The Endocannabinoid System: A New Perspective for Cardiometabolic Risk Control

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The prevalence of many cardiovascular risk factors has been significantly reduced in the last forty years[1]. Therapeutic advance gained from lipid-lowering agents, anti-hypertensive drugs, and anti-diabetic oral agents have acted as invaluable tools to reduce the heavy burden imposed by cardiovascular and metabolic risk factors on public health. Despite this evidence, cardiovascular diseases still remain a major cause of death in many countries in the Western World - whether due to inappropriate control of diseases such as diabetes mellitus and hypertension, and the smoking habit[1], or by the emergence of new risk factors such as abdominal obesity[2], reduced levels of HDL-C, hypertriglyceridemia and higher proportion of small and dense LDL particles[3], all recognized to act as contributory elements for cardiovascular risk as a whole.

Although clinical trials have demonstrated significant reduction in the number of events by the use of highly effective therapeutic regimens, a major residual risk still remains, leaving a considerable number of treated patients vulnerable to cardiovascular and metabolic morbidity[4-6]. This is particularly alarming for individuals reporting multiple risk factors.

Obesity – especially visceral adiposity – is an ongoing pandemic affecting the populations of both developed and developing countries, as Brazil[7,8], in a similar way. In our days, visceral adipose tissue is seen as a potentially diabetogenic, pro-hypertensive, pro-inflammatory, and pro-atherosclerosis endocrine organ[9]. Changes in the expression and secretion of adipocytokines and inflammatory mediators explain the association of abdominal adiposity to insulin resistance, atherogenic dyslipidemia, and hypertension. These factors are included as syndrome components, in the different definitions, of Metabolic Syndrome — ATP III[11], *World Health Organization*[12] and *International Diabetes Federation (IDF)*[13].

Recent studies have identified the molecular basis, the neuronal circuits, and the metabolic pathways involved in food intake regulation. A considerable number of neuropeptides has been characterized in distinctive hypothalamic nuclei as interacting with signals originated at peripheral organs, which suggests that there is a complex network participating not only in appetite and satiety control, but also in energy balance modulation and body constitution[14].

The endocannabinoid system is an endogenous signaling system with physiological action on energy homeostasis regulation as well as on lipids and carbo hydrates metabolism[15]. Endocannabinoid system hyperactivation not only results in body weight increase[15] but can also induces dyslipidemic and dysglycemic phenotypes[16]. A number of clinical and experimental studies have shown that pharmacological intervention in the system has proven to be a promising therapeutic perspective to control obesity, dyslipidemia, insulin resistance and atherosclerosis[17,18].

The endocannabinoid system

History

Cannabis Sativa (marijuana) is the most widely consumed illegal drug in the world as of the 1960's[19]. Having been cultivated for over five thousand years for the fibers it provides for materials manufacturing process, *Cannabis* had been prescribed by the Chinese as from 2600 BC to treat cramps, rheumatic and menstrual pain[20]. However, not until 1964 was its active ingredient Δ^9-tetrahydrocannabinol (THC) isolated and its chemical structure characterized[21]. In our days, a considerable number of *Cannabis Sativa* analogues have been prescribed as antiemetic and appetite stimulants to oncology patients on chemotherapy. Dronabinol – a THC synthetic compound – was approved by the FDA over fifteen years ago as ancillary management for advanced stages of AIDS and cancer patients that develop anorexia and cachexia[22-24].

The first cannabinoid receptor was identified[25] in 1988. In 1993, that receptor was called CB_1, after that same year a second receptor had been characterized and named CB_2[26]. Both receptors are coupled to $G_{i/o}$ proteins and belong to a wide and diverse family of proteins coupled to the cellular membrane. Tissue distribution in those structures explains most of the psychotropic effects of THC accounted to CB_1[27] receptors. The effects of CB_2 peripheral receptors are more closely associated to immune response[28].

The first cannabinoid receptors endogenous ligands – the endocannabinoids – were isolated in 1992[29]. Presently, anandamide (*N*-arachidonoyl ethanolamine) and 2-araquidonoil glycerol (2-AG) are the most extensively studied among all endogenous cannabinoids. "Ananda" comes from Sanscrit and means serene happiness or eternal happiness[20]. Both endocannabinoids are CB_1 and CB_2 receptors agonists. 2-AG cell and tissue levels are higher than those of anandamide as a result of their higher involvement in different metabolic pathways. Cannabinoid receptors, endocannabinoids, as well as the enzymes that catalyze their biosynthesis and degradation constitute the endocannabinoid system.

CB_1 and CB_2 Receptors

The cannabinoid receptors that have been characterized to this day regulate the activity of adenylate cyclases (which they

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inhibit) and *MAPK* (*Mitogen-Activated Protein Kinases*) – which they stimulate. As for CB_1 receptors, specifically, modulation is carried out on voltage activated Ca^{2+} channels (which they inhibit) and K^+ channels (which they stimulate)[30]. The primary function of those receptors is the transduction of extra cellular stimuli in intracellular signals.

Among the G Protein-coupled membrane receptors, the CB_1 are the more abundant so far identified in the central nervous system, although they are also found in peripheral nervous system[31]. Through their receptors, major actions can be seen by endogenous cannabinoids on central nervous system, such as cognitive and emotional function regulation at neuronal circuits in the cortex, in the hippocampus and amygdala, and in boosting substances effects that lead to chemical dependence in the mesolimbic system: cocaine[32], heroin[33], anphetamine[34] and alcohol[35].

Some studies have shown the important role played by the endocannabinoid system in modulating nicotine dependence. In $CB_1^{-/-}$ animals, nicotine rewarding effects are abolished[36] and the administration of a selective CB_1 antagonist – rimonabant – reduces their search for the alkaloid[37].

CB_2 receptors are located in structures associated to immune system and hematopoiesis modulation. Stimulation of those structures by Δ^9-tetrahydrocannabinol results in an immunosuppressant phenotype[38].

Formation and inactivation of endocannabinoids. Retrograde Neurotransmission Process

Most endocannabinoids identified so far are by-products of PUFAs –long chain polyunsaturated fatty acids, especifically arachidonic acid (Figure 1). Therefore, anandamide and 2-AG are formed by phospholipid independent pathways; the synthesis enzymes are N-acylphosphatidylethanolamine-selective phospholipase D (NAPE-PLD) and diacylglycerol lipase (DAG Lipase), respectively[39,40].

Although most endocannabinoids act under demand or need, in response both to physiological (neuronal depolarization) and pathological[15] stimuli, evidence has shown that their primary activation takes place in some areas in the brain where energy balance is controlled, thus suggesting an ongoing tonus that favors energy intake and storage[41].

Both anandamide and 2-AG have their action interrupted by a neuronal reuptake process, followed by their metabolism. This stage seems to take place by mere diffusion and/or through a process facilitated by a carrier protein. Both endocannabinoids are quickly metabolized and hydrolized by FAAH (*Fatty Acid Amide Hydrolase*) and MAG lipase (*Monoacyl Glycerol*), respectively, in inactive compounds[16,42].

The multiple functions of the endocannabinoid system

Clinical and experimental studies have demonstrated that endogenous cannabinoids and the concurrent activation of their CB_1 receptors result in a plethora of effects, among them: 1) involvement in antinociceptivity (reduction in painful stimuli), movement control and short term memory inhibition[43]; 2) inhibition of prolactin secretion and growth hormone, and increased ACTH[44] secretion; 3) ansyolitic effects through action on hypothalamus-hypophysis-adrenal axis[45]; 4) immune and inflammatory response modulation[46]; 5) increase in heart rate, vasodilation and bronchodilation[47,48]; 6) inhibition of testosterone secretion, anovulation and uterine

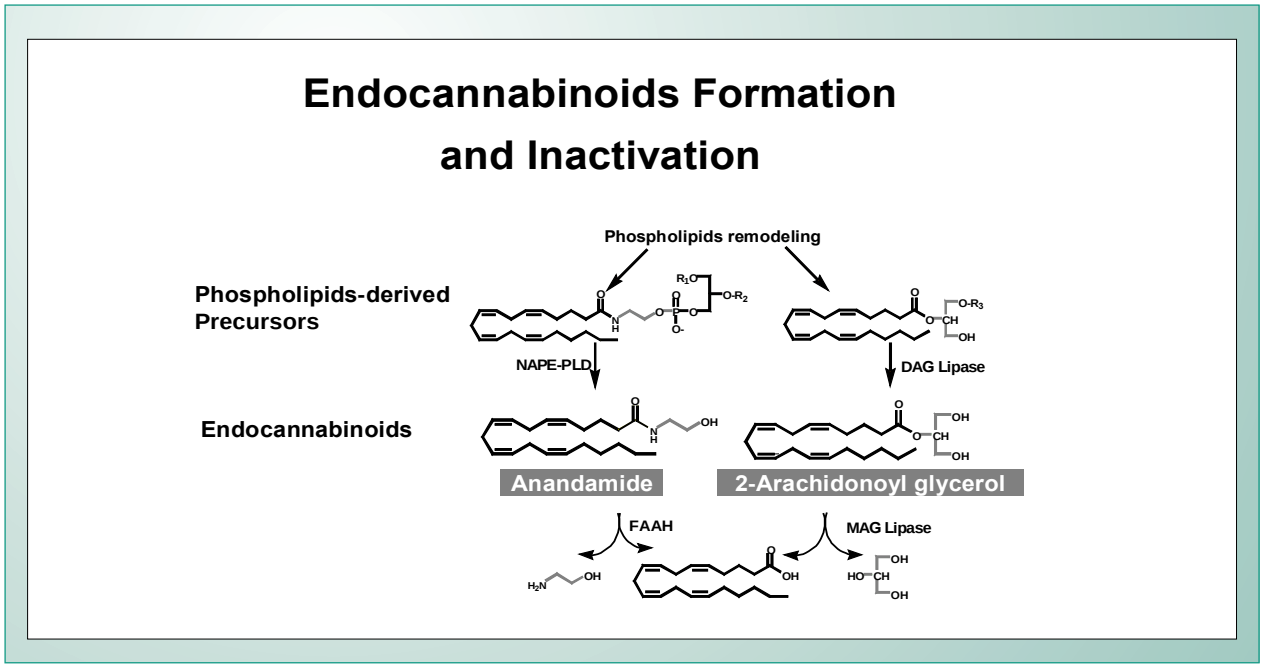

Fig. 1 - *Most endocannabinoids are long-chain polyunsaturaded fatty acids by-products. Anandamide and 2-arachidonoyl glycerol (2-AG) are produced from the remodeling of phospholipids through pathways that use NAPE-PLD (N-acylphosphatidylethanolamine-seletive phospholipase D) and DAG (Diacylglycerol) lipase synthesis enzymes. They are rapidly metabolized and hydrolized by FAAH (Fatty Acid Amide Hydrolase) and MAG L (Monoacyl Gycerol Lipase) enzymes. Endocannabinoids have local action and are produced on demand. Adapted from: Di Marzo V et al.*[52]

relaxation[9]; 7) antitumoral activity[50]; 8) neuroprotection against trauma and hypoxia conditions[51]; 9) food intake modulation due to its effects on the release of peptides and hypothalamic hormones and to their regulation by steroids[31]. All of those pleiotropic effects have been summarized by Di Marzo and cols[52] in one statement: "The endocannabinoid system reduces the feeling of pain; controls movement, memory, sleep, and appetite; and is protective."

The tonic activation of cardiac and vascular CB_1 receptors seems to limit blood pressure increase. Recently, Kunos and cols[53] have observed that when spontaneously hypertensive rats (SHR) were treated with an anandamide degradation inhibitor their hypertension condition was controlled. Such effect was reversed by the administration of CB_1 antagonists. In addition to reducing SHR blood pressure, endocannabinoids inactivation blocking concurrently reduced left ventricle contractile performance, although the same parameters were not shown to have been affected in normal animals[53].

Another extremely intriguing observation – this time in regard to CB_2 receptors function – is that their immunosuppressant properties would have a beneficial, protective effect on the inflammatory milieu of the atherosclerotic lesions. While working with apoliprotein E receptors knock-out mice fed with a cholesterol-rich diet, Stefens and cols[54] have observed significant regression of the atherogenic plaques that are peculiar to that model when the animals were treated with small oral doses of THC. A plausible explanation would be that CB_2 receptors expressed in the atherosclerotic lesions, but not in normal arteries, would be activated by THC.

Food Intake Regulation by Endocannabinoids

Central effects of CB_1 receptors activation are ultimately reflected on energy balance modulation and appetite control. A body of evidence from experimental studies with obese animals (*ob*/*ob* and *db*/*db* mice and obese Zuker rats) and normal murine models has shown that: 1) the activation of CB_1 receptors by endogenous cannabinoids or THC and the injection of endocannabinoid directly in the hypothalamus or in the mesolimbic region stimulate food intake[55,56]; 2) in contrast, animals whose CB_1 receptors genes had been suppressed ($CB_1^{-/-}$) have lower food intake and exhibit a slim phenotype resistant to diet-induced body weight increase[57]; 3) under normal conditions, the intake of nutrients reduces endocannabinoids levels in the hypothalamus and in limbic forebrain, while fasting has the opposite effect, significantly increasing them[55].

Figure 2 shows that food deprived rats have consistently increased 2-AG tissue levels both in the limbic forebrain and in hypothalamus, which are areas strongly associated to motivation and the pleasure of eating[55]. The 2-AG levels are shown to decrease when animals are being fed.

In another experiment, when anandamide was administered to mice, food intake increased 44% and was shown to be significantly associated to hypothalamic concentrations of norepinephrine, dopamine, and serotonin[58].

In 2003, Cota and cols[38] demonstrated that $CB_1^{-/-}$ animals – although slimmer – did not report any change in their locomotor activity, body temperature or energy expenditure when compared to wild akin. Such data show that the decrease

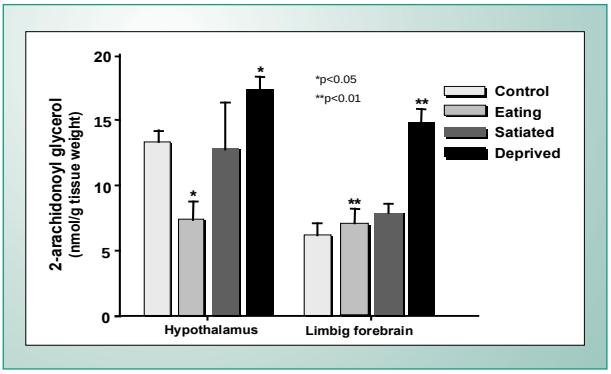

Fig. 2 - *2-AG levels measured directly at the hypothalamus and in limbic forebrain show different values in food-deprived animals, whose levels are significantly higher when compared to animals being fed. No change was observed in satiated animals. Adapted from: Kirkham TC et al.*[55]

in central orexigenic stimulus, as a result of the absence of the CB_1 receptor, explains the differences between those animals rather than the changes in their locomotor activity or their energy expenditure.

The administration of rimonabant – the first CB_1 selective antagonist, described in 1994 by Rinaldi-Carmona and cols[59] – to diet induced obese mice, led to sustained body weight reduction when compared to control animals. CB_1 blocker persistent effects on body weight reduction in these animals – in contrast with transitory decrease in food intake – suggest that other mechanisms would contribute for rimonabant long-lasting effects in addition to caloric intake[60] (Figure 3).

These data suggest that the endocannabinoid system controls energy intake at two levels. Firstly, by tonically accentuating and incentivating motivation for food search and intake, possibly from interacting with the mesolimbic pathways (nucleus accumbens) involved in reward mechanisms. Secondly, the system is activated on demand in the hypothalamus – after a short period of food deprivation – to then transiently modulate the levels and/or the action of other orexigenic and anorexigenic mediators for the purpose of appetite induction. The assumption of a dual action in the mesolimbic and hypothalamic regions has been proven by the demonstration that food intake in rodents[42] is stimulated when endocannabinoids are injected in those encephalic areas.

In the hypothalamus, endocannabinoid levels changes are inversely correlated to leptin plasma concentrations, the hormone secreted by the adipocyte which plays a key role in food intake and energy expenditure regulation. Leptin reduces endocannabinoid levels in the hypothalamus, similarly to what it does to other orexigenic mediators. Additionally, obese mice genetically defficient in leptin signalling pathways exhibit increased concentrations of endocannabinoids in the hypothalamus[57].

Co-expression of CB_1 receptors with anorexigenic and orexigenic mediators

The endocannabinoid system is a major modulator in energy intake as a result of its regulation action over the expression or the action of different anorexigenic or orexigenic mediators in different areas of the hypothalamus. Experimental studies

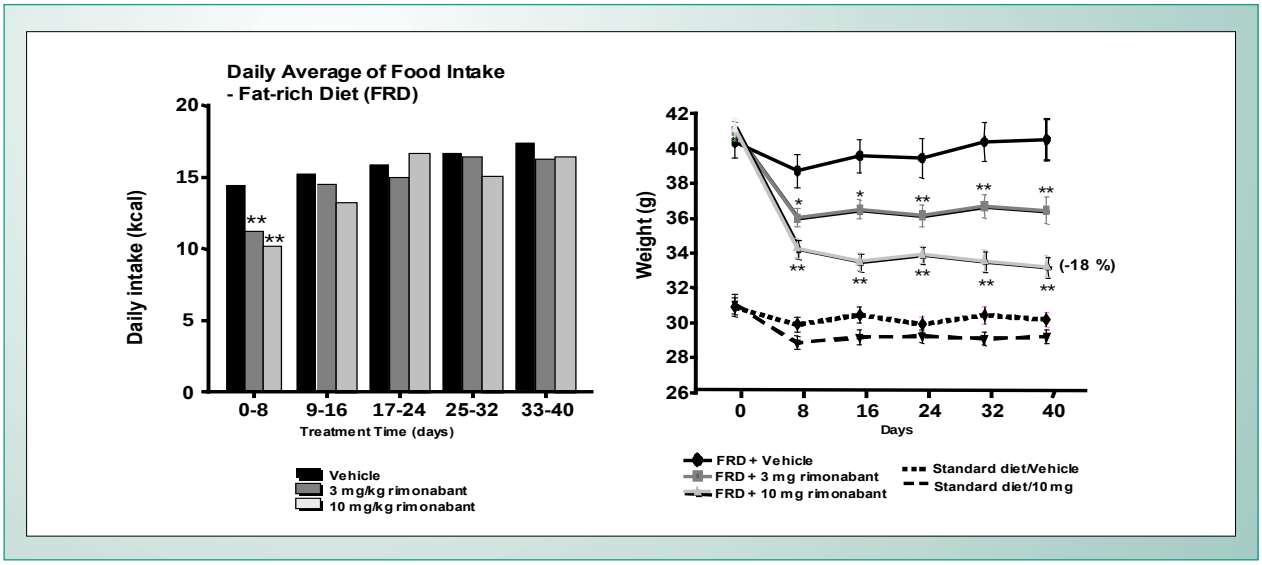

Fig. 3 - *Effects of rimonabant on food intake (to the left) and obese mice weight (to the right) after fat rich diet intake. It should be pointed out that sustained effects on drug induced weight loss contrast with lower intake as observed only in the first treatment week. Adapted from: Ravinet Trillou C et al.*[60]

have shown that CB_1 receptors are co-expressed: 1) at the paraventricular nucleus with the anorexigenic mediator CRH (*Corticotropin Release Hormone*)[61], where the endocannabinoids act in a retrograde way reducing the glutamatergic transmission of pre-synaptic neurons, thus attenuating CRH release[62]; 2) at hypothalamus lateral nucleus with orexigenic mediator MCH (*Melanin-Concentrating Hormone*)[61]; 3) at the arcuate nucleus with CART (*Cocaine Amphetamine Regulated Transcript*)[61] expressing cells; and 4) at ventromedial hypothalamus with prepro-orexin[61]. The genetic deletion of CB_1 receptors increases CRH expression, thus reflecting the tonic inhibition of that mediator by endocannabinoids[63].

A positive and direct correlation can be observed between endocannabinoid system tonus and ghrelin circulating levels after food deprivation. That peptide – secreted by the digestive tract – acts locally and interacts with endocannabinoids at vagal afferent terminations, thus increasing food intake. Those effects are blocked by rimonabant[64].

As for the mesolimbic system, there is evidence showing that endocannabinoids would increase the yearning for food while inducing higher dopamine release at the nucleus accumbens or by synergic action with opioids through yet unkonwn mechanisms[65].

Another important aspect in satiety control is the relationship between endocannabinoid system and vagal terminations connecting the gastrointestinal tract to the medulla and brainstem nuclei area. Endocannabinoids reduce satiety through their action on the vagus. Such effects may be reversed by the destruction of capsaicin-sensitive vagal terminations that modulate the effects of cholecystokinin on satiety[66]. On the other hand, cholecystokinin inhibits the expression of CB_1 receptors through vagal afferent neurons[67].

Those data suggest that reduced endocannabinoid activity may mediate the induction of satiety activity by cholecystokinin. As a counterpart, fasting overcomes satiety by stimulating the secretion of endocannabinoids in the small intestine, thus releasing vagal CB_1 from cholecystokinin inhibition.

Peripheral Effects of CB_1 Receptors Activation

The endocannabinoid system plays an effective role in lipogenesis modulation. When stimulated, CB_1 receptors increase lipoprotein lipase expression and reduce adiponectin[44]. As a counterpart, CB_1 receptor blocking has resulted in increased expression of adiponectin – both *in vitro* and *in vivo*. Adiponectin has a crucial role in reducing the expression of enzymes involved in lipogenesis[69,70], with potential major role in atherogenic dyslipidemia and disglycemia. Additionally, the activation of CB_1 receptors in hepatocytes is translated into increased *de novo* synthesis of fatty acids by these cells, as a result of the higher genic expression of the lipogenic transcription factor SREBP-1c (*Sterol Regulatory Element-Binding Protein 1c*), as well as of associated enzymes: FAS (*Fatty-Acid Synthase*) and Acyl-CoA C1 (*Acetyl-CoA Carboxilase-1*). Contrarily, $CB_1^{-/-}$ mice are resistant to those changes and to the development of hepatic steatosis[71].

As for glycemic homeostasis, $CB_1^{-/-}$ mice shows lower glucose levels after insulin intraperitoneal administration when feeding a fat-rich diet, as compared with wild animals[72]. They also exhibited a reduction in leptin and insulin plasma concentrations, thus suggesting higher sensitivity to these two hormones.

It has recently been demonstrated that rimonabant increases oxygen consumption and glucose uptake by the solear muscle of *ob/ob*[73] mice, thus showing the favorable effects of the drug on thermogenesis and insulin sensitivity.

The pathophysiologic consequences of endocannabinoid system hyperactivity

Studies in animals have suggested that the endocannabinoid system would be transitorily activated after short term fasting and/or exposure to palatable foods, which would stimulate appetite and attenuate satiety, in addition to

increasing lipogenesis and reducing energy expenditure[42]. This is consistent with the concept that increased levels of endocannabionoids – inevitable in the presence of stimuli associated to stress – would act as a strategy in helping superior organisms for the purpose of homeostasis retrieval.

As a counterpart, results from pre-clinical and clinical trials have clearly indicated that the system also contributes for the modulation of conditions accompanied by hyperphagia and adipose mass accumulation, and that its pharmacological blocking would reverse this scenario. Sustained hyperactivity of the system in tissues that control energy balance would, therefore, play a key role not only in the development of obesity but in the emergence of the consortium of cardiometabolic risk factors[15] (Figure 4).

The question that must be asked is: Which causal factors would be involved in the changes from a system that acts "on demand" to a sustained hyperactivity system? Apparently, such hyperactivity would be associated to fat-rich diets that make polyunsaturated fatty acids available for the biosynthesis of the endocannabinoids[42]. Additionally, obese rats' adipocyte has shown higher expression of CB_1 receptor when compared to the adipocyte of slim rats or immature adipocytes[70]. A fat-rich diet also results in higher anandamide synthesis by the hepatocytes with higher expression of CB_1 receptors[71]. Against to this scenario, openly pro-orexigenic, significant resistance to leptin anoretic actions would take place[57].

A *missense* polymorphism in homozygote genotype FAAH 385 A/A has recently been identified in overweight and obese individuals, showing potentially inadequate functioning of one of the key enzymes in endocannabinoids[74] degradation pathways, which would be an additional explanation for system hyperactivity.

Indeed, the assumption of endocannabinoid system sustained hyperactivity in certain circumstances has been the *leitmotiv* to sponsor the indication of CB_1 selective antagonists for the management of obesity and its consequences.

Effects of CB_1 receptors selective blocking on cardiovascular risk factors. The RIO (*Rimonabant In Obesity*) Program

The blocking of CB_1 receptors with selective antagonists, as rimonabant, seems to be a promising perspective for the reduction of cardiovascular diseases that persist even after therapeutic regimens, considered highly effective, have been unfavorable.

Rimonabant pharmacokinetic studies have shown that the drug has rapid oral absorption, with a terminal half-life of 9 days in healthy individuals, and of 16 days in obese individuals. It is metabolized by the CYP3A and amidohydrolase, and eliminated by biliary pathways, with insignificant renal excretion. No rimonabant dosage adjustments are required for mild to moderate renal and hepatic failure patients. Co-administration of rimonabant with food or orlistat showed to be of minimal impact on the drug pharmacokinetics[75].

Data from pre-clinical phase were confirmed by RIO-Europe[76], RIO-Lipids[17], RIO-North America[18], and RIO-Diabetes[77] phase III clinical trials –that enrolled overweight or obese patients, with or without associated comorbidities. These three multicenter, randomized, double-blind, placebo-controlled trials included 6,627 patients – both males and females – under the following profile: Mean age 45-56 years old, mean body mass index between 33-38kg/m^2, and waist circumference > 88cm for women and > 102cm for men. From all patients randomized in the clinical trials, 53% to 66% completed the 12 – month period of the studies.

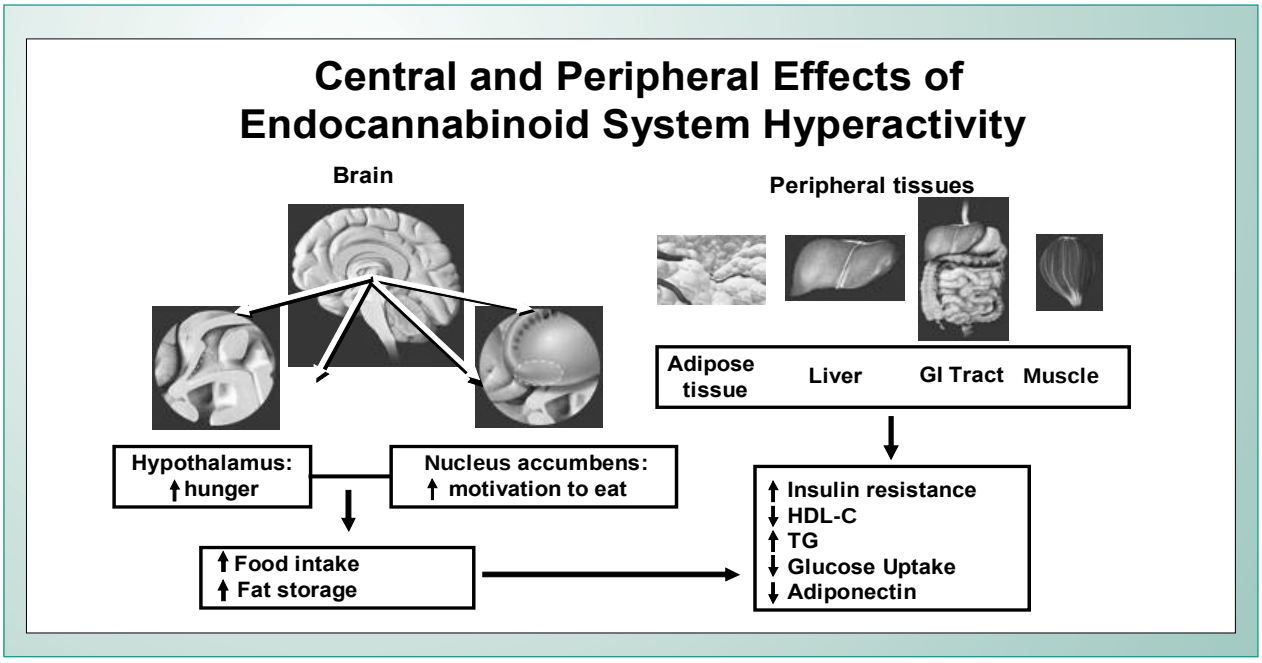

Fig. 4 - *Repercussions of endocannabinoid system hyperactivity at central sites responsible for hunger and motivation for food, as well as in peripheral tissues. Sustained hyperactivity contributes for the development of overweight and the emergence of cardiometabolic risks that are aggregated under the metabolic syndrome denomination. Adapted from: Di Marzo V, Matias I [15], Pagotto U et al.[16]*

Dyslipidemia rate ranged from 55.7% in the RIO-Diabetes to 100% in the RIO-Lipids. Metabolic syndrome rate ranged from 34.7% in the RIO-North America to 79.3% in the RIO-Diabetes. Hypertension percentage ranged from 61.2% in the RIO-Diabetes to 27.2% in the RIO-Lipids. The trials were carried out in the United States, Canada and Europe.

RIO-Lipids trial included 1,033 patients who were overweight or obese, and had non-treated dyslipidemia. Diabetics were excluded. The trial was one-year long[17]. The RIO-Europe trial included 1,507 overweight or obese individuals, with or without comorbidities. Diabetics were also excluded in this two-year long[76] trial. The RIO-North America trial included 3,040 obese or overweight individuals, with or without associated comorbidities. The study also excluded diabetics and had two phases: the first was 12-month long; the second, with patients who had been on rimonabant and randomized to an arm that used placebo and another that kept the same dosing for rimonabant[18]. The RIO-Diabetes randomized 1,047 patients – all of them overweight, obese, and with type 2 diabetes. This trial last one-year[77].

Significant waist circumference (-8,5cm) and body weight (-8,6kg) reduction could be seen after one year in the three studies that were published, with rimonabant, 20mg/day (Figure 5). Prevention against weight and abdominal circumference regain was evaluated in RIO-North America patients, who were re-randomized to the 20mg/20mg rimonabant arm.

As for rimonabant effect on cardiometabolic risk factors, the following results were observed:

1) HDL-C, triglycerides, small and dense LDL, and LDL-C levels

Rio-Europe reported significant changes in triglycerides (-6,8%) and HDL-C (22.3%) concentrations after one year under treatment (*vs* placebo) in the group on 20mg rimonabant. The changes in those two parameters were very similar in RIO-Lipids and were maintained after two years on the drug in RIO-North America. Rimonabant had no appreciable effect on cholesterol and LDL-C levels in any of the three studies. In RIO-Lipids, there was a significant reduction in the proportion of LDL small and dense particles, in the Rimonabant 20 mg group (Figure 6), when compared to placebo. Logistic regression models and/ or ANCOVA using body weight loss as covariable, showed that after 20mg of rimonabant, both HDL-C and triglycerides reported changes partially independent of weight loss (Figure 7).

2) Changes in glycemic parameters.

An analysis of the three clinical trials that were published characterized a subgroup of pre-diabetic patients (n=1,290) whose glucose fasting levels ranged between equal to or higher than 100mg/dl and lower than 126mg/dl. The results showed that 46.5% of pre-diabetic patients who were administered 20mg/day of rimonabant for one year had fasting glucose levels back to normal values (under 100mg/dl).

As for drug effects on glycosylated hemoglobin values, RIO-Diabetes showed that 43% of patients on 20mg of rimonabant had this parameter reversed to normal values (under 6.5% after one year of treatment, when compared to the placebo group, where that change was reported for 21% of patients).

Significant improvement was also reported in insulin fasting concentration, as well as in insulin resistance calculated by HOMA, when results were compared with the placebo group. After one year of treatment with placebo, and 5mg and 20mg/day of rimonabant, metabolic syndrome prevalence in those groups was 48.1%, 46.4% and 32.3% ($p=0.30$ and $p<0.001$ *vs* placebo, respectively).

3) RIO-Lipids showed that adinopectin levels increased by 57.7% with the administration of 20mg of rimonabant. That difference was significant when compared to the placebo group (Figure 8). It is important to point out that over 50% of such increase happened irrespective of body weight loss. Additionally, adiponectin levels had a positive and significant correlation with changes in HDL-C and Apo-A1. The same trial showed that leptin levels significantly decreased with the administration of rimonabant - both at 5mg and 20mg regime. Plasma concentrations of C-reactive protein were significantly reduced in the rimonabant group, thus showing favorable drug interference in that inflammatory marker (Figure 6). Systolic and diastolic pressure were significantly reduced (-2.1mmHg and -1.7mmHg, respectively). Hypertensive patients showed a higher reduction.

4) Drug primary efficacy analysis was applied to the ITT (*Intention-To-Treat*) population and based on LOCF (*Last Observation Carried Forward*). As general, 20mg dosing of rimonabant was well tolerated, and adverse effects – mild to moderate – were mostly limited to depression episodes [2.9% *vs* 0.6% (placebo)], anxiety [1.7% *vs* 0.6% (placebo)] and nausea [1.2% *vs* 0% (placebo)]. Serious adverse effects that led to study discontinuation were reported by 5.2%, 4.0% and 2.3% among patients who were on 20mg, 5mg and placebo, respectively[17], for one year. Patients receiving the same treatment for two years reported comparable discontinuation rate due to adverse effects (4% placebo, 6.3% 5mg, 4.2% 20mg), thus suggesting that they occur early, and that 5mg and 20mg of rimonabant exhibit tolerability and safety profile similar to placebo[18].

5) Based on RIO-Program, no relevant interaction was reported between anti- hypertensive agents, vastatins, oral anti-diabetic drugs, fibrates and rimonabant[17,18,76,77].

In addition to the clinical trials included in the RIO-Program, other studies are being carried out to investigate whether the improvement in cardiometabolic risk profile by the administration of rimonabant is translated by changes in coronary atherosclerotic plaque volume, as measured by intravascular ultrasound (STRADIVARIUS Study)[79]. The possible benefits of rimonabant on fatal and non-fatal outcomes, as a result of acute myocardial infarction episodes and stroke, are being evaluated in a prospective, randomized, and controlled trial (CRESCENDO Study), currently in patient recruiting phase.

Considering that quitting smoking is associated to a significative decrease in cardiovascular events, rimonabant has being tested – both in the United States and in Europe (STRATUS Study)[81], with the purpose of investigating the possible effects of this drug on cigarette smoking abstinence rate. Partial results from STRATUS-US have shown that this rate was significantly higher in individuals who used 20mg of rimonabant when compared to those who used placebo.

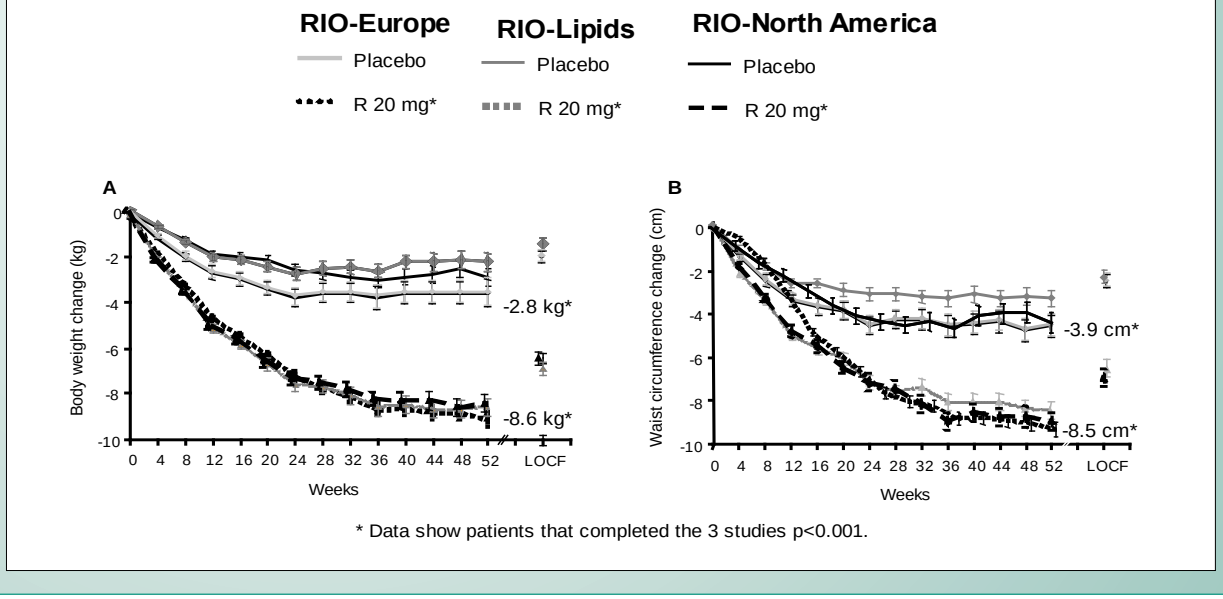

Fig. 5 - *Effects of rimonabant (20 mg/day) on body weight (A) and waist circumference (B) after one year of treatment. Data show patients who have completed the three studies: RIO-Lipids, RIO-North America and RIO-Europe. Adapted from: Després JP et al 17, Pi-Sunyer FX et al [18], Van Gaal LF et al. [75]*

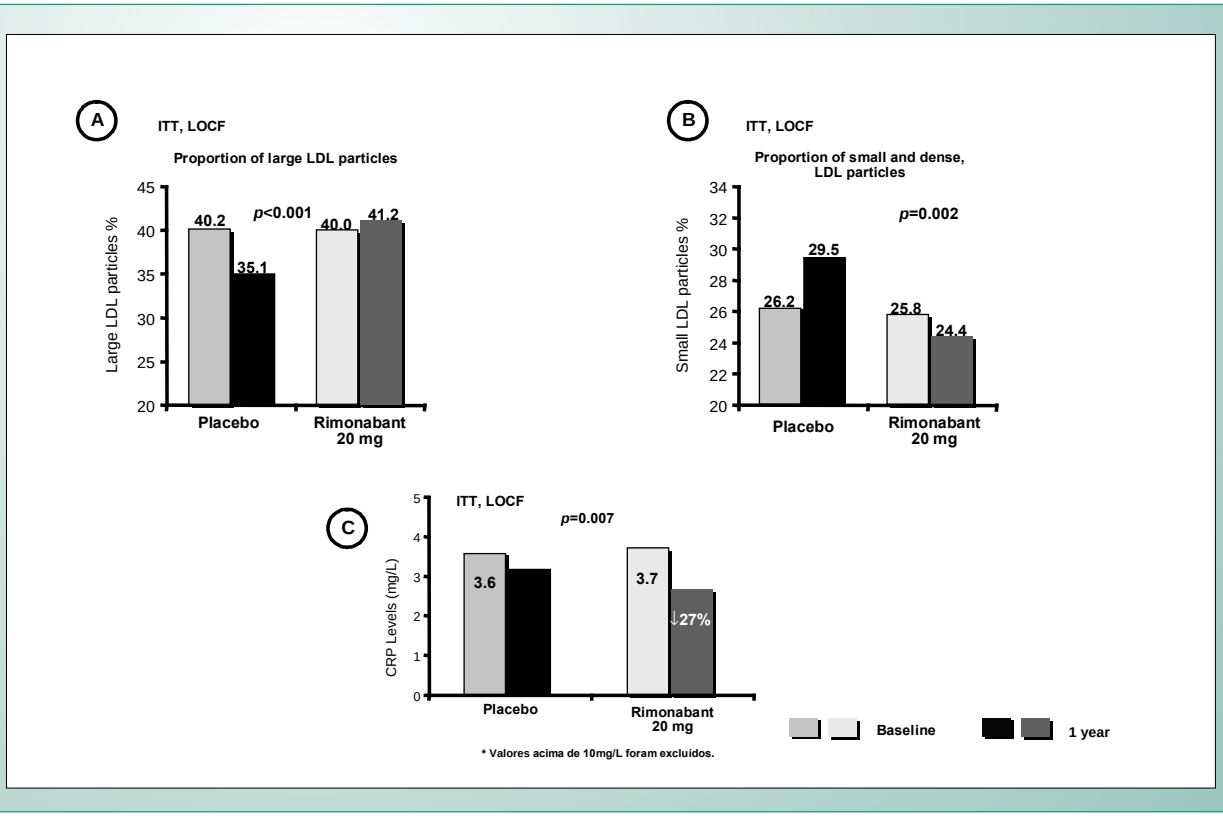

Fig. 6 - *In the RIO-Lipids, LDL particle distribution showed that the larger ones were found in the group that received 20mg of rimonabant (A), when compared to placebo. This resulted in significant 4.7% reduction in the proportion of small and dense LDL particles (B). Significant reduction was also shown (0.6 mg/l) in ultrasensitive CRP (C). P values refer to the differences between the 20mg rimonabant group vs placebo group. Adapted from: Després JP et al.[17]*

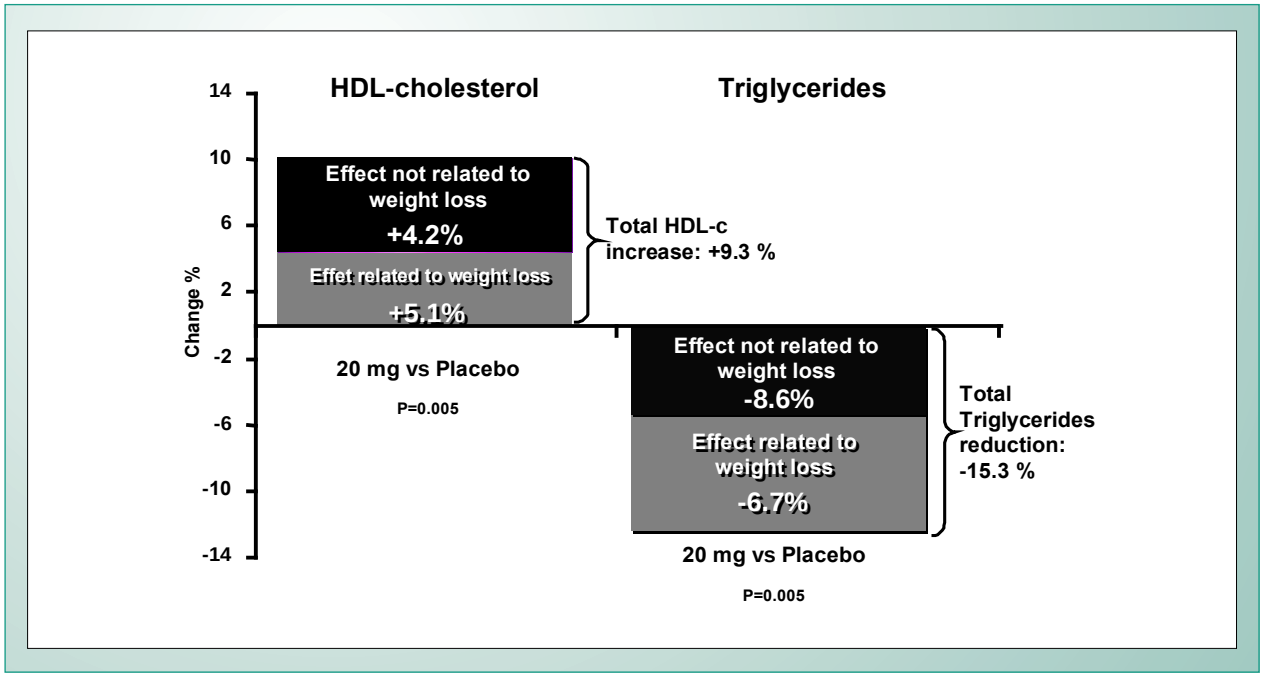

Fig. 7 - Changes observed in HDL-c concentrations and triglycerides with the administration of rimonabant 20 mg were partially independent of weight loss. Data from RIO-Europe. Logistic regression models were used, using weight loss as co-variable. Adapted from: Van Gaal LF et al.[75]

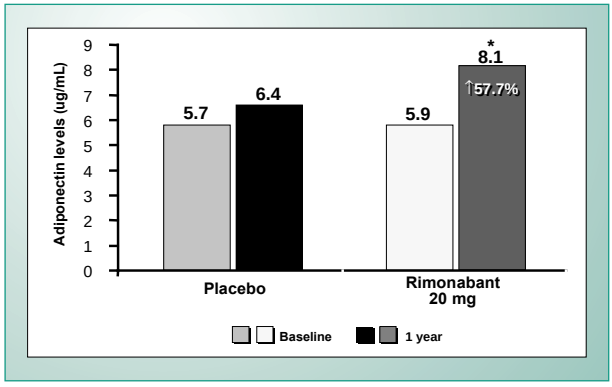

Fig. 8 - RIO-Lipids data show plasma adiponectin levels increased over 57% with the administration of rimonabant when compared to baseline levels. It is relevant that over 55% of such increase was independent of weight loss. Asterisk represents P<0.01 (difference between rimonabant 20mg vs placebo). Adapted from: Després JP et al.[17]

Data analysis of these clinical trials shows that pharmacological intervention on endocannabinoid system is not only an innovative alternative, but also very promising for the treatment of cardiometabolic risk factors associated to abdominal obesity, and possibly a truly potential tool for the prevention of atherosclerosis and its consequences. It also points towards a 2-year extension period for these effects, while emphasizing that the metabolic profile improvement was partially independent from body weight loss.

The treatment of obesity with drugs that antagonize the CB_1 receptors goes beyond body weight loss or merely aesthetic purposes. It is meant for high risk patients, most exhibiting excessive intra-abdominal fat associated to cardiovascular and metabolic risk factors. Actions aiming at changes in lifestyle must always be implemented. Additionally, identifying the dyslipidemic and dysglycemic phenotypes, as mentioned earlier, by measuring abdominal waist circunfrrence, may change the residual risk that is still reported by a significant number of patients.

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