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Review

Endocannabinoids and cardiovascular prevention: real progress?

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ABSTRACT: The prevalence of obesity continues to increase and represents one of the principal causes of cardiovascular morbidity and mortality. After the discovery of a specific receptor of the psychoactive principle of marijuana, the cannabinoid receptors and their endogenous ligands, several studies have demonstrated the role of this system in the control of food intake and energy balance and its overactivity in obesity. Recent studies with the CB1 receptor antagonist rimonabant have demonstrated favorable effects such as a reduction in body weight and waist circumference and an improvement in metabolic factors (cholesterol, triglycerides, glycemia etc). Therefore, the antagonism of the endocannabinoid (EC) system, if recent data can be confirmed, could be a new treatment target for high risk overweight or obese patients. Obesity is a growing problem that has epidemic proportions worldwide and is associated with an increased risk of premature death (1-3). Individuals with a central deposition of fats have elevated cardiovascular morbidity and mortality (including stroke, heart failure and myocardial infarction) and, because of a growing prevalence not only in adults but also in adolescents, it was reclassified in AHA guidelines as a "major modifiable risk factor" for coronary heart disease (4, 5). Although first choice therapy in obesity is based on correcting lifestyle (diet and physical activity) in patients with abdominal obesity and high cardiovascular risk and diabetes, often it is necessary to use drugs which reduce the risks. The EC system represents a new target for weight control and the improvement of lipid and glycemic metabolism (6, 7). (Heart International 2007; 3: 27-34)

KEY WORDS: Endocannabinoid system, Obesity, CB1 antagonists

The endocannabinoid system

The identification, in the mid-1960s, of the major psycochoactive component of *Cannabis sativa* and marijuana (Δ9-tetrahydrocannabinol, Δ9 THC) and the discovery of its membrane receptors, paved the way to reveal a whole endogenous signaling system known as the endocannabinoid (EC) system (7).

Several studies have demonstrated the role of the EC system in physiological functions, such as homeostasis and stress response. EC have neuroprotective and analgesic properties, controlling movement and some memory processes (8, 9). Moreover, the EC system modulates immune, endocrine and inflammatory response. Finally, EC influence the pulmonary and cardiovascular system, controlling blood pressure and heart rhythm and having bronchodilatating properties (6-9).

Endocannabinoids

Anandamide (N-arachidonoyl-ethanolamine (AEA)) and 2-arachidonoyl-glycerol (2-AG) are the most studied EC, but recently new synthetic and non-synthetic molecules have been proposed as cannabinoid receptor agonists (Fig. 1) (6, 7).

It is well established that AEA and 2-AG are not prestored in secretory vesicles but are de novo biosynthesized following an increase in the intracellular concen-

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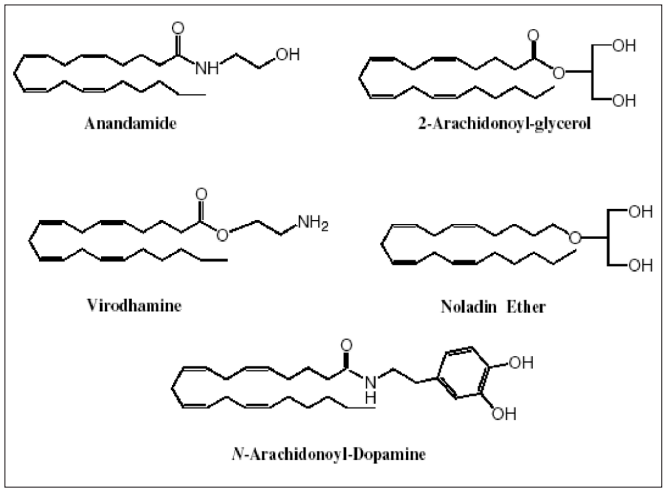


Fig. 1 - *Endocannabinoids.*

tration of calcium, within a framework of a metabolic reaction involving phospholipid precursors (N-acyl-phosphatidyl-ethanolamine and diacylglycerol) and specific calcium-sensitive enzymes (phospholipasis D and diacylglycerol lipasis). Therefore, the whole EC production is triggered "on demand". EC are released from the cell immediately after their biosynthesis and then quickly removed from the extracellular space by a rapid and selective cellular reuptake mechanism (not yet clear). In particular, EC transport is not leaded by transmembrane ionic gradients, but to passive diffusion through a transporter not yet identified. Instead, EC degradation consists of the hydrolysis of AEA to ethanolamine and arachidonic acid by fatty acid amide hydrolase (FAAH) and of 2-AG by monoacyl-glycerol lipases (6, 7).

Cannabinoid receptors

To date, only two cannabinoid receptors have been cloned: the CB1 and CB2 receptors (8, 9).

At first the CB1 receptor was thought to be expressed just in the cortical brain region (neocortex, hippocampus, amigdala), in the basal ganglia, in the mesolimbic system, in the thalamus and hypothalamus and in the cerebellum: neurons of these areas regulate the expression of orexigenic and anorexigenic signals (10, 11).

Recent studies have demonstrated that CB1 receptors are expressed in peripheral cells and tissues controlling energy homeostasis, including gut, liver adipocytes, skeletal muscle and pancreas.

CB2 receptors are present in several immune cells and blood cells where they participate in the regulation of cytokine release and function (10). Nevertheless, CB2 receptors also develop functions in other cells such as the cheratinocitis, the osteoclastis and the endocrine pancreas.

Since CB2 agonists have no psychological effects on central nervous system they have become the object of numerous studies in the therapeutic use of cannabinoids, particularly as regards the analgesic, anti-inflammatory and antineoplastic effects.

Endocannabinoids act primarily at cannabinoid CB1 and/or CB2 receptors with different efficacy. Synthetic cannabinoids have been created so as to act as highly selective agonists or antagonists for CB1 or CB2 receptors. Δ9-tetrahydrocannabinol and 2-AG approximately have similar affinity for CB1 and CB2 receptors, while the AEA has a marginal selectivity for CB1 receptors. However, the effectiveness of the Δ9-THC and the AEA is lower in CB2 receptor than in CB1 receptor (7).

Both receptors belong to the family of the receptors coupled to the G protein (GPCRs). The intracellular signaling events include:

1. inhibition of stimulus-induced adenylate cyclase and subsequent impairment of cAMP/protein kinase A-mediated short and long-term effects;
2. stimulation of mitogen-activated protein kinase signaling;
3. in case of CB1 receptors, inhibition of voltage-gated Ca^{2+} channels and stimulation of inwardly rectifying G protein-coupled K^+ channels;
4. in case of CB1, stimulation of phosphatidylinositol 3-kinase and of intracellular Ca^{2+} mobilization (7).

Endocannabinoid system and modulation of energetic balance

The EC system participates in the modulation of the so-called mechanisms of pleasure and the manipulation of this system influences appetite. Elevated CB1 expression in cerebral areas involved in the control of pleasure indicates a strong involvement of this system in different psychological functions, regulated by these regions of the brain, including appetite (10).

Under these circumstances, the ingested food that acts on the nervous fibers which connect the hindbrain

and the midbrain to the hypothalamus influences dopamine, opioids, serotonine and noradrenaline modulating appetite and satisfaction.

The most remarkable route of pleasure is represented by the mesolimbic dopaminergic system. The study of this system clearly shows the existence of an increase of dopamine extracellular levels inside the accumbens nucleus after the ingestion of tasty food. Psychoactive drugs such as marijuana and ethanolol, but also pleasant stimuli or tasty foods, are known to induce the release of dopamine in specific cerebral regions. At this level, the existence of a relationship has also been shown between the EC system and the oppioid and serotoninergic system (12).

It is now widely accepted that EC released from depolarized post-synaptic neurons retrogradely activate presynaptic CB1 receptors, thereby reducing both inhibitory (GABA mediated) and excitatory (glutamate mediated) neurotransmission. This property seems to participate in EC regulation of the hypothalamic networks and of the anorexigenic (arcuate nucleus expressing cocaine-amphetamine regulated transcript – CART and paraventricular nucleus expressing corticotrophin-releasing hormone) and orexigenic signals (lateral hypoyhalamus neurons containing melanin-concentrating-hormone and orexins) (13-15).

Signals coming from various peripheral organs such as the liver, the gut and the adipose tissue direct hormonal and biochemical signals to the hypothalamus to notify the central nervous system about nutritional state. An example of this peripheral control is leptin, a hormone produced only by adipose tissue able to interact with specific receptors located in the hypothalamus to carry an anorexigenic signal. The EC system is modulated by leptin too; it was demonstrated that acute treatment with leptin reduces hypothalamic levels of AEA and 2-AG in normal mice, but above all it was underlined that, in mice made obese and hyperphagic by a defect of the leptin signal, EC hypothalamic levels are permanently and pathologically elevated (14, 15).

Finally, some experimental evidence indicated that EC and CB1 receptors regulate energetic metabolism through a peripheral action at the level of the adipose tissue, liver and pancreas (16-18).

Cota et al (11) demonstrated for the first time that wild-type rats exhibit significantly higher amounts of fat mass than CB1 receptor deficient rats. In addition, CB1 receptors stimulate lipoprotein lipase; as a consequence they activate lipogenesis, suggesting that EC contribute to the accumulation of body fat not only stimulating food intake, but also acting directly at the adipose tissue level.

Recent studies *in vitro* on adipose cell cultures underlined that CB1 receptors blockage induces an arrest of adipocytis proliferation, while chronic stimulation of these receptors induces differentiation of preadipocytis to adipocytis, shown by the early appearance of the differentiation marker PPAR (peroxisome proliferator activated receptor). These results confirm that EC actively participate in adipogenesis and fat accumulation; however, it was observed that AEA is able to act on PPAR receptors, independently from CB1 receptors (7).

In the liver, CB1 receptors are expressed around the centrolobular vein, where, stimulating the expression of SREBP-1c (steroid regulatory element binding protein 1-c) and its target (acetilCoA-carboxylase 1 and fatty acid synthase), they activate lipogenesis and fatty acid synthesis (7).

More recently, it was observed that EC are able to regulate insulin levels, peripheral glucose uptake; and therefore, to increase glucose tolerance. EC through CB1 activation, would seem to be able to modify the secretion of Ca^{2+} and, therefore, of insulin (19). Nevertheless, further studies will be necessary to define the role of the EC system in the pancreas.

Endocannabinoid overactivity in obesity

Obesity is probably a condition associated to the hyperactivity of the EC system. This recent hypothesis is supported by a series of studies developed on animal models of obesity; nevertheless, it still needs to be demonstrated in humans.

It was demonstrated that the CB1 receptor is overexpressed in tissues which control energetic metabolism such as the liver (18), skeletal muscles (9) and the adipose organ (12, 16) when animals are made obese by a high fat diet. In addition, the most important demonstration about metabolism, is that the hyperactivation of the EC system induces in adipocyte cultures a reduction in the levels of adiponectin and an increase in visfatin, two adipokines having opposing roles. Adiponectin, a hormone produced only by adipose tissue, plays an important role in the modulation of fat and glucose metabolism, because

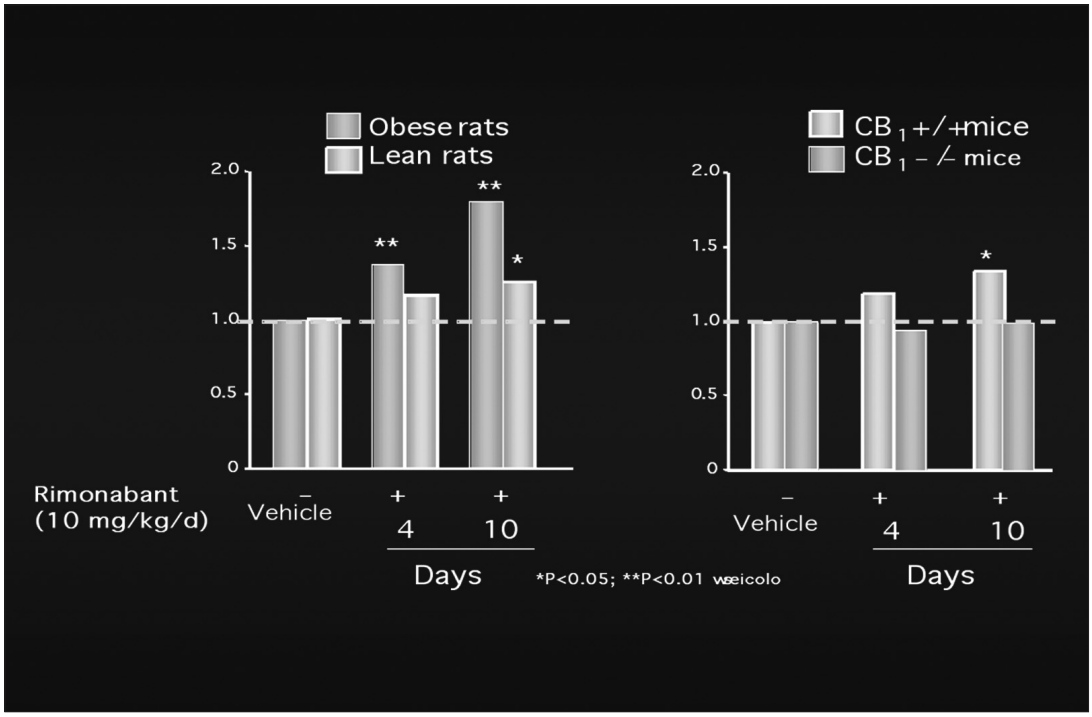


Fig. 2 - *Effects of rimonobant on adiponectin mRNA levels in lean and obese rats and in CB1 knockout mice.*

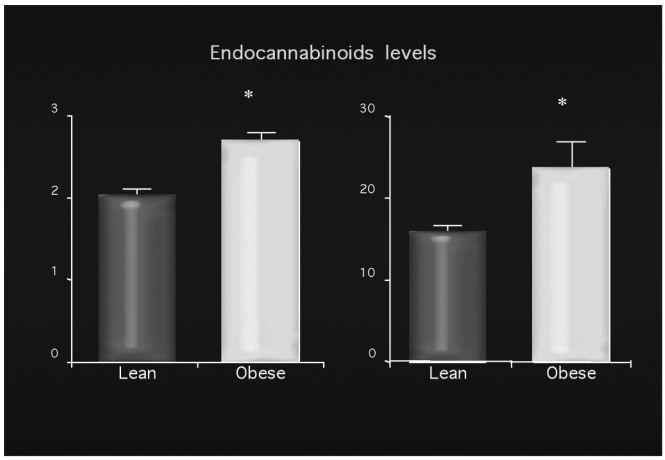


Fig. 3 - *Association between obesity and overactivity of the EC system.*

it inhibits hepatic gluconeogenesis and controls free fatty acid production, through the suppression of lipogenesis and the activation of fatty acid oxidation (16) (Fig. 2).

It is still premature to express a judgment on the clinical meaning of circulating levels of EC in humans and the mechanisms that would stimulate overactivity of the EC system in obesity remain unclear.

In obese women, with no others comorbidities, and whose cause of obesity was correlated to alimentary disorders or to the menopause, AEA and 2-AG levels were significantly greater than in the control group (17) (Fig. 3). In addition, in patients affected by diabetes mellitus type 2, EC levels were significantly greater than in non-diabetic controls (20).

According to a recent study (21), a possible mechanism of EC system hyperactivation would be represented by a reduced degradation of AEA; the authors identified in a population of obese subjects a polymorphism, at the level of the FAAH sequence, the enzyme appointed to the degradation of AEA, which would behave as reduced enzymatic activity (21).

Endocannabinoid system antagonists in the treatment of obesity

The increasing evidence of the role of the EC system in the regulation of food intake and energetic balance has stimulated the development of CB1 receptor blockers, whose the most studied one in the treatment of obesity is rimonabant (Fig. 4).

An extensive study of experimental phase III denominated RIO (rimonabant in obesity), was conducted on

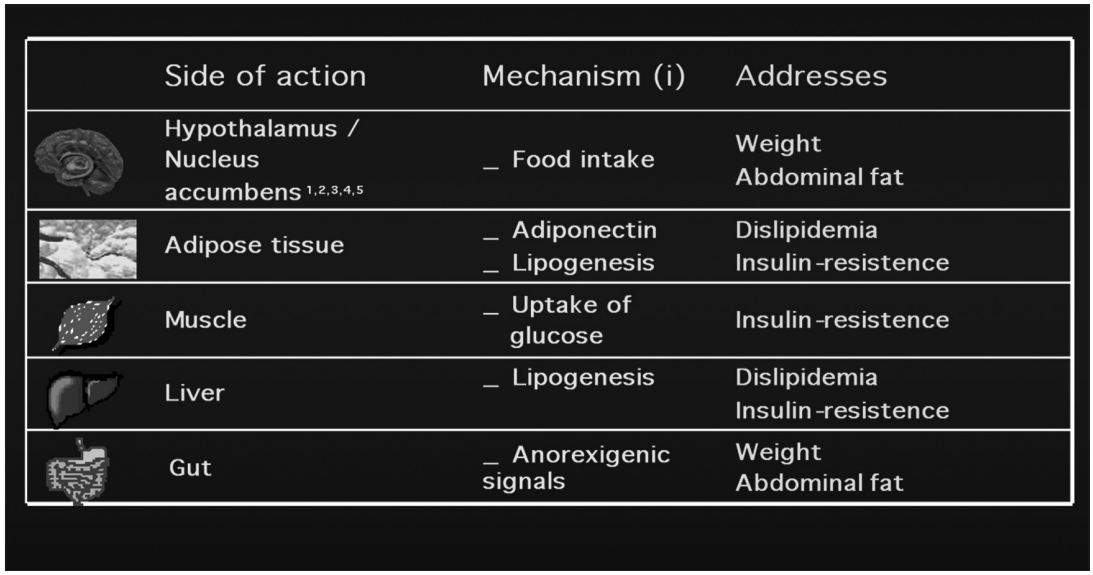


Fig. 4 - *Possible action mechanisms of CB1 receptor blockers and rimonabant effects.*

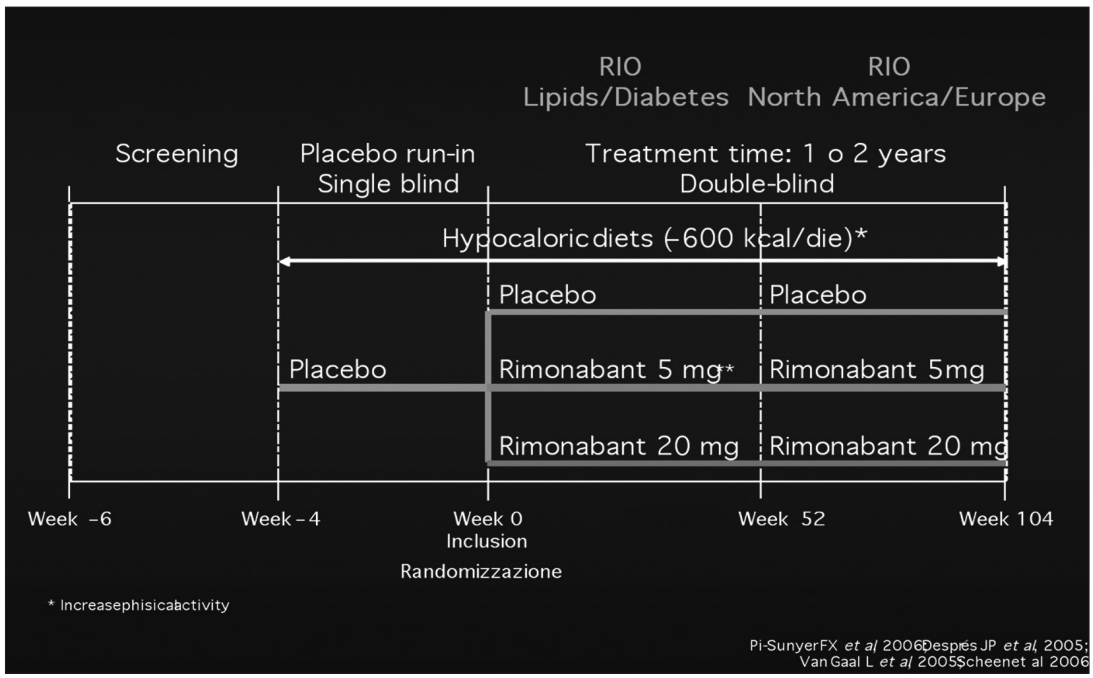
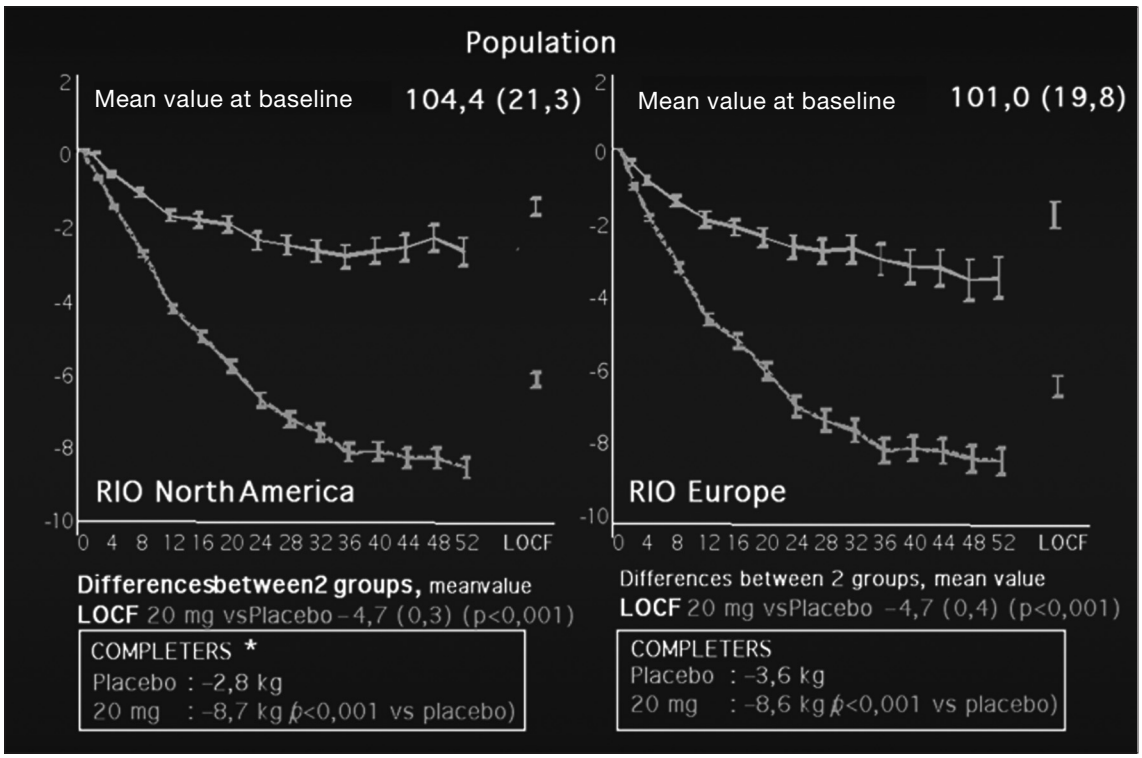


Fig. 5 - *Study design of RIO (rimonabant in obesity).*

about 6600 obese or overweight patients (22). This experiment was composed of four substudies (RIO-North America, RIO-Europe, RIO-Lipids and RIO-Diabetes) directed to identify the effectiveness of rimonabant on bodyweight as a primary end-point and on various metabolic alterations such as secondary end-point.

The pharmacological treatment, of 5 or 20 mg of rimonabant vs. placebo, was associated with a reduction in energy intake of 600 calories in respect of basal metabolic rate and to an increase in physical activity (Fig. 5). In RIO-North America (23) compared to RIO-Europe (24), patients enrolled after the first year were re-



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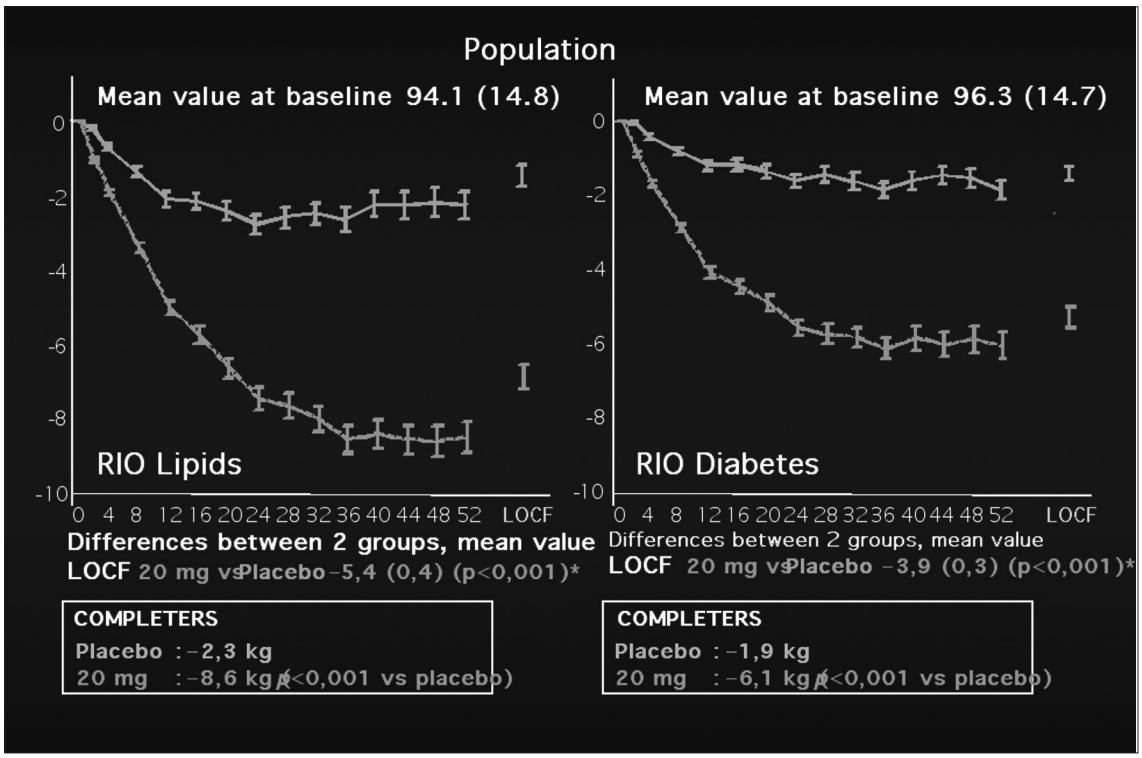

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Fig. 6 - *Results of RIO: reduction in body-weight in rimonabant vs. placebo group.*

randomized to placebo or rimonabant, for monitoring possible resumption of body weight at the end of the drug treatment. RIO-Lipids (25) and RIO-Diabetes (26) were programmed for investigating the improvements due to the administration of rimonabant, in patients that were associated with obesity or with overweight diabetes and dyslipidemia, respectively.

In all studies, treatment with rimonabant 20 mg produced results significantly greater than 5 mg and the results were equivalent for primary and secondary endpoints.

The treatment with 20 mg rimonabant determined in comparison to the placebo group a significant reduction in body weight (8.7 vs. 2.8 kg in RIO-North America, 8.6 vs. 3.6 kg in RIO-Europe, 8.6 vs. 2.3 kg in RIO-Lipids, 6.1 vs. 1.9 kg in RIO-Diabetes) and of waist circumference (8.5 vs. 4 cm in RIO-North America, 9 vs. 3 cm in RIO-Europe, 9 vs. 4 cm in RIO-Lipids, 5.2 vs. 1.9 cm in RIO-Diabetes) (Fig. 6). In the group treated with rimonabant a significant improvement in the lipidic profile was also observed, with an increase in high-density lipoprotein (HDL) cholesterol, a reduction in triglycerides, and an improvement in glycemia and insulinemia during oral glucose tolerance test.

In RIO-Lipids, changes in leptin and adiponectin levels were observed, demonstrating a significant decrease of leptin and an increase of the circulating levels of adiponectin in patients treated with rimonabant. Multivariate analysis showed that rimonabant had independent positive effects from weight loss on lipidic profile and adiponectin levels. In RIO-Europe around 50% of the variation in HDL cholesterol and in triglycerides was independent from weight loss, while in the RIO-Lipids the increase of 57% in adiponectin did not seem justifiable on the basis of caloric reduction only, but rather related to the peripheral action of rimonabant.

In RIO-Diabetes, the HbA1 levels were lower in both rimonabant groups compared to the placebo group and showed a persistent reduction in the rimonabant 20 mg group. In addition, it was possible to show that the reduction of HbA1 levels, that was two-fold higher than the reduction due to weight loss, was independent from the reduction in body weight, related to a peripheral action of rimonabant.

Finally, in RIO-North America during the second year of treatment in the group that continued with rimonabant, a continuous and progressive reduction in body weight was observed, while in the group randomized to placebo a recovery of most of the weight lost during the first year was associated with an increase in triglycerides and a reduction in HDL cholesterol.

Rimonabant was generally well tolerated and the most frequent side effects were gastrointestinal (nausea and diarrhea) and humoral (anxiety and depression).

CONCLUSIONS

The prevalence of obesity is continuously increasing and represents one of the principal causes of cardiovascular morbidity and mortality. The recent discovery of the role of the EC system in the control of energetic metabolism and the presence of the overactivity of this system in obesity, has enabled the development of new drugs, antagonists of CB1 receptors such as rimonabant. These drugs are able not only to determine a reduction in body weight, but also to promote favorable effects on lipidic and glycemic profiles, independently from weight reduction.

Therefore, CB1 antagonists could represent a new therapeutic option for the treatment of obesity and the comorbidities related to it, when the currently available data will be confirmed by further clinical studies.

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