correlation of the ECS with the regulation of skeletal and bone remodeling has derived upon several preclinical observations and deeper understanding of the ECS itself (Zhao, 2008; Bab, 2008; Tam, 2008). For instance, in the case of bone formation and bone mass, the central production of 2-AG, is subject to negative regulation by leptin, while traumatic head injury stimulates both bone formation and central 2-AG production. On the other hand bone metabolism is subject to biochemical pathways involving signaling by the hypothalamic receptors of leptin and neuropeptide Y, which are regulated by the ECS.

Several preclinical data confirm the expression of CB receptors in bones.

Osteoblast progenitors exhibit very low levels of both CB_1 and CB_2 receptors. Nevertheless, under certain experimental conditions osteoblast CB_2 mRNA expression increases progressively in parallel to the expression of osteoblastic marker genes such as tissue non-specific alkaline phosphatase (TNSALP), parathyroid hormone receptor 1 (PTHRc1) and the osteoblastic master regulatory gene, RUNX2. In osteoclasts, CB_1 is also mildly expressed but CB_2 mRNA transcripts are increased. In vivo, CB_2 protein has been found in trabecular osteoblasts, osteocytes and osteoclasts. CB_1R is highly expressed in skeletal sympathetic nerve terminals(Zhao, 2008; Bab, 2008; Tam, 2008).

The presence of endocannabinoids has also been substantiated in the skeleton. AEA and 2-AG levels in bones are equally high to their hypothalamic levels, while there is evidence of their synthesis by osteoblastic cells in culture. Additionally, DAGLa and DAGLb (diacylglycerol lipase), which are essential for the biosynthesis of 2AG, are expressed in osteoblasts, osteocytes and bone-lining cells.

According to Bab (2008), 2-AG activates CB_1Rs in the sympathetic nerve terminals, whereas AEA affects bone cells by binding to CB_2 receptors.

Tam (2006) has reported on data confirming the implication of ECS in bone metabolism. This group found that CB_2 deficient mice have a low bone mass (LBM) phenotype and that specific activation of CB_2 receptors attenuates ovariectomy-induced bone loss possibly by restraining osteoclastogenesis and stimulating bone formation.

An interesting aspect is that the activation of CB_1Rs is differentiated according to the age and gender. CB_1Rs control osteoblast function through negative regulation of norepi-nephrine release from sympathetic nerve terminals near these cells (Zhao, 2008; Bab, 2008; Tam, 2008). The age and gender link has not been shown for CB_2 receptors, whose main involvement seems to be the preservation of bone remodeling.

The available data, although still experimental and controversial, suggest that the CBR is a contributor of human osteoporosis pathogenesis.

Ofek (2006) found that CB_2 knockout mice experience accelerated age-related trabecular bone loss and cortical expansion accompanied by increased activity of trabecular osteoblasts, increased numbers of osteoclasts, and decreased numbers of diaphyseal osteoblast precursors. The corresponding administration of selective CB_2 agonist HU-308, but not the CB_1 agonist noladine ether, weakened ovariectomy-induced bone loss and stimulated cortical thickness. Furthermore, HU-308 dose dependently increased the number and activity of endocortical osteoblasts and restrained trabecular osteoclastogenesis by inhibiting proliferation of osteoclast precursors. These results attribute a potential role for CB_2 receptors in osteoporosis progression and have been confirmed by a genetic association study.

On the other hand, Eleftheriou (2008) showed that CB_1 knockout mice and those treated with antagonists of CB_1 or CB_2 receptors were protected from ovariectomy-induced bone loss. Simultaneously, evidence supporting osteoclast apoptosis promotion, osteoclast activity inhibition and a decrease in the production of osteoclast survival factors in vitro were presented.

Antiproliferative functions

THC was recognized as a potential anti-cancer almost 30 years ago, while cannabinoids have been known to afford palliative effects in cancer patients including appetite stimulation, chemotherapy-related nausea inhibition (Nabilone, a synthetic derivative of THC, has been launched under this indication), pain and insomnia relief as well as mood elevation (Guzman, 2003; Hall, 2005; Pertwee, 2005; Pacher, 2006,Onaivi, 2006).

Plentiful studies have associated cannabinoids with cancer growth inhibition in many cancer types but in most cases in a cell type specific mechanism. Cannabinoids are known to interact with tumor cells

cycles resulting in growth arrest, apoptosis inhibition, angiogenic activity and reduced tumor cell migration. The mechanisms for these activities are not yet clear since cannabinoid receptors have been found to interfere with various intracellular signaling pathways (Flygare, 2008).

Endocannabinoids levels vary according to cell type and malignancy. For example, AEA concentration in normal colon tissue triples upon malignant transformation

Given that the cellular uptake of endocannabinoids is very rapid both *in vivo* and *in vitro,* the only way to investigate their antitumor effect is by locally increasing their concentrations at the tumor cell surface by blocking endocannabinoid transport and inactivating enzymes FAAH and MAGL. In this way anti-tumor effects of CB receptors signaling have been induced in thyroid, brain and prostate cancer. Using a xenograft model of thyroid cancer, endocannabinoid degradation inhibitors have been found to increase AEA and 2-AG levels in tissue and reduced tumor growth (Sarfaraz, 2008).

The interaction with CB_1/CB_2-and VR_1 receptors is another mechanism through which AEA, 2-AG, and some endocannabinoid transport inhibitors induce apoptotic cell death and inhibit cell proliferation in glioma, oligodendroglioma, astrocytoma, neuroblastoma, pheochromocytoma, colon carcinoma, uterine cervix carcinoma, leukemia, and lymphoid tumors (Pushkarev, 2008).

In brain cancer (glioma and glioblastoma), cannabinoids inhibit cell proliferation through stimulation of ceramide synthesis in a tumor selective way. Velasco (2007) reported on a clinical study evaluating the effect of intracranial THC administration in patients with glioblastoma.

Estrogens and prolactin regulate breast cancer, which is a hormone-dependent malignancy. *In vitro*, cannabinoids have been found to arrest cell cycle through down-regulation of prolactin receptors. The same effect applied when FAAH was blocked, meaning that breast cancer cells are capable of producing endocannabinoids that inhibit proliferation (Maccarrone, 2003; Bari, 2006).

In prostate cancer, CB_1 and CB_2 receptors are overexpressed in comparison to benign prostatic epithelium. Cannabinoids inhibit cancer cells proliferation *in vitro*. Several mechanisms have been proposed to support this effect but the most accepted include prolactin receptors inhibition, down regulation of the androgen, PSA and EGFR receptor (Bifulco, 2008).

In opposition, Hart (2004) published on how THC, AEA, HU-210, and WIN 55,212-2 induce EGF receptor and metalloprotease-dependent cancer cell proliferation in lung, kidney, squamous cell and bladder carcinoma, glioblastoma and astrocytoma. In the same study micromolar concentrations of cannabinoids induced cancer cell apoptosis, in agreement with previous reports.

These results alone substantiate the dual actions of cannabinoids on cancer cell growth. Their effect may be the combination of direct toxic effects on cells, increased cannabinoid signaling and effects mediated by cannabinoid metabolites since low concentrations are pro-proliferative and high concentrations exhibit antiproliferative action (Flygare, 2008).

The correlation of the immune system with cancer pathogenesis may be another link of cannabinoids to anticancer effects since cannabinoids bear well-established immunosuppressant effects. It is indicative that approximately 50% of primary AML (acute myeloid leukemia) expresses CB_2Rs while MCL (mantle cell lymphoma) demonstrate high expression of both CB_1 and CB_2 receptors. On the other hand, THC enhances breast and lung cancer growth and metastasis by suppressing CB_2R-mediated antitumor immune responses, leading to infections susceptibility (Pertwee, 2005, 2008; Kofalvi, 2007; Flygare, 2008; Sarafaz, 2008).

Very recently, tumor angiogenesis was associated with cannabinoids activity. For example, cannabinoids inhibit VEGF production in glioma, skin and thyroid cancer, while at the same time, they target directly the tumor vasculature (Bifulco, 2008; Flygare, 2008). Cannabinoids acting on CB_1R, also inhibit bFGF induced endothelial cell proliferation, tube formation and sprouting, inducing apoptosis in endothelium *in vitro* and *in vivo*.

The current findings are encouraging implying that cannabinoids may be the next therapeutic target to inhibit tumor growth.

Rimonabant reverts the effect of CBR agonists in *vivo* and *in vitro* at nM and low μM concentrations (Flygare, 2008). This effect leaves a question mark on whether this class of drugs could enhance carcinogenesis, but so far no such data have been published. Rimonabant has been also found to inhibit growth on breast, colon thyroid cancer cell lines, as well as on primary cells and malignant lymphoma cell lines (Bifulco, 2008; Flygare, 2008).

Reproductive functions

There are several data indicating that the ECS is involved in reproductive functions in both male and female animals and humans ***(figure 4)***.

Blastocysts, spermatozoa, uterus and testis synthesize enocannabinoids and mainly AEA, which is present throughout the whole pregnacy period. Paria (2000, 2002) has shown that uterine AEA levels fluctuate during the progression of pregnancy, and are lower in the receptive uterus on day 4 of pseudopregnancy, compared with the levels in the non-receptive uterus on days 5 and 6. The author concluded that an increase in AEA levels is associated with embryotoxic effects and inhibition of embryo development.

Table 5: Distribution and expression of CB_2 receptors in reproductive cells and tissues *Adjusted from Maccarrone, 2008*

Preclinical data	Sex affected
CB_2 is weakly expressed in boar sperm cells and normal and malignant human prostatic epithelium	♂
$CB_2^{-/-}$ mice present an early non-synchronous embryo development in vivo	♀
CB_2 mRNA is expressed in human uterus and myometrium	♀
CB_2 mRNA is expressed in rat testis	♂
CB_2 mRNA and protein are expressed in first trimester human placenta	♀
CB_2 mRNA is expressed during the early stages of mouse embryo development and throughout the preimplantation period	♀
CB_2 mRNA and protein are expressed in mouse Sertoli cells bearing anti-apoptotic properties	♂

Impact of fertility-related hormones and cytokines on FAAH activity and expression *Adjusted from Battista, 2008*

Increase of FAAH levels	Decrease of FAAH levels
IL-4	IL-2
IL-10	IL-12
Progesterone	IFN-g
Leptin	NK
LIF	TNF-a

The enzymes FAAH and NAPE-PLD also play a crucial role in the regulation of both AEA levels and signalling pathways of human reproduction, regulating the oocyte transfer for the ovaries to the implantation site (Taylor, 2007, Sun, 2008).

Wang (2007) provided evidence that FAAH and NAPE-PLD levels are noticeably high on days 1–4 of pregnancy in the non-receptive uterus and at the inter-implantation sites, while FAAH is highly expressed in the epithelium at the ampullary region. FAAH is present at the morula and blastocyst stages but NAPE-PLD is expressed in embryos at all stages, suggesting a more regulatory activity in preimplantation. FAAH activity facilitates AEA disposal at the implantation site and there is data showing its inactivation delays implantation.

The CB_1R involvement in embryo development has also been demonstrated. Wang (2006) claims that rimonabant administration is able to resume the exact on-time implantation *per se* and reverse the negative effects of endocannabinoids on the development of two-cell embryos into blastocysts in culture.

$CB_1^{-/-}$ knockout mice show pregnancy loss attributed to maternal loss of the receptor. In addition, the normal CB_1 accounts for the ehancement of the oviduct-to-uterus transport of embryos. Therefore, its deficiency results in embryo retention, ectopic pregnancy and reduced fertility in this kind of mice.

Paria (1995, 1996, 1999) have conducted an intersting experiment trying to confirm whether embryos deficient in CB receptors respond to endocannabinoids *in vitro*. Therefore, two-cell wild-type or mutant embryos were cultured in the presence or absence of AEA. A comparable development of wild-type and mutant embryos was observed in the absence of AEA, while $CB_1^{-/-}$ and $CB_1^{-/-}/CB_2^{-/-}$ mutant embryos, but not $CB_2^{-/-}$ or wild-type embryos, were resistant to the inhibitory action of AEA. This observation reinforces the assumption that CB_1 is the functional receptor for ensuring embryo growth and differentiation to blastocysts. In all, mouse models provide evidence that embryonic CB_1 primarily contributes to normal embryo development, while oviductal CB_1 directs the timely oviductal transport of embryos.

CB_2Rs seems to hold a role only in embryo development in a non-fully understood way (Maccarrone, 2008). CB_2 mRNA is present at the one-cell stage and is maintained through the blastocyst stage. CB_2 receptors are only restricted to the ICM cells and potentially affect embryo development via ICM cell lineage determination (Fride, 2008) ***(table 5)***.

The expression of CBRs has also been investigated in human uterus during pregnancy in order to determine the endocannabinoid impact on myometrial contractility. Dennedy (2004) showed that AEA and THC exert a direct, relaxant effect on myometrial contractility *in vitro*. Rimonabant was found to prevent this effect, while an experimental CB_2 antagonist (SR144528) did not, although human endometrium expresses both receptor subtypes.

Helliwell (2004) in his own experiments using RT-PCR, detected no CB_1 mRNA in human placenta from first trimester tissues, while CB_2 mRNA was found in the majority of samples. CB_1 positive samples were immune inactive, while the CB_2-positive cells immunoreacted to anti-CD14 antibodies. The authors concluded that human placenta only expresses the CB_2 receptor, which may be a placentation process mediator and a regulator of normal immune responses during early pregnancy.

This last observation attracts further interest given the established role of CB_2 receptors in immune fuc-

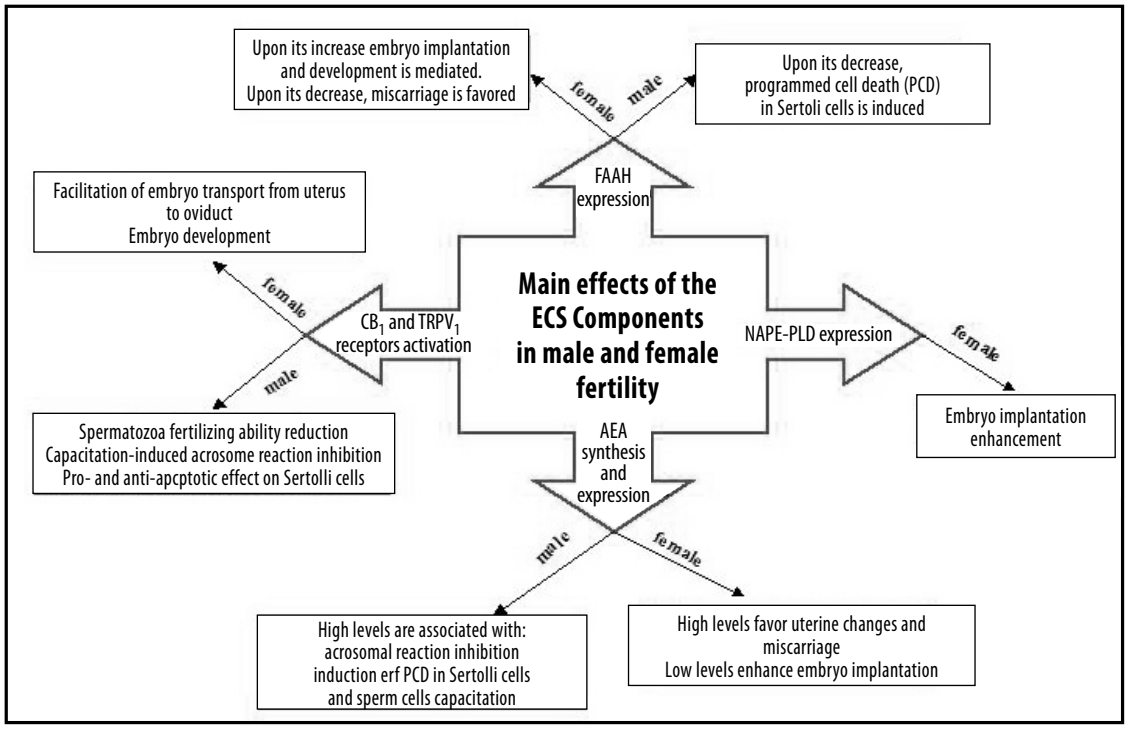

Figure 4: Main effects of the ECS components in male and female fertility

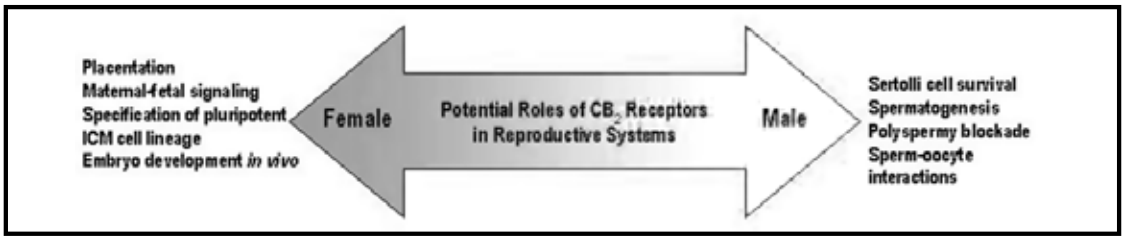

Figure 5: Potential roles of CB_2 receptors in reproductive systems

tions in combination with the immunoregulation to which mammalian reproduction subjected to ***(figure 5)***.

Among others, peripheral CB_2 expression (Piomelli, 2005; Pagotto, 2006; Wang, 2006; Battista, 2007; Maccarrone, 2008; Battista, 2008) is known to:

- Stimulate MCP-1 and IL-8 gene expression
- Reduce human monocytic cell (neuro) toxicity and cytokine secretion
- Modulate TNF-α gene expression
- Negatively regulate IL-12p40 production in murine macrophages

In a similar way, normal gestation is based on an early immunological adaptation involving peripheral T lymphocytes of pregnant women, producing Th1 and Th2 cytokines. Th1 cytokines (IL-2, IL-12 and IFN-γ) impair gestation by damaging the Tr, via stimulation of NK cells and enhancement of TNF-α secretion. On the other hand, Th2 cytokines (IL-3, IL-4 and IL-10) favour blastocyst implantation and pregnancy by promoting Tr growth through the NK cells activity inhibition and the stimulation of natural suppressor cells. Added to that, one should also consider that FAAH expression in T-lymphocytes is regulated by Th1/Th2 cytokines: IL-4 and IL-10 enhance FAAH activity, while IL-2 and INF-γ attenuate it (Maccarrone, 2002).

Based on the above, one may further conclude on the CB_2 impact on the cytokine network regulating the maternal/foetal interactions.

Regarding male fertility, only a limited number of both experimental and *in vivo* studies are available on the involvement of ECS (Wang, 2006; Battista, 2008; Cacciola, 2008; Maccarrone, 2008; Rossato, 2008).

It is known that males smoking cannabis exert decreased plasma LH levels, leading to decreased plasma testosterone. FSH secretion is also modulated by cannabinoids and the combined effects on the gonadotrophins lead to decreased sperm count in regular cannabis smokers in comparison to occasional ones. The effects of cannabinoids on gonadotrophin secretion are localised upstream to the pituitary, and in particular, at the pre-optic area of the hypothalamus where both CB_1Rs and LHRH-secreting neurones are found. Cannabinoids negatively modulate the activity of LHRH-

secreting neurones by direct and indirect mechanisms. The identification of CB_1Rs at the anterior pituitary indicate their effect on LH and FSH secretion (Park, 2004).

N-acylethanolamines (NAEs) are expressed in human reproductive fluids, in rodent testis, in Sertoli cells and in boar spermatozoa. The binding of AEA to CB receptors of sea urchin spermatozoa reduces their fertilising capacity, whereas AEA affects the capacitation process and acrosomal reaction of spermatozoa in mammals. Recently, AEA has been shown to reduce human sperm motility by reducing mitochondrial activity and to inhibit capacitation-induced acrosome reaction, by triggering CB_1 signalling. It has been sugegsted that this progresses via a cAMP-dependent pathway triggered by CB_1R activation (Schuel, 2002).

Sertoli cells synthesize both AEA and 2-AG. These cells express functional CB_2 receptors on their surface, and the levels of this receptor remain stable for at least 16 days. Conversely, FAAH activity and the uptake of AEA have been found to decrease in an age-dependent manner, owing to downregulation of gene expression (Wang, 2006; Battista, 2008; Maccarrone, 2002, 2008; Rossato, 2008).

The CB_2R has been recently described in Sertoli cells as a modulator of their apoptosis. Moreover, Leydig cells express the CB_1R, which, when activated, induces a reduction of testosterone production, thus further inhibiting the spermatogenetic process.

Additionally, both FSH and CB_2 receptors expressed by Sertoli cells have a protective role against the toxic effects of AEA. It can be therefore suggested that altered levels of FSH during testis development may control the proapoptotic potential of AEA, thus contributing to the differentiation process (Wang, 2006; Battista, 2008; Maccarrone, 2002, 2008).

It was as early as 1981, when Perez first reported that cannabinoids negatively influence sperm motility in different mammalian species. Nevertheless, it was very recently, when these inhibitory effects were attributed to the CB_1 activation receptor. Cobellis (2006) confirmed this observation with his experiment on $CB_1^{-/-}$ knockout mice, which showed that sperm from these mice exert a dramatic increase in motility percentages in the caput epididymis. In any case, the limited number of clinical data as well as the lack of mechanisms fully explaining the effect reguires further research before safe conclusions are drawn on the CB_1 activation effect on sperm motility.

In addition, cananbinoids have been found to interact with sperm capacitation (Rossato, 2008). In a recent experiment, sperm pre-incubation with rimonabant has been found to enhance the signalling pathways through which, sperm capacitation is achieved.

Regarding sperm acrosome reaction, CB_1R activation reduces the ability of sperm to undergo acrosome reaction both in vertebrates and invertebrates, further confirming the inhibitory effects of cannabinoids on sperm fertilising ability.

The existing data suggest that sperm function is regulated by endocannabinoids via a dual stage-dependent effect. On the one hand, AEA, prevents premature capacitation in freshly ejaculated sperm via a CB_1 (and potentially CB_2)-mediated mechanism, contributing to the maintainance of a suitable environment for the sperm to transfer through the uterine tract. On the other, when sperm reaches the oviduct, this inhibition effect degrades, allowing the sperm to be exposed to a progressively reduced concentration of AEA in the proximal female genital tract(Wang, 2006; Battista, 2008; Maccarrone, 2002, 2008, Rossato, 2008).

In all, the preclinical and clinical data create a cautionary environment around women of reproductive age regarding long-term use or abuse of marijuana or other endocannabinoid system-orientated drugs.

Although weight loss is often advised for women trying to get pregnant, the potential side effects of CB_1 antagonists on fertility should be well considerd before their administration in this particular group of obese patients.

Given the recent launch of CB_1 antagonists in order to treat obesity, we cannot neglect the influence of adipokines and enterokines in human reproduction. The ECS impact on the reproductive system is not only directly expressed via the above described activities but is also occurring via the regulation of adipokines and enterokines levels, that are associated with fertility.

Hormones regulating energy metabolism exert different effects on several reproductive events. For example, insulin and insulin-like growth factors affect ovarian steroidogenesis, folliculogenesis, and ovulation physiology. Insulin resistance is known to contribute to the pathogenesis of polycystic ovary syndrome (PCOS) (Vignesh, 2007).

In addition, the adipose tissue is implicated on estrogen production and metabolism and regulates SHBG levels, thus contributing to the HPA axis sensitivity. Adipokines like leptin, adiponectin, resistin and enterokines like ghrelin, and peptide YY3-36 have been found to interact with both energy homeostasis and reproductive functions. ***Table 3*** summarizes the metabolic and concurrent reproductive effects of several adipokines and enterokines (Gosman, 2006; Mircea, 2007).

Nutritional disorders of all kinds disrupt gonadotropins and gonadal hormones and therefore interfere with menstrual pattern and fertility potential. Many clinical studies have proved that medium weight loss (<10%) is capable of improving PCOs pathological parameters and increase fertility rates (Pasquali, 2006; Balen, 2007; Metwally, 2007).

As already mentioned, leptin is an important component in the long-term regulation of body weight and energy expenditure (Mitchell, 2005; Pasquali, 2006; Crown, 2007; Loucks, 2007). Its effects on reproductive physiology lies on several different aspects like puberty,

HPA axis function maintenance, menopause and pregnancy outcome, endometriosis, PCOs, ART treatment.

Leptin mainly acts as a catalyst in the initiation of pulsatile GnRH secretion and maturation of the reproductive axis. Leptin levels increase is one of the earliest signs of puberty initiation and contributes to the HPA axis activation, resulting in increased sex steroid production and subsequent activation of the GH–insulin-like growth factor I axis (Grisaru-Granovsky, 2008).

Several preclinical data support this hypothesis (Mircea, 2007, Gosman, 2006; Paraskevas, 2006; Henson, 2006). For example, early onset of reproductive function is observed in normal female mice after leptin treatment. In leptin deficient (*ob/ob*) mice, exogenous leptin administration reverses anovulation and sterility. Leptin-gene knockout mice exert normal sexual development but remain prepubertal without ovulating. Leptin administration restores their fertility. Prepubertal mice treated with leptin become thin but also reach early reproductive maturity in comparison to control mice. Female transgenic skinny mice overexpressing leptin have accelerated onset of puberty. In prepubertal male rats, leptin administration causes a dose-related stimulatory effect on GnRH secretion as well as an increase in the frequency of pulsatile GnRH secretion before puberty and an increase in amplitude after puberty.

Similar results have been drawn from studies in humans. For example, Matkovic (1997) showed that higher levels of leptin correspond to earlier menarche. Grinspoon (1996), Grasemann (2004) and Matejek (1999), have found low leptin levels in anorectic women, children with delayed puberty and athletes. Strobel (1998) has associated leptin missense mutation with hypogonadism and morbid obesity. In his own study, Mantzoros (2007) found that leptin levels are sex-related during progressive pubertal stages. Girls show a steady rise in serum leptin levels throughout puberty, whereas boys appear to peak just before puberty. The authors suggest that the suppressive effect of testosterone on leptin production may account for the decrease in serum leptin levels in boys after the initial prepubertal rise, whereas estrogen increase in girls may account for their higher serum leptin levels during the late stages of puberty.

Leptin is also known to facilitate GnRH secretion via indirect mechanisms, acting through interneurons secreting neuropeptides such as cocaine and amphetamine-regulated transcript peptide and/or melanocortin-concentrating hormone. In addition, there is evidence that leptin may be increasing NO release from adrenergic interneurons, which then induces GnRH release from GnRH neurons by activating guanylate cyclase and COX-1. Leptin contributes to the hypothalamic control of pulsatile GnRH secretion, influences the regulation of GnRH, FSH, LH, ACTH, cortisol, and GH concentrations and accelerates GnRH pulsatility in arcuate hypothalamic neurons in a dose-dependent manner. Other studies have shown that estrogen administration stimulates leptin secretion, whereas hyperinsulinemia, hyperandrogenism, and hyperprolactinemia inhibit its circulating levels (Gosman, 2006; Mircea, 2007; Crown, 2007).

In a very interesting study, Di Carlo (2007) reported on the increased serum leptin levels and body fat composition in menopausal women not receiving hormonal therapy. The authors correlated the development of abdominal obesity with serum leptin levels in untreated postmenopausal women and proved that hormonal therapy restores adipose tissue levels.

The role of circulating leptin levels during the menstrual cycle remains highly controversial and several authors have reported on its levels fluctuation. The only common observation is the increase in leptin concentrations during a natural menstrual cycle, which correlates with LH at several time points.

Finally, regarding embryonic implantation, Budak (2006), showed that human endometrial leptin expression might be activated *in vitro* by leptin of embryonic origin and that leptin mRNA expression occurs at the blastocyst stage of the preimplantation embryo, and not in earlier stages of embryonic development.

Ghrelin. Ghrelin has been recently linked to human reproduction functions and is expressed in the pituitary as well as in the hypothalamus (Gosman, 2006; Budak, 2006; Mircea, 2007). Ghrelin and its receptor have been localized in various reproductive organs, such as placenta, testis Leydig cells, rat ovary, mouse embryo and endometrium. The ghrelin gene is also expressed in rat ovary throughout the estrous cycle and its mRNA levels vary according to cycle stages. Caminos (2003) detected ghrelin mRNA in rat ovary throughout pregnancy. Higher levels were detected in early pregnancy compared with lower expression during the later part of gestation.

On the other hand, Kawamura (2003) detected ghrelin and its receptor in endometrial tissue and preimplantation embryos and suggested that enterokines interfere with preimplantation.

Ghrelin and its receptor have been also localized in uterine fluid, mouse preimplantation embryos, mouse morula, blastocyst, and hatched blastocyst-stage embryos, inner cell mass and trophectoderm cells.

Increased ghrelin levels may diminish reproductive function by inhibiting LH and stimulating prolactin secretion. Ghrelin affords embryotoxic effects and inhibits the development of mouse embryos *in vitro*. Furthermore, it inhibits testosterone secretion by testicular Leydig cells. Ghrelin knockout mice have no distinguishable metabolic or reproductive phenotype compared with wild type, having normal size, body distribution, behaviour and fertility patterns (Gosman, 2006; Budak, 2006; Mircea, 2007).

Adiponectin. Adiponectin is expressed in human and rat placenta and its levels are higher in females than males ***(table 4)***. Mazaki-Tovi (2005) showed that its serum levels might be a predicting factor of the newborn length and birthweight. In adolescent boys, adiponectin levels are negatively affected by androgen concentrations.

Adiponectin levels have been linked to the PCOs pathogenesis and progression although results remain contradictory. Ardawi (2005) as well as Orio (2003) have described low levels of adiponectin in obese and lean women with PCOs. However, the study by Panidis (2003) found no difference in serum adiponectin levels between different BMI groups. Nishizawa (2002) also concluded that there is an inverse correlation between observed adiponectin and androgen levels, which is consistent with its reduced concentration in women with PCOs.

It is speculated that adiponectin interacts with ovarian steroidogenesis given the negative effects of testosterone on circulating adiponectin in humans and mice. Such an interaction has also been demonstrated for resistin, which stimulates testosterone production on cultured human theca cells. Furthermore, resistin dose-dependently increases testosterone production by cultured rat testis

The only data showing expression of adiponectin receptors in ovarian tissue or oocytes has been shown by Lord (2005) in pig ovaries. The authors suggested that adiponectin might be involved in regulation of oocyte nutrient-sensing via the AMPK pathway.

Rimonabant, the first CB_1 selective antagonist

Based upon preclinical data showing that food intake and nicotine addiction share a common pathogenic component, that of a hyperactive ECS, rimonabant was initially intended as a dual-purpose aiming in smoking cessation and weight management. However, results from Phase III clinical trials STRATUS-US and STRATUS-Europe (rimonabant effect on smoking cessation) were not further pursued given the clinical superiority of rimonabant on food intake, food-reinforced behaviours, weight-loss, and cardiometabolic parameters (Johnsson, 2006).

Rimonabant is a CB_1R antagonist with a greater than 1000-fold selectivity for the CB_1 *vs.* CB_2R. It also acts as a CB_1R inverse agonist and it affects endocannabinoid signal transduction in the absence of CB_1R stimulation. Rimonabant binds to the vanilloid receptor, which is involved in some of its neuroprotective effects. Animal studies have shown that rimonabant reduces food intake and body weight in treated rodents and alters adipose tissue metabolic activity inducing adiponectin gene expression (DiMarzo, 2001, 2004; 2008; Pacher, 2006; Cota, 2007; Despres, 2007; Matias, 2007; Engeli, 2008).

Rimonabant's mechanism of action involves the mediation of both central and peripheral pathways by the CB_1R. Central actions include the inhibition of CB_1R transmission in the mesolimbic and central melanocortin systems, which result in decreased motivation for palatable food (nucleus accumbens) and anorexigenic effect (hypothalamus). In addition, a CCK potentiation of vagal satiety signals is enhanced in the brainstem (DiMarzo, 2001, 2004; 2008; Cota, 2007; Despres, 2007; Engeli, 2008) ***(figure 3)***.

In the periphery, rimonabant inhibits hepatic and adipose tissue lipogenesis, enhances skeletal-muscle fatty acid oxidation, increases glucose uptake by the muscles. Furthermore, it noticeably increases adiponectin-circulating levels.

Obesity-related pre-clinical studies

Genetically obese rodents have increased hypothalamic levels of AEA, suggesting their ECS over activation. The activation of CB_1R has been related to the increased expression of hypothalamic orexigenic appetite mediators like orexin-1 receptor (DiMarzo, 2001; 2004; 2008; Pacher, 2006; Cota, 2007; Despres, 2007; Matias, 2007; Engeli, 2008). In contrast, hypothalamic endocannabinoid levels are suppressed by leptin.

In the periphery, the activation of CB_1R modulates body weight through its functions in adipose tissue, GI tract, liver and muscles. CB_1Rs in adipocytes have been found upregulated in obese mice, stimulating lipoprotein lipase activity and fat accumulation (Wang, 2007). Hyperactivation of CB_1Rs in the liver has been correlated with increased serum lipid production, fatty liver disease and diet-induced obesity.

Further indications that the ECS is involved in food and weight regulation derive from CB_1^-/CB_1^- knockout mice, which are lean and resistant to diet-induced obesity, showing enhanced insulin and leptin sensitivity. These rodents fail to put on weight even when their caloric intake is similar to that of wild-type mice on a high-fat diet (DiMarzo, 2001, 2004; 2008; Cota, 2007; Despres, 2007; Matias, 2007).

Other studies show that blockade of CB_1Rs with rimonabant, reduces motivation for highly palatable food in diet-induced obese mice. Weight reduction has also been attributed to rimonabant's peripheric actions. For example, the increase of adiponectin expression in adipocytes and the inhibition of the enzymic activity involved in fat accumulation is an indirect way to favour the metabolic profile of these rodents.

An interesting study by Jbilo and Ravinet-Trillou (2005) showed that rimonabant reverses the phenotype of obese adipocytes and attains reduction of adipose mass. This effect has been mainly attributed to the regulation of the enzymic activity of the β-oxidation and tricarboxylic (citric) acid cycle, which enhances lipolysis. Additionally, rimonabant increases oxygen consumption and soleus muscle glucose uptake in Lepob/Lepob

mice, suggesting that other factors besides anorexia may be contributing to the weigh loss effect.

Clinical program

Rimonabant was initially launched in EU in 2004 under the indication of treating overweight and obese patients with co-morbidities like dyslipidaemia and/or diabetes.

The so-called RIO ("Rimonabant In Obesity") Program has recruited populations in USA, Canada and Europe comparing placebo, 5 mg and 20 mg for up to 2 years. Obese without or without co-morbidities, non-treated dyslipidemics and inadequatelly controlled diabetic patients were included in this prorgram aiming to accumulate data on the drug's efficacy and safety profile (Pi-Sunyer, 2006; VanGaal, 2005; Despres, 2005; Scheen, 2006; Nissen, 2008).

Throughout the program, consistent effects on weight loss, waist circumference decrease and therapeutic alterations in lipid and glucose metabolism were recorded thus the metabolic syndrome prevalence was significantly reduced.

Rimonabant showed a profound effect on HDL-C, triglycerides and VLDL-C. Regression analysis suggests that the effect on lipids is only half way attributed to the achievable weight-loss and therefore the remaining result is directly related to the drug's periprheric action (suppression of hepatic lipogenesis, enhanced lipid oxidation etc). The results were maintained for up to 2 years (Scheen, 2006,2008; Johnson, 2007; Kinttscher, 2008) ***(table 6)***.

Table 6: Cardiometabolic effects of Rimonabant independent of the achievable weight loss: Half of the rimonabant effect is due to direct CB_1 blockade on peripheral tissues

Cardiometabolic parameter	Overall effect*	Effect independent of weight loss *	Overall effect beyond that attributed to the weigh loss alone*
HDL-C (%)	8.0	3.6	45%
Triglycerides (%)	-14.0	-6.5	46%
Adiponectin (μg/ml)	1.5 (0.2)	0.85	57%
HbA1c (%)	-0.67	-0.37	55%
Fasting insulin (μIU/ml)	-2.74	-1.34	49%

Mean difference versus placebo at year 1, p<0.001 for all comparisons
Linear regression analysis between metabolic changes and body weight loss (RIO trials)
Analysis using ANCOVA to estimate the change in metabolic parameters not explained by weight loss
Relationship with weight loss in the placebo group is estimated using regression

HDL-C, Triglycerides: RIO- Europe, RIO-North America, RIO-Lipids, RIO-Diabetes
Adiponectin: RIO-Lipids
HbA1c: RIO-Diabetes
Fasting insulin: RIO- Europe, RIO-North America, RIO-Lipids

Regarding the drug's safety profile, (Pi-Sunyer, 2006; VanGaal, 2005; Despres, 2005; Scheen, 2006; Nissen, 2008) the predominant adverse effects recorded were nausea, dizziness, diarrhoea, gastrointestinal disorders. CNS related side effects like anxiety, mood alterations and depressive disorders mainly occurred in patients with a history of major depression.

Despite the drug's large clinical program, the non-conclusive discussion regarding its CNS-related side-effects and mainly its potential to grow depression or suicidality thoughts in general population has led EMEA to withdraw it from the market on October 2008. The differentiation among the studied populations provoked confusion (Mitchell, 2007; Astrup, 2008; Bluher, 2008; Despres, 2008) in the interpretation of safety data. Further meta-analyses concluded negatively on the drug's cost-benefit ratio, mainly because most patients were not staying long enough to the therapy for the benefits to outweigh the potential risks.

CONCLUSIONS

The endocannabinoid system represents a complex, regulatory, messenger system active on many different organs and tissues. It is therefore raising expectations for the development of new drugs for diseases that are so far inadequatelly treated or not treated at all. Its pleiotropic functions bound the system to dysregulation from several different pathways, thus making it more time-consuming to fully comprehend its exact profile and optimal applications.

To date, apart from the direct or indirect agonists and antagonists of CBRs, a number of enzymes involved in the system's functions are also being targeted aiming to produce beneficial effects with minimum psychotropic side effects.

To sum up, there are three basic therapeutic strategies involving the ECS:

- ECS activation (CB_2Rs)

The emerging data on the CB_2Rs anti-inflammatory and analgesics properties raises expectations for the treatment of diseases whose pathology is related to ECS activation. The virtual absence of CB_2Rs from the brain is an indulging factor regarding lack of potential CNS side effects.

- ECS signalling (CB_1Rs)

The other class of indications is related to ECS enhanced signalling and includes addictive (e.g. smoking, drug and alcohol abuse) and metabolic (e.g. obesity, fatty liver disease and diabetes) diseases. These disorders all share a common appetitive component, which may be treatable with selective CB_1R antagonists.

This category of drugs is more familiar to the medical community but is also known to afford CNS-specific side effects. Therefore, the development of new drugs prerequisites further *in vivo* pharmacological profiling studies in order to determine those structure-activity relationships between the candidate agents and the targeted CBRs that will ensure the minimization of psychotropic side effects. Moreover, given the abundance of the CB_1Rs in the brain, it is absolutely essential to explore the consequences of the long-term inhibition of CBRs before launching any other new agents. Finally, the discovery of non-CB_1/non-CB_2Rs in cardiovascular and other tissues as well as the data showing that ECs act on vanilloid and other receptors, constitute a solid proof on the potential of the ECS in managing several pathological diseases after the system will have been sufficiently comprehended.

- FAAH and other ECS enzymes inhibition

Among others, FAAH generates the transmembrane concentration of cellular AEA uptake and catalyzes its degradation in a way that decisively controls endocannabinoid signalling. This is why FAAH is an attractive therapeutic target among known ECS enzymes.

To conclude with, it lies within the author's strong beliefs that the potential of the endocannabinoid system in medical practice will continuously grow in the coming years. Updates on its applications should be therefore embraced and taken seriously into consideration while practicing clinical medicine. The engagement of the international research community with ECS constitutes a major breakthrough and can be sufficiently described by Horace's incitement in his play, Odes: *Carpe Diem, Quam Minimum Credula Postero (Seize the day, trusting little in the future)* – or in this case, seize the system, trusting little in the past……

REFERENCES

1 Antuna-Puente B, Feve B, Fellahi S, Bastard JP (2008). Adipokines: the missing link between insulin resistance and obesity. Diabetes Metab. **34**(1): 2–11.
2 Ardawi MS, Rouzi AA (2005). Plasma adiponectin and insulin resistance in women with polycystic ovary syndrome. Fertil Steril. **83**(6): 1708–1716.
3 Ashton JC, Smith PF (2007). Cannabinoids and cardiovascular disease: the outlook for clinical treatments. Curr Vasc Pharmacol. **5**(3): 175–185.
4 Astrup A, Christensen R, Bartels E, Bliddal H (2008). Efficacy and safety of the weight-loss drug rimonabant – Authors' reply. Lancet. **371**(9612): 556–557.
5 Bab I, Ofek O, Tam J, Rehnelt J, Zimmer A (2008). Endocannabinoids and the Regulation of Bone Metabolism. J Neuroendocrinol. **20**(Suppl 1): 69–74.
6 Bab I, Zimmer A (2008). Cannabinoid receptors and the regulation of bone metabolism. Br J Pharmacol. **153**: 182–188.
7 Balen AH, Anderson RA (2007). Policy and practice of the BFS. Impact of obesity on female reproductive health: British Fertility Society, Policy and Practice Guidelines. Hum Fertil. **4**: 195–206.
8 Bari M, Battista N, Fezza F, Gasperi V, Maccarrone M. 2006. New insights into endocannabinoid degradation and its therapeutic potential. Mini Rev Med Chem. **6**(3): 257–268.
9 Basavarajappa BS (2007). Critical enzymes involved in endocannabinoid metabolism. Protein Pept Lett. **14**(3): 237–246.
10 Batkai S, Pacher P, Osei-Hyiaman D, Radaeva S *et al* (2004). Endocannabinoids acting at cannabinoid-1 receptors regulate cardiovascular function in hypertension. Circulation. **110**(14): 1996–2002.
11 Battista N, Bari M, Rapino C, Trassati F *et al* (2007). Regulation of female fertility by the endocannabinoid system. Hum Fertil. **10**(4): 207–216.
12 Battista N, Rapino C, DiTommaso M, Pasquariello N *et al* (2008). Regulation of male fertility by the endocannabinoid system. Mol Cell Endocrinol. **286**(1–2 Suppl 1): S17–S23.
13 Battista N., N. Pasquariello, M. Di Tommaso, M. Maccarrone (2008). Interplay Between Endocannabinoids, Steroids and Cytokines in the Control of Human Reproduction. J Neuroendocrinol. **20** (Suppl 1): 82–89.
14 Bellocchio L, Mancini G, Vicennati V, Paquali R *et al* (2006).Cannabinoid receptors as therapeutic targets for obesity and metabolic diseases. Curr Opin Pharmacol. **6**(6): 586–591.
15 Bełtowski J, Jamroz-Wiśniewska A, Widomska S (2008). Adiponectin and its role in cardiovascular diseases. Cardiovasc Hematol Disord Drug Targets. **8**(1): 7–46.
16 Bensaid M, Gary-Bobo M, Esclangon A, Maffrand JP *et al* (2003). The cannabinoid CB1 receptor antagonist SR141716 increases Acrp30 mRNA expression in adipose tissue of obese fa/fa rats and in cultured adipocyte cells.Mol Pharmacol. **63**(4): 908–914.
17 Bermundez-Silva FJ, Sanchez-Vera I, Suarez J, Serrano A *et al* (2007). Role of cannabinoid CB2 receptors in glucose homeostasis in rats. Eur J Pharmacol. **565**(1–3): 207–211.
18 Bermundez-Silva FJ, Suarez J, Baixeras E, Cobo N *et al.* (2008). Presence of functional cannabinoid receptors in human endocrine pancreas. Diabetologia. **51**(3): 476–487.
19 Bifulco M, Malfitano A, Pisanti S, Laeazz C (2008). Endocannabinoids in endocrine and related tumours. Endocr Relat Cancer. **15**(2): 391–408.
20 Bisogno T (2008). Endogenous Cannabinoids: Structure and Metabolism. J Neuroendocrinol. **20**(1): 1–9.
21 Bluher M, Engeli S, Kloting N, Berndt J *et al* (2006). Dysregulation of the peripheral and adipose tissue endocannabinoid system in human abdominal obesity. Diabetes. **55**(11): 3053–3060.
22 Blüher M (2008). Efficacy and safety of the weight-loss drug rimonabant. Lancet. **371**(9612): 555–556.
23 Blüher S, Mantzoros CS. 2007. Leptin in reproduction. Curr Opin Endocrinol Diabetes Obes. **14**(6): 458–464.
24 Bouaboula M, Hilairet S, Marchand J, Fajas L *et al.* 2005. Anandamide induced PPARgamma transcriptional activation and 3T3-L1 preadipocyte differentiation. Eur J Pharmacol. **517**(3): 174–181.
25 Brown AJ (2007). Novel cannabinoid receptors. Br J Pharmacol. **152**(5): 567–575.
26 Budak E, Fernández Sánchez M, Bellver J, Cerveró A, Simón C, Pellicer A (2006). Interactions of the hormones leptin, ghrelin, adiponectin, resistin, and PYY3-36 with the reproductive system. Fertil Steril. **85**(6): 1563–1581.
27 Burstein S (2005). PPAR-gamma: a nuclear receptor with affinity for cannabinoids. Life Sci. **77**(14): 1674–1684.
28 Cabral GA, Raborn ES, Griffin L, Dennis J, Marciano-Cabral F. (2008). CB2 receptors in the brain: role in central immune function. Br J Pharmacol. **153**(2): 240–251.
29 Cacciola G, Chioccarelli T, Ricci G, Meccariello R *et al* (2008). The endocannabinoid system in vertebrate male reproduction: a comparative overview. Mol Cell Endocrinol. **286**(1–2 Suppl 1): S24–S30.
30 Caminos JE, Tena-Sempere M, Gaytan F, Sanchez-Criado JE *et al* (2003). Expression of ghrelin in the cyclic and pregnant rat ovary. Endocrinology. **144**(4): 1594–602.
31 Capasso R, Matias I, Lutz B. Borrelli F *et al* (2005). Fatty acid amide hydrolase controls mouse intestinal motility in vivo. Gastroenterology. **129**(3): 941–951.

32 Capasso R, Izzo A (2008). Gastrointestinal Regulation of Food Intake: General Aspects and Focus on Anandamide and Oleoylethanolamide. J Neuroendocrinol. **20**(Supp1): 39–42.
33 Caraceni P, Domenicali M, Bernardi M (2008). The Endocannabinoid System and Liver Diseases. J Neuroendocrinol. **20**(Supp1): 47–52.
34 Cobellis G, Cacciola G, Scarpa D, Meccariello R *et al* (2006). Endocannabinoid system in frog and rodent testis: type-1 cannabinoid receptor and fatty acid amide hydrolase activity in male germ cells. Biol Reprod. **75**(1): 82–89.
35 Cota D, Marsciano G, Tschop M, Grubler Y *et al*. 2003. The endogenous cannabinoid system affects energy balance via central orexigenic drive and peripheral lipogenesis. J Clin Invest. **112**(3): 423–431.
36 Cota D, Marsicano G, Lutz B, Vicennati V *et al* (2003). Endogenous cannabinoid system as a modulator of food intake. Int J Obes Relat Metab Disord. **27**(3): 289–301.
37 Cota D, Tschop M, Horvath T, Levine A (2006).Cannabinoids, opioids and eating behavior: the molecular face of hedonism? Brain Res Rev. **51**(1): 85–107.
38 Cota D (2007). CB1 receptors: emerging evidence for central and peripheral mechanisms that regulate energy balance, metabolism, and cardiovascular health. Diabetes Metab Res Rev. **23**: 507–517.
39 Cota D (2008). The Role of the Endocannabinoid System in the Regulation of Hypothalamic-Pituitary-Adrenal Axis. J Neuroendocrinol. **20**(Suppl 1): 35–38.
40 Croci T, Landi M, Galzin AM, Marini P (2003). Role of cannabinoid CB1 receptors and tumor necrosis factor-alpha in the gut and systemic anti-inflammatory activity of SR 141716 (rimonabant) in rodents. Br J Pharmacol. **140**(1): 115–122.
41 Crown A, Clifton DK, Steiner RA (2007). Neuropeptide signaling in the integration of metabolism and reproduction. Neuroendocrinology. **86**(3): 175–182.
42 Dennedy MC, Friel AM, Houlihan DD, Broderick VM *et al* (2004). Cannabinoids and the human uterus during pregnancy. Am J Obstet Gynecol. **190**(1): 2–9.
43 DePetrocellis L, Marini P, Matias I, Moriello AS (2007). Mechanisms for the coupling of cannabinoid receptors to intracellular calcium mobilization in rat insulinoma beta-cells. Exp Cell Res. **313**(14): 2993–3004.
44 Despres J, Golay A, Sjostrom L (2005). Effects of rimonabant on metabolic risk factors in overweight patients with dyslipidemia. N Engl J Med. **353**(20): 2121–34.
45 Despres J (2007). The endocannabinoid system. A new target for the regulation of energy balance and metabolism. Crit Pathways in Cardiol **6**: 46–50.
46 Després JP (2007). The endocannabinoid system: a new target for the regulation of energy balance and metabolism. Crit Pathw Cardiol. **6**(2): 46–50.
47 Després JP, Gaal LV, Pi-Sunyer X, Scheen A. 2008. Efficacy and safety of the weight-loss drug rimonabant. Lancet. **371**(9612): 555.
48 Di Marzo V, Goparaju SK, Wang L, Batkai S *et al* (2001). Leptin-regulated endocannabinoids are involved in maintaining food intake. Nature. **410**(6830): 822–825.
49 Di Marzo V, Bifulco M, De Petrocellis L (2004). The endocannabinoid system and its therapeutic exploitation. Nature Reviews, Drug Discovery. **3**: 771–784.
50 Di Marzo V (2008). Targeting the endocannabinoid system: to enhance or reduce? Nat Rev Drug Discov. **7**(5): 438–455.
51 Di Marzo V (2008). The endocannabinoid system in obesity and type 2 diabetes. Diabetologia DOI: 10.1007/s00125-008-1048-2.
52 DiCarlo C, Tommaselli GA, DiSpiezio A, Sammartino A *et al* (2007). Longitudinal evaluation of serum leptin and bone mineral density in early postmenopausal women. Menopause. **14**(3 Pt 1): 450–454.
53 Elefteriou F (2008). Regulation of bone remodeling by the central and peripheral nervous system. Arch Biochem Biophys. **473**(2): 231–236.
54 Engeli S, Bohnke J, Feldpausch M, Gorzelniak K *et al* (2005). Activation of the peripheral endocannabinoid system in human obesity. Diabetes. **54**(10): 2838–2843.
55 Engeli S (2008). Dysregulation of the Endocannabinoid System in obesity. J Neuroendocrinol **20**(Suppl 11): 110–115.
56 Fajardo G, Bernstein D (2007). Endocannabinoid inhibition: a new cardioprotective strategy against doxorubicin cardiotoxicity. J Am Coll Cardiol. **50**(6): 537–539.
57 Ferré P. 2004. The biology of peroxisome proliferator-activated receptors: relationship with lipid metabolism and insulin sensitivity. Diabetes. **53**(Suppl 1): S43–50.
58 Flygare J, Sander B (2008). The endocannabinoid system in cancer – Potential therapeutic target? Sem Cancer Biol. **18**: 176–189.
59 Földy C, Neu A, Jones MV, Soltesz I (2006). Presynaptic, activity-dependent modulation of cannabinoid type 1 receptor-mediated inhibition of GABA release. J Neurosci. **26**(5): 1465–1469.
60 Ford WR, Honan SA, White R, Hiley CR (2002). Evidence of a novel site mediating anandamide-induced negative inotropic and coronary vasodilatator responses in rat isolated hearts. Br J Pharmacol. **135**(5): 1191–1198.
61 Fowler CJ (2007). The pharmacology of the cannabinoid system – a question of efficacy and selectivity. Mol Neurobiol. **36**(1): 15–25.
62 Fride E (2008). Multiple Roles for the Endocannabinoid System During the Earliest Stages of Life: Pre- and Postnatal Development. J Neuroendocrinol. **20** (Suppl 1): 75–81.
63 Gamber KM, Macarthur H, Westfall TC (2005). Cannabinoids augment the release of neuropeptide Y in the rat hypothalamus. Neuropharmacology. **49**(5): 646–652.
64 Gasperi V, Fezza F, Pasquariello N, Bari M *et al* (2007). Endocannabinoids in adipocytes during differentiation and their role in glucose uptake. Cell Mol Life Sci. **64**(2): 219–229.
65 Gomez R, Navarro M, Ferrer B, Trigo JM *et al* (2002). A peripheral mechanism for CB1 cannabinoid receptor-dependent modulation of feeding. J Neurosci. **22**(21): 9612–9617.
66 Gonthier MP, Hoareau L, Festy F, Matias I *et al* (2007). Identification of endocannabinoids and related compounds in human fat cells. Obesity. **15**(4): 837–845.
67 Gosman G, Katcher H, LegroR (2006). Obesity and the role of gut and adipose hormones in female reproduction. Human Reproduction Update, **12**(5): 585–601.
68 Grasemann C, Wessels HT, Knauer-Fischer S, Richter-Unruh A (2004). Increase of serum leptin after short-term pulsatile GnRH administration in children with delayed puberty. Eur J Endocrinol. **150**(5): 691–698.
69 Grassi F, Quarti-Trevano G, Seravalle F, Arenare G *et al* (2008). Blood Pressure Lowering Effects of Rimonabant in Obesity-related Hypertension. J Neuroendocrinol. **20**(Supp1): 63–68.
70 Grinspoom S, Gulick T, Askari H, Landt M *et al,* 1996. Serum leptin levels in women with anorexia nervosa. J Clin Endocrinol Metab. 81(11): 3861–3863.
71 Grisaru-Granovsky S, Samueloff A, Elstein D (2008). The role of leptin in fetal growth: a short review from conception to delivery. Eur J Obstet Gynecol Reprod Biol. **136**(2): 146–150.
72 Guzman M, Lo Verme J, Fu J, Oveisi F, Blazquez C. *et al* (2004). Oleoylethanolamide stimulates lipolysis by activating the nuclear receptor peroxisome proliferator-activated receptor alpha (PPAR-alpha). J Biol Chem. **279**(27): 27849–2754.
73 Guzman M (2003). Cannabinoids: potential anticancer agents. Nat Rev Cancer. **3**(10): 745–755.
74 Hall W, Christie M, Currow D (2005). Cannabinoids and cancer: causation, remediation, and palliation. Lancet Oncol. **6**(1): 35–42.
75 Hart S, Fischer OM, Ullrich A. 2004. Cannabinoids induce cancer cell proliferation via tumor necrosis factor alpha-converting enzyme (TACE/ADAM17)-mediated transactivation of the epidermal growth factor receptor. Cancer Res. **64**(6): 1943–1950.
76 Hashimotodani Y, Ohno-Shosaku T, Kano M (2007). Endocannabinoids and synaptic function in the CNS. Neuroscientist. **13**(2): 127–137.

77 Helliwell RJ, Chamley LW, Blake-Palmer K, Mitchell MD *et al* (2004). Characterization of the endocannabinoid system in early human pregnancy. J Clin Endocrinol Metab. **89**(10): 5168–5174.
78 Henson MC, Castracane VD.2006. Leptin in pregnancy: an update. Biol Reprod. **74**(2): 218–229.
79 Hentges ST, Low MJ, Williams JT (2005). Differential regulation of synaptic inputs by constitutively released endocannabinoids and exogenous cannabinoids. J Neurosci. **25**(42): 9746–9751.
80 Hermann A, Kaczocha M, Deutsch DG. 2006. 2-Arachidonoylglycerol (2-AG) membrane transport: history and outlook. AAPS J. 8(2): E409–412.
81 Hiley CR, Kaup SS (2007). GPR55 and the vascular receptors for cannabinoids. Br J Pharmacol. **152**(5): 559–561.
82 Hill MN, Patel S, Carrier EJ, Rademacher DJ *et al* (2005). Down-regulation of endocannabinoid signaling in the hippocampus following chronic unpredictable stress. Neuropsychopharmacology. **30**(3): 508–515.
83 Hill MN, Miller GE, HO WS, Gorzalka BB *et al* (2008). Serum endocannabinoid content is altered in females with depressive disorders: a preliminary report. Pharmacopsychiatry. **41**(2): 48–53.
84 Hillard CJ. 2000. Biochemistry and pharmacology of the endocannabinoids arachidonylethanolamide and 2-arachidonylglycerol. Prostaglandins Other Lipid Mediat. **61**(1–2): 3–18.
85 Ho WS, Hillard CJ (2005). Modulators of endocannabinoid enzymic hydrolysis and membrane transport. Handb Exp Pharmacol.**168**: 187–207.
86 Howlett AC, Breivogel CS, Childers SR, Deadwyler SA *et al* (2004). Cannabinoid physiology and pharmacology: 30 years of progress. Neuropharmacology **47**: 345–358.
87 Izzo AA, Camilleri M (2008). Emerging role of cannabinoids in gastrointestinal and liver diseases: basic and clinical aspects. Gut. **57**(8): 1140–1155.
88 Jbilo O, Ravinet-Trillou C, Arnone M, Buisson I *et al.* 2005. The CB1 receptor antagonist rimonabant reverses the diet-induced obesity phenotype through the regulation of lipolysis and energy balance. FASEB J. **19**(11): 1567–1569.
89 Jbilo O, Ravinet-Trillou C, Arnone M, Buisson I *et al* (2005). The CB1 receptor antagonist rimonabant reverses the diet-induced obesity phenotype through the regulation of lipolysis and energy balance. FASEB J. **19**(11): 1567–1569.
90 Jellinger PS (2007). Metabolic consequences of hyperglycemia and insulin resistance. Clin Cornerstone. **8**(Suppl 7): S30–42.
91 Jensen DP, Andreasen CH, Andersen MK, Hansen L *et al* (2007). The functional Pro129Thr variant of the FAAH gene is not associated with various fat accumulation phenotypes in a population-based cohort of 5,801 whites. J Mol Med. **85**(5): 445–449.
92 Jesudason, D, Wittert G. 2008. Endocannabinoid system in food intake and metabolic regulation. Curr Opin Lipidol. **19**(4): 344–348.
93 Jhaveri MD, Richardson D, Chapman V (2007). Endocannabinoid metabolism and uptake: novel targets for neuropathic and inflammatory pain. Br J Pharmacol. **152**(5): 624–632.
94 Jonsson, K Holt S Fowler C (2006). The endocannabinoid system: current pharmacological research and therapeutic possibilities. Bas Clin Pharmacol Toxicol **98**: 124–134.
95 Johnson RA, Vettor R, Rossato M, Fallo F, Pagano C (2007). The blockade of the endocannabinoid CB1 receptors and its influence on cardiometabolic risk: Lesson from rimonabant in obesity (RIO) trials. Clin Cornerstone. **8**(3): 82.
96 Juan-Pico P, Fuentes E, Bermudez-Silva FJ, Javier Diaz-Molina F *et al* (2006). Cannabinoid receptors regulate Ca(2+) signals and insulin secretion in pancreatic beta-cell. Cell Calcium. **39**(2): 155–162.
97 Kawada T, Goto T, Hirai S, Kang MS *et al* (2008). Dietary regulation of nuclear receptors in obesity-related metabolic syndrome. Asia Pac J Clin Nutr. **17**(Suppl 1): 126–130.
98 Kawamura K, Sato N, Fukuda J, Kodama H *et al* (2003). Ghrelin inhibits the development of mouse preimplantation embryos in vitro. Endocrinology. **144**(6): 2623–2633.
99 Kintscher U. 2008. The cardiometabolic drug rimonabant: after 2 years of RIO-Europe and STRADIVARIUS.doi: 10.1093/eurheartj/ehn255.
100 Kirkham TC, Williams CM. 2004.Endocannabinoid receptor antagonists: potential for obesity treatment. Treat Endocrinol. **3**(6): 345–360.
101 Kofalvi A (2007). Cannabinoids and the Brain. Springer (eds).
102 Kong AP, Chan NN, Chan JC (2006). The role of adipocytokines and neurohormonal dysregulation in metabolic syndrome. Curr Diabetes Rev. **2**(4): 397–407.
103 Kuusisto J, Andrulionyte L, Laakso M. (2007). Atherosclerosis and cardiovascular risk reduction with PPAR agonists. Curr Atheroscler Rep. **9**(4): 274–280.
104 Kyrou I, Valsamakis G, Tsigos C (2006). The endocannabinoid system as a target for the treatment of visceral obesity and metabolic syndrome. Ann N Y Acad Sci. **1083**: 270–305.
105 Labar G, Michaux C (2007). Fatty acid amide hydrolase: from characterization to therapeutics. Chem Biodivers. **4**(8): 1882–1902.
106 Lafontan M, Piazza PV, Girard J (2007). Effects of CB1 antagonist on the control of metabolic functions in obese type 2 diabetic patients. Diabetes Metab. **33**(2): 85–95.
107 Laviolette SR, Grace AA (2006). The roles of cannabinoid and dopamine receptor systems in neural emotional learning circuits: implications for schizophrenia and addiction. Cell Mol Life Sci. **63**(14): 1597–613.
108 Lenman A, Fowler CJ (2007). Interaction of ligands for the peroxisome proliferator-activated receptor gamma with the endocannabinoid system. Br J Pharmacol. **151**(8): 1343–1351.
109 López-Moreno JA, González-Cuevas G, Moreno G, Navarro M (2008). The pharmacology of the endocannabinoid system: functional and structural interactions with other neurotransmitter systems and their repercussions in behavioral addiction. Addict Biol. **13**(2): 160–187.
110 Lord E, Ledoux S, Murphy BD, Beaudry D *et al* (2005). Expression of adiponectin and its receptors in swine. J Anim Sci. **83**(3): 565–578.
111 Loucks AB (2007). Energy availability and infertility. Curr Opin Endocrinol Diabetes Obes. **14**(6): 470–474.
112 Maccarrone M, Falciglia K, Di Rienzo M, Finazzi-Agrò A. 2002. Endocannabinoids, hormone-cytokine networks and human fertility. Prostaglandins Leukot Essent Fatty Acids. **66**(2–3): 309–317.
113 Maccarrone M. Finazzi-Agró A (2003). The endocannabinoid system, anandamide and the regulation of mammalian cell apoptosis. Cell Death Differ. **10**(9): 946–955.
114 Maccarrone M (2006). Fatty acid amide hydrolase: a potential target for next generation therapeutics. Curr Pharm Des. **12**(6): 759–772.
115 Maccarrone M (2008). CB2 receptors in reproduction. Br J Pharmacol. **153**(2): 189–198.
116 Mackie K (2008). Cananbinoid Receptors: where they are and what they do. J Neuroendocrinol. **20**(1): 10–14.
117 Mackie K (2008). Signaling via CNS cannabinoid receptors. Mol Cell Endocrinol. **286**(1–2 Suppl 1): S60–S65.
118 Mallat A, Lotersztajn S (2006). Endocannabinoids as novel mediators of liver diseases. J Endocrinol Invest. **29**(3 Suppl): 58–65.
119 Martínez-Orgado J, Fernández-López D, Lizasoain I, Romero J (2007). The seek of neuroprotection: introducing cannabinoids. Recent Patents CNS Drug Discov **2**(2): 131–139.
120 Massa F, Monory K (2006). Endocannabinoids and the gastrointestinal tract. J Endocrinol Invest. **29**(3 Suppl): 47–57.
121 Matejek N, Weimann E, Witzel C, Mölenkamp G. 1999. Hypoleptinaemia in patients with anorexia nervosa and in elite gymnasts with anorexia athletica. Int J Sports Med. **20**(7): 451–456.
122 Mathieu P, Pibarot P, Larose E, Poirier P, Marette A, Després JP (2008). Visceral obesity and the heart. Int J Biochem Cell Biol. **40**(5): 821–836.
123 Matias I, Gonthier MP, Orlando P, Martiadis V *et al* (2006). Regulation, function, and dysregulation of endocannabinoids in models of adipose and beta-pancreatic cells and in obesity and hyperglycemia. J Clin Endocrinol Metab. **91**(8): 3171–3180.
124 Matias I, Di Marzo V (2007). Endocannabinoids and the control of energy balance. Trends Endocrinol Metab. **18**(1): 27–37.

125 Matias I, Gonthier MP, Petrosino S, Docimo L, Capasso R *et al*. 2007.Role and regulation of acylethanolamides in energy balance: focus on adipocytes and beta-cells. Br J Pharmacol. **152**(5): 676–690.
126 Matias I, Cristino L, Di Marzo V (2008). Endocannabinoids: some like it fat (and sweet too). J Neuroendocrinol. **20**(1): 100–109.
127 Matias I, Petrosino S, Racioppi A, Capasso R, Izzo AA *et al* (2008). Dysregulation of peripheral endocannabinoid levels in hyperglycemia and obesity: Effect of high fat diets. Mol Cell Endocrinol. **286**(1–2 Suppl 1): S66–78.
128 Matias I, Vergoni AV, Petrosino S, Ottani A *et al* (2008). Regulation of hypothalamic endocannabinoid levels by neuropeptides and hormones involved in food intake and metabolism: insulin and melanocortins. Neuropharmacology. **54**(1): 206–212.
129 Matkovic V, Ilich JZ, Skugor M, Badenhop Ne *et al* (1997). Leptin is inversely related to age at menarche in human females.J Clin Endocrinol Metab. **82**(10): 3239–3245.
130 Mazaki-Tovi S, Kanety H, Sivan E (2005). Adiponectin and human pregnancy.Curr Diab Rep. **5**(4): 278–281.
131 Metwally M, Li TC, Ledger WL (2007). The impact of obesity on female reproductive function. Obes Rev. **8**(6): 515–253.
132 Mircea CN, Lujan ME, Pierson RA. 2007. Metabolic fuel and clinical implications for female reproduction. J Obstet Gynaecol Can. **29**(11): 887–902.
133 Mitchell M, Armstrong DT, Robker RL, Norman RJ (2005). Adipokines: implications for female fertility and obesity. Reproduction. **130**(5): 583–597.
134 Mitchell PB, Morris MJ (2007). Depression and anxiety with rimonabant. Lancet. **370** (9600): 1671–1672.
135 Monteleone P, Matias I, Martiadis V, DePetrocellis L etal (2005). Blood levels of the endocannabinoid anandamide are increased in anorexia nervosa and in binge-eating disorder, but not in bulimia nervosa. Neuropsychopharmacology. **30**(6): 1216–1221.
136 Moreira FA, Lutz B (2008). The endocannabinoid system: emotion, learning and addiction. Addict Biol. **13**(2): 196–212.
137 Müller-Vahl KR, Emrich HM (2008). Cannabis and schizophrenia: towards a cannabinoid hypothesis of schizophrenia. Expert Rev Neurother. **8**(7): 1037–1048.
138 Nakada M, Yada T (2008). Cannabinoids inhibit insulin secretion and cytosolic Ca2+ oscillation in islet beta-cells via CB1 receptors. Regul Pept. **145**(1–3): 49–53.
139 Nieswandt B, Aktas B, Moers A, Sachs UJ (2005). Platelets in atherothrombosis: lessons from mouse models. J Thromb Haemost. **3**(8): 1725–1736.
140 Nishizawa H, Shimomura I, Kishhida K, Maeda N *et al* (2002). Androgens decrease plasma adiponectin, an insulin-sensitizing adipocyte-derived protein. Diabetes. **51**(9): 2734–2741.
141 Nissen SE, Nicholls SJ, Wolski K, Rodes-Cabau J *et al* (2008). Effect of rimonabant on progression of atherosclerosis in patients with abdominal obesity and coronary artery disease: the STRADIVARIUS randomized controlled trial. JAMA. **299**(13): 1547–60.
142 Nixon PJ (2006). Health effects of marijuana: a review. Pac Health Dialog. **13**(2): 123–129.
143 Ofek O, Karsak M, Leclerc N, Fogel M *et al* (2006). Peripheral can-nabinoid receptor, CB2, regulates bone mass. Proc Natl Acad Sci U S A. **103**(3): 696–701.
144 Onaivi E., Sugiura T., Di Marzo V (2006). Endocannabinoids. The Brain and Body's marijuana and beyond. CRC Press. Taylor and Francis Group (eds).
145 Orio F, Palomba S, Cascella T, Milan G *et al* (2003). Adiponectin levels in women with polycystic ovary syndrome.J Clin Endocrinol Metab. **88**(6): 2619–2623.
146 Osei-Hyiaman D, DePetrillo M, Patcher P, Liu J *et al* (2005). Endocannabinoid activation at hepatic CB1 receptors stimulates fatty acid synthesis and contributes to diet-induced obesity. J Clin Invest. **115**(5): 1298–1305.
147 Osei-Hyiaman D, Harvey-White J, Bátkai S, Kunos G (2006).The role of the endocannabinoid system in the control of energy homeostasis. Int J Obes. **30** (Suppl.1): S33–S38.
148 O'Sullivan SE (2007). Cannabinoids go nuclear: evidence for activation of peroxisome proliferator-activated receptors. Br J Pharmacol. **152**(5): 576–582.
149 Pacher P, Batkai S, Osei-Hyiaman D, Offertaler L *et al* (2005). Hemodynamic profile, responsiveness to anandamide, and baroreflex sensitivity of mice lacking fatty acid amide hydrolase. Am J Physiol Heart Circ Physiol. 289(2): H533–541.
150 Pacher P, Bátkai S, Kunos G (2006). The endocannabinoid system as an emerging target of pharmacotherapy. Pharmacol Rev. **58**(3): 389–462.
151 Pagano C, Pilon C, Calcagno A, Urbanet R. *et al* (2007). The endogenous cannabinoid system stimulates glucose uptake in human fat cells via phosphatidylinositol 3-kinase and calcium-dependent mechanisms. J Clin Endocrinol Metab. **92**(12): 4810–4819.
152 Pagotto U, Marsicano G, Cota D, Lutz B, Pasquali R (2006).The emerging role of the endocannabinoid system in endocrine regulation and energy balance. Endocr Rev. **27**(1): 73–100.
153 Panidis D, Kourtis A, Farmakiotis D, Mouslech T, Rousso D, Koliakos G (2003). Serum adiponectin levels in women with polycystic ovary syndrome. Hum Reprod. **18**(9): 1790–1796.
154 Paradisi A, Oddi S, Maccarrone M (2006).The endocannabinoid system in ageing: a new target for drug development. Curr Drug Targets. **7**(11): 1539–1552.
155 Paraskevas KI, Liapis CD, Mikkhailidis DP (2006). Leptin: a promising therapeutic target with pleiotropic action besides body weight regulation. Curr Drug Targets. **7**(6): 761–771.
156 Paria BC, Das SK, Dey SK (1995). The preimplantation mouse embryo is a target for cannabinoid ligand-receptor signaling. Proc Natl Acad Sci U S A. **92**(21): 9460–9464.
157 Paria BC, Deutsch DD, Dey SK (1996). The uterus is a potential site for anandamide synthesis and hydrolysis: differential profiles of anandamide synthase and hydrolase activities in the mouse uterus during the periimplantation period. Mol Reprod Dev. **45**(2): 1 83–192.
158 Paria BC, Zhao X, Wang J, Das SK, Dey SK (1999). Fatty-acid amide hydrolase is expressed in the mouse uterus and embryo during the periimplantation period. Biol Reprod. **60**(5): 1151–1157.
159 Paria BC, Dey SK (2000). Ligand-receptor signaling with endocannabinoids in preimplantation embryo development and implantation. Chem Phys Lipids **108**(1–2): 211–220.
160 Paria BC, Wang H, Dey SK (2002). Endocannabinoid signaling in synchronizing embryo development and uterine receptivity for implantation. Chem Phys Lipids. **12**(1–2): 201–210.
161 Park B, McPartland JM, Glass M (2004). Cannabis, cannabinoids and reproduction. Prostaglandins Leukot Essent Fatty Acids. **70**(2): 189–197.
162 Pasquali R, Gambineri A, Pagotto U (2006). The impact of obesity on reproduction in women with polycystic ovary syndrome. BJOG. **113**(10): 1148–1159.
163 Pasquali R, Gambineri A (2006). Metabolic effects of obesity on reproduction. Reprod Biomed Online. **12**(5): 542–551.
164 Patcher P, Batkai S, Kunos G (2005). Blood pressure regulation by endocannabinoids and their receptors. Neuropharmacology. **48**: 1130–1138.
165 Perez LE, Smith CG, Asch RH (1981). Δ9-tetrahydrocannabinol inhibits fructose utilization and motility in human, rhesus monkey, and rabbit sperm in vitro. Fertil Steril. **35**(6): 703–705.
166 Pertwee RG (2005). Pharmacological actions of cannabinoids. Handb Exp Pharmacol.**168**: 1–51.
167 Pertwee RG (2006). The pharmacology of cannabinoid receptors and their ligands: an overview. Int J Obes. **30** (Suppl 1): S13–8.
168 Pertwee RG (2007). GPR55: a new member of the cannabinoid receptor clan? Br J Pharmacol. **152**(7): 984–986.
169 Pertwee RG. 2008. Ligands that target cannabinoid receptors in the brain: from THC to anandamide and beyond. Addict Biol. **13**(2): 147–159.
170 Pertwee RG (2008). The diverse CB1 and CB2 receptor pharmacology of three plant cannabinoids: delta9-tetrahydrocannabinol, cannabidiol and delta9-tetrahydrocannabivarin. Br J Pharmacol.**153**(2): 199–215.

171 Petersen G, Sorensen C, Schmid PC, Artmann A *et al* (2006). Intestinal levels of anandamide and oleoylethanolamide in food-deprived rats are regulated through their precursors. Biochim Biophys Acta. **1761**(2): 143–150.
172 Piomelli D (2005). The endocannabinoid system: a drug discovery perspective. Curr Opin Investig Drugs. **6**(7): 672–679.
173 Pi-Sunyer FX, Aronne LJ, Heshmati HM, Devin J *et al* (2006). Effect of rimonabant, a cannabinoid-1 receptor blocker, on weight and cardiometabolic risk factors in overweight or obese patients: RIO-North America: a randomized controlled trial. JAMA. **295**(7): 761–75.
174 Poso A, Huffman JW (2008). Targeting the cannabinoid CB2 receptor: modelling and structural determinants of CB2 selective ligands.Br J Pharmacol. **153**(2): 335–346.
175 Prostaglandins Other Lipid Mediat. **83**(1–2): 62–74.
176 Puffenbarger RA (2005). Molecular biology of the enzymes that degrade endocannabinoids. Curr Drug Targets CNS Neurol Disord. **4**(6): 625–631.
177 Pushkarev VM, Kovzun OI, Tronko MD (2008). Antineoplastic and apoptotic effects of cannabinoids. N-acylethanolamines: protectors or killers? Exp Oncol. **30**(1): 6–21.
178 Randall MD (2007). Endocannabinoids and the haematological system. Br J Pharmacol. **152**(5): 671–675.
179 Ravinet Trillou C, Delgorge C, Menet C, Arnone M, Soubrié P (2004). CB1 cannabinoid receptor knockout in mice leads to leanness, resistance to diet-induced obesity and enhanced leptin sensitivity. Int J Obes Relat Metab Disord. **28**(4): 640–648.
180 Reggio PH (2005). Cannabinoid receptors and their ligands: ligand-ligand and ligand-receptor modeling approaches. Handb Exp Pharmacol. **168**: 247–281.
181 Roche R, Hoareau L, Bes-Houtmann S, Gonthier MP *et al*. 2006. Presence of the cannabinoid receptors, CB1 and CB2, in human omental and subcutaneous adipocytes. Histochem Cell Biol. **126**(2): 177–187.
182 Rossato M (2008). Endocannabinoids, sperm functions and energy metabolism. Mol Cell Endocrinol. **286**(1–2 Suppl 1): S31–S35.
183 Rossato M., C. Pagano, R. Vettor (2008). The Cannabinoid System and Male Reproductive Functions. J Neuroendocrinol. **20** (Suppl 1): 90–93.
184 Russo P, Strazzullo P, Cappuccio FP, Tregouet DA *et al* (2007). Genetic variations at the endocannabinoid type 1 receptor gene (CNR1) are associated with obesity phenotypes in men. J Clin Endocrinol Metab. **92**(6): 2382–2386.
185 Saario SM, Laitinen JT (2007). Monoglyceride lipase as an enzyme hydrolyzing 2-arachidonoylglycerol. Chem Biodivers. **4**(8): 1903–1913.
186 Saario SM, Laitinen JT (2007). Therapeutic potential of endocannabinoid-hydrolysing enzyme inhibitors. Basic Clin Pharmacol Toxicol. **101**(5): 287–293.
187 Sarfaraz S, Adhami VM, Syed DN, Afaq F *et al* (2008). Cannabinoids for cancer treatment: progress and promise. Cancer Res. **68**(2): 339–342.
188 Sarzani R (2008). Endocannabinoids, Blood Pressure and the Human Heart. J Neuroendocrinol. **20**(Supp1): 59–62.
189 Scheen AJ, Finer N, Hollander P, Jensen MD *et al* (2006). Efficacy and tolerability of rimonabant in overweight or obese patients with type 2 diabetes: a randomised controlled study. Lancet. **368**(9548): 1660–72.
190 Scheen AJ. 2008. CB1 receptor blockade and its impact on car-diometabolic risk factors: overview of the RIO programme withrimonabant. J Neuroendocrinol. **20** (Suppl 1): 139–46.
191 Schuel H, Burkman LJ, Lippes J, Crickard K *et al* (2002). N-Acylethanolamines in human reproductive fluids. Chem Phys Lipids. **121**(1–2): 211–227.
192 Siegmund SV, Schwabe RF (2008). Endocannabinoids and liver disease. II. Endocannabinoids in the pathogenesis and treatment of liver fibrosis. Am J Physiol Gastrointest Liver Physiol. **294**(2): G357–362.
193 Sipe JC, Waalen J, Gerber A, Beutler E (2005). Overweight and obesity associated with a missense polymorphism in fatty acid amide hydrolase (FAAH). Int J Obes **29**(7): 755–759.
194 Smita K, Kumar SV, Premendran JS (2007). Anandamide: an update. Fundam Clin Pharmacol. **21**(1): 1–8.
195 Starowicz KM, Cristino L, Matias I, Capasso R Racioppi A *et al* (2008). Endocannabinoid dysregulation in the pancreas and adipose tissue of mice fed with a high-fat diet. Obesity. **16**(3): 553–565.
196 Steffens S, Veillard NR, Arnaud C, Pelli G *et al* (2005). Low dose oral cannabinoid therapy reduces progression of atherosclerosis in mice. Nature. **434**(7034): 782–786.
197 Stein EA, Fuller SA, Edgemond WS, Campbell WB (1996). Physiological and behavioural effects of the endogenous cannabinoid, arachidonylethanolamide (anandamide), in the rat. Br J Pharmacol. **119**(1): 107–114.
198 Stern E, Lambert DM (2007). Medicinal chemistry endeavors around the phytocannabinoids. Chem Biodivers. 4(8): 1707–1728.
199 Storr MA, Keeman CM, Emmerdinger D, Zhang H *et al* (2008). Targeting endocannabinoid degradation protects against experimental colitis in mice: involvement of CB(1) and CB (2) receptors. J Mol Med. In press.
200 Strobel A, Issad T, Camoin L, Ozata M, Strosberg AD (1998). A leptin missense mutation associated with hypogonadism and morbid obesity. Nat Genet. **18**(3): 213–215.
201 Sun Y, Alexander SP, Garle MJ, Gibson CL *et al* (2007).Cannabinoid activation of PPAR alpha; a novel neuroprotective mechanism. Br J Pharmacol. **152**(5): 734–743.
202 Sun X, Dey SK (2008). Aspects of endocannabinoid signaling in periimplantation biology. Mol Cell Endocrinol. **286**(1–2 Suppl 1): S3–S11.
203 Tam J, Ofek O, Fride E, Ledent C, Gabet Y *et al*. (2006). Involvement of neuronal cannabinoid receptor CB1 in regulation of bone mass and bone remodeling. Mol Pharmacol. **70**(3): 786–792.
204 Tam J, Trmbovler V, DiMarzo V, Petrosino S *et al* (2008). The cannabinoid CB1 receptor regulates bone formation by modulating adrenergic signaling. FASEB J. **22**(1): 285–294.
205 Taylor AH, Ang C, Bell SC, Konje JC (2007). The role of the endocannabinoid system in gametogenesis, implantation and early pregnancy. Hum Reprod Update. **13**(5): 501–513.
206 Tyler K, Hillard CJ, Greenwood-Van Meerveld B (2000). Inhibition of small intestinal secretion by cannabinoids is CB1 receptor-mediated in rats. Eur J Pharmacol. **409**(2): 207–211.
207 Valassi E, Scacchi M, Cavagnini F (2008). Neuroendocrine control of food intake. Nutr Metab Cardiovasc Dis.**18**(2): 158–168.
208 Van Gaal LF, Rissanen AM, Scheen AJ, Ziegler O, *et al* (2005). Effects of the cannabinoid-1 receptor blocker rimonabant on weight reduction and cardiovascular risk factors in overweight patients: 1-year experience from the RIO-Europe study. Lancet. **365**(9468): 1389–97.
209 Vandevoorde S (2008). Overview of the chemical families of fatty acid amide hydrolase and monoacylglycerol lipase inhibitors. Curr Top Med Chem. **8**(3): 247–267.
210 Vaughan CW, Christie MJ (2005). Retrograde signalling by endocannabinoids. Handb Exp Pharmacol. **168**: 367–383.
211 Velasco G, Carracedo A, Blazquez C, Lorente M *et al* (2007). Cannabinoids and gliomas. Mol Neurobiol. **36**(1): 60–67.
212 Verty AN, McFarlane JR, McGregor IS, Mallet PE (2004). Evidence for an interaction between CB1 cannabinoid and melanocortin MCR-4 receptors in regulating food intake. Endocrinology. **145**(7): 3224–3231.
213 Vignesh JP, Mohan V (2007). Polycystic ovary syndrome: a component of metabolic syndrome?J Postgrad Med. **53**(2): 128–134.
214 Vinod K, Arango V, Xie S, Kassir SA *et al* (2005). Elevated levels of endocannabinoids and CB1 receptor-mediated G-protein signaling in the prefrontal cortex of alcoholic suicide victims. Biol Psychiatry. **57**(5): 480–486.
215 Vinod KY, Hungund BL (2005). Endocannabinoid lipids and mediated system: implications for alcoholism and neuropsychiatric disorders. Life Sci. **77**(14): 1569–1583.
216 Vinod KY, Hungund BL (2006). Role of the endocannabinoid system in depression and suicide. Trends Pharmacol Sci. **27**(10): 539–545.

217 Viso A, Cisneros JA, Ortega-Gutiérrez S (2008). The medicinal chemistry of agents targeting monoacylglycerol lipase. Curr Top Med Chem. **8**(3): 231–246.
218 Wang H, Dey SK, Maccarrone M (2006). Jekyll and hyde: two faces of cannabinoid signaling in male and female fertility. Endocr Rev. **27**(5): 427–448.
219 Wang H, Xie H, Sun X, Kingsley PJ *et al* (2007). Differential regulation of endocannabinoid synthesis and degradation in the uterus during embryo implantation. **83**(1–2): 62–74.
220 Wang J, Natsuo U (2007). Role of the endocannabinoid system in metabolic control. Curr Opin Nephrol Hypertens. **17**(1): 1–10.
221 Watanabe S, Doshi M, Hamazaki T (2003). n-3 Polyunsaturated fatty acid (PUFA) deficiency elevates and n-3 PUFA enrichment reduces brain 2-arachidonoylglycerol level in mice. Prostaglandins Leukot Essent Fatty Acids. **69**(1): 51–59.
222 Woelkart K, Salo-Ahen OM, Bauer R (2008). CB receptor ligands from plants. Curr Top Med Chem **8**(3): 173–186.
223 Wolf SA, Ullrich O (2008). Endocannabinoids and the Brain Immune System: New Neurones at the Horizon? J Neuroendocrinol. **20** (Suppl 1): 15–19.
224 Wright KL, Duncan M, Sharkey KA (2008). Cannabinoid CB2 receptors in the gastrointestinal tract: a regulatory system in states of inflammation. Br J Pharmacol. **153**(2): 263–270.
225 Xie S, Furjanic MA, Ferrara JJ, McAndrew NR *et al* (2007). The endocannabinoid system and rimonabant: a new drug with a novel mechanism of action involving cannabinoid CB1 receptor antagonism – or inverse agonism – as potential obesity treatment and other therapeutic use J Clin Pharm Therap **32**: 209–231.
226 Yan ZC, Liu DY, Zhang LL, Shen CY *et al* (2007). Exercise reduces adipose tissue via cannabinoid receptor type 1 which is regulated by peroxisome proliferator-activated receptor-delta. Biochem Biophys Res Commun. **354**(2): 427–433.
227 Zhao LJ, Jiang H, Papasian CJ, Maulik D *et al* (2008). Correlation of obesity and osteoporosis: effect of fat mass on the determination of osteoporosis. J Bone Miner Res. **23**(1): 17–29.