# The leptin receptor gene, its action mechanism, polymorphisms and obesogenic clinical associations

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**Amanda Sihnel Cortez da Silva**
Medical Student
Institution: Universidade Paranaense (UNIPAR)

**Maria Luiza Marchi Silva**
Medical Student
Institution: Universidade Paranaense (UNIPAR)

**Luciano Seraphim Gasques**
PhD in Biology of Organic Interactions
Institution: Universidade Paranaense (UNIPAR)

**ABSTRACT**
With the significant increase in obesity cases worldwide, there is also an increase in research to understand its causes. Known that the predisposing factors may be environmental and / or genetic, the study focused on the Leptin Receptor (LEPR) gene, its action on satiety and the pathophysiology when the presence of polymorphisms, as well as their interaction with comorbidities that are directly related to obesity and increased adipose tissue. In addition, the motivators that influence a greater or lesser production and action of leptin, its pathway through the central nervous system and how this mechanism sends information on the body's lipid stock, thus regulating the body's energy metabolism, were highlighted. The main result of the LEPR gene mutation is leptin resistance, which consequently leads the individual to not have an adequate perception of satiety and body fat stores, the main effect of which is hyperphagia.

**Keywords:** Obesity, Genetics, Comorbidities, LEPR gene, Polymorphisms.

## 1 INTRODUCTION

In terms of biostatistics, the number of obese individuals is gradually rising, reaching epidemic levels, a basis validated by the NCD *Risk Factor Collaboration (NCD-RisC*, 2017), an organization responsible for a network of global scientists dedicated to providing rigorous and timely data on risk factors for NCDs in 200 different countries and territories. Of these, between 1975 and 2016 the prevalence of obesity increased from 69 million to 390 million in females and from 31 million to 281 million in males. Still, the WHO (2020) adds the subject versing that since the year 2000 there has been a 1.5-fold increase in the number of cases of the disease.

The etiology of obesity is multifactorial, and two main factors are listed: genetic and environmental conditions. The literature contextualizes genes as pillars of intervention and balance of body fat, and their action occurs through control of efferent and afferent pathways, as well as central mechanisms. That said, it is worth mentioning that the energy balance is regulated approximately 40% through genetic inheritance, mainly through genes of the Central Nervous System (SNC) (MARQUES-LOPES, *et al*., 2004).

Regarding genetics as a factor notably intervening in the regulation of body weight, Machado, Monteiro and Pinto (2015) review that one of the highlighted genes is leptin (LEP) and leptin receptor (LEPR), whose scope is to regulate food intake, in addition to body temperature, energy expenditure and cardiac function. The relationship of leptin - etymology from the Greek "*leptos*", which means lean - with obesity was discovered in 1974, thus being described as the "satiety hormone", since it is a peptide (similar to cytokines, being composed of 167 amino acids) that signals the energy balance and is the most important peripheral signal for energy homeostasis.

In this context, the parallelism between obesity and the LEP/LEPR genes also spreads in other spheres of the health area, fostering discussions tangent to the burdens caused by this interaction. These clinical associations raised by obesogenic polymorphisms are due to their strong risk factor implicated in other pathologies, are comorbidities, as well summarized Lima, Glaner and Taylor (2010).

In view of the above, the study aims to clarify the system that makes up the mechanism of action of the LEPR gene, establishing a link between its polymorphisms and the obese pathophysiology, in addition to exposing the links that may have repercussions of this obesity-LEPR relationship. In addition, the study intends to indicate potential treatments used for the obesogenic case.

## 2 DEVELOPMENT

According to Fernandes, Fujiwara and Melo (2011), although the substantial cause of obesity is associated with environmental aspects, the manifestation will occur mainly in those genetically predisposed. It is pertinent to clarify that phenotypic heredity is presented through a certain amount of genes and their variation. In fact, Serrasqueiro (2018) reveals that among the most known and studied genes are FTO, LEP and LEPR, BDNF, MC4R, MC3R, PCSK1, SH2B1, POMC and ADRB3.

Because the topic is broad and there is an extensive variety of genes, research and identification of genetic variants related to obesity have been carried out, aiming basically to identify the mechanisms of action and single nucleotide *polymorphisms (*SNPs) associated with the pathology in question.

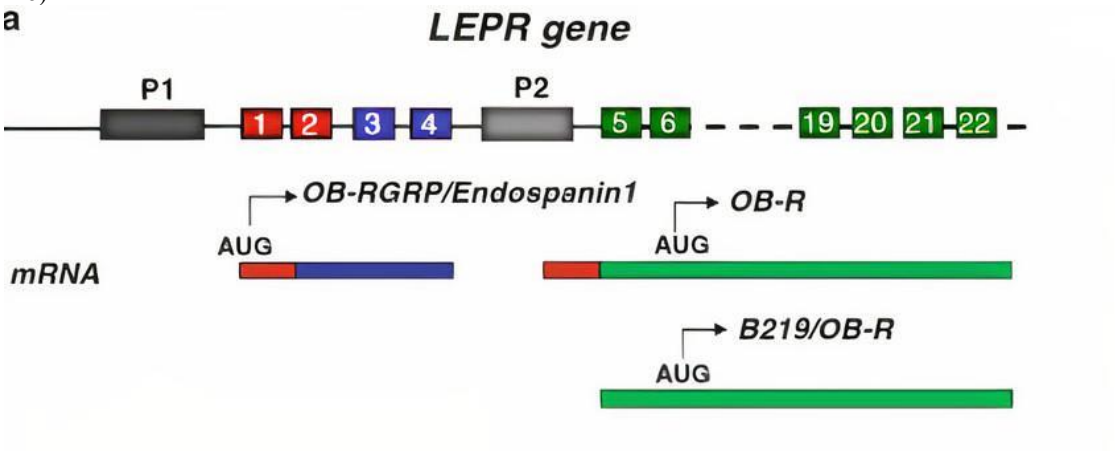
In general, Dagogo-Jack (2015) recommends that the history of obesogenic research began to deserve attention in 1949, in the United States, in studies implemented by the Jackson laboratory (independent and non-profit organization of biomedical research), when observing rats already overweight early in life, but with later obese development. Experiments in reproduction revealed that the syndrome was caused by a single autosomal recessive gene (represented by the gene symbol "*ob*"), located on chromosome 6. Years later, in 1965, in the same experiment, another gene was identified in the laboratory: it was a mutation inherited as an autosomal recessive gene, located on chromosome 4. This was called the diabetes genes, represented by the symbol "*db*". In both gene cases, the mice manifested infertility, hyperphagia, and early-onset obesity.

Meanwhile, Dagogo-Jack (2015) undertakes to point out that after the discovery of the *ob* gene, a long scientific journey was trodden and its climax was reached with the discovery of leptin by Douglas L. Coleman, a researcher at the Jackson laboratory. In this tuning fork, Coleman proposed that while *db* genes produced excessive satiety but could not respond to *it, ob genes* recognized and responded to satiety but could not produce it. Years passed and with the advancement of molecular biology, the scholar proved correct when there was the possibility of cloning leptin and the perception of its receptor, LEPR, which also presented mutations in the evaluated rats. Despite the vast array of information concerning the structure and functioning of LEPR, its molecular mechanisms of leptin resistance are still somewhat uncertain.

Regarding the basic architecture of the leptin receptor, Machado, Monteiro and Pinto (2015), certify that the gene is composed of three functional parts: 1. Extracellular: which performs the interactions; 2. Intracellular: if activated, stimulates cellular events, determining the action of leptin on target cells and; 3. Transmembrane: binds receptor to cell membrane. As for its location, Dias, *et. al.* (2012), define that the LEPR is installed in the short arm of chromosome 1, in position 1p31, and is composed of 18 exons and 17 introns.

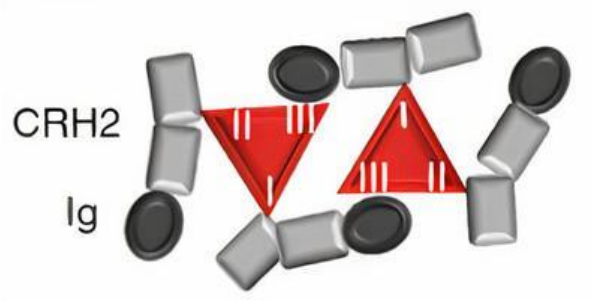
Following the teachings of Dagogo-Jack (2015), it is essential to address the genomic organization of the leptin receptor, which is structured when the activity of a promoter (region of DNA that initiates the transcription of the gene) begins, which produces two different transcriptions through an *alternative splicing* (process in which exons of a first transcript are cleaved at different locations in the newly synthesized RNA), giving rise to distinct proteins without homology in the sequence of amino acids. Important to the discussion of the study is the isoform OB-R (also called Ob-Rb), which is now named LEPR. It should be noted that in addition to this, five other isoforms are originated during *splicing,* all of which are identical in the extracellular medium, differing, however, in size and sequence. However, as well guided by Negrão and Licínio (2000), only the OB-R variant dominates the ability to transmit the signal to introduce leptin into the cell. The formulation of this gene can be presented as shown in Figure 1.

Figure 1: Representative scheme of the LEPR gene. LEPR expression is controlled by two promoters: P1 and P2. The first of these gives rise to two divergent transcriptions through an alternative splicing: LEPROT and LEPR. This generates two distinct proteins without homology in the amino acid sequence: OB-RGRP/endospanin-1 and LEPR in their short and long isoforms. In turn, the second promoter (P2) allows the expression of B219 OBR in its short isoform. (DAGOGO-JACK, 2015, p. 16)




The mechanism by which the leptin receptor is activated occurs at the moment it binds to LEP on the cell surface. In this perspective, Dagogo-Jack (2015) argues that the binding of LEPR to LEP is mediated by two domains: 1. One of the cytokine receptors allocated at the end of the polypeptide chain (called n-terminal), which is called CRH2.; 2. An immunoglobulin known as Ig, whose function is to separate the cytokine receptors CRH2 and CRH1. Thus, CRH2 and Ig bind to LEP, being also required in its activation. The consequence of this interaction is perceived after binding of two leptin molecules to LEPR, when a conformational change is triggered in the extracellular medium, causing dimers to be grouped and a hexameric complex to be formed. The composition of this complex therefore results in two leptin molecules and four LEPR promoters. Each leptin molecule binds to LEPR molecules through the binding sites, with whom they have affinity, whether high or low. For further clarification, Figure 2 is presented, which represents this succession of events:

Figure 2: Hexameric complex formed by the LEP-LEPR bond. It deals with a compound with two LEP molecules and four LEPR promoters. Each LEP binds to LEPR through the binding site: the binding site II connects to CHR2, with whom it has high affinity; binding site I is also related to CRH2, but has low affinity; finally, the link site III is associated with the IG domain, and interaction is also of low affinity (DAGOGO-JACK, 2015, p. 18)



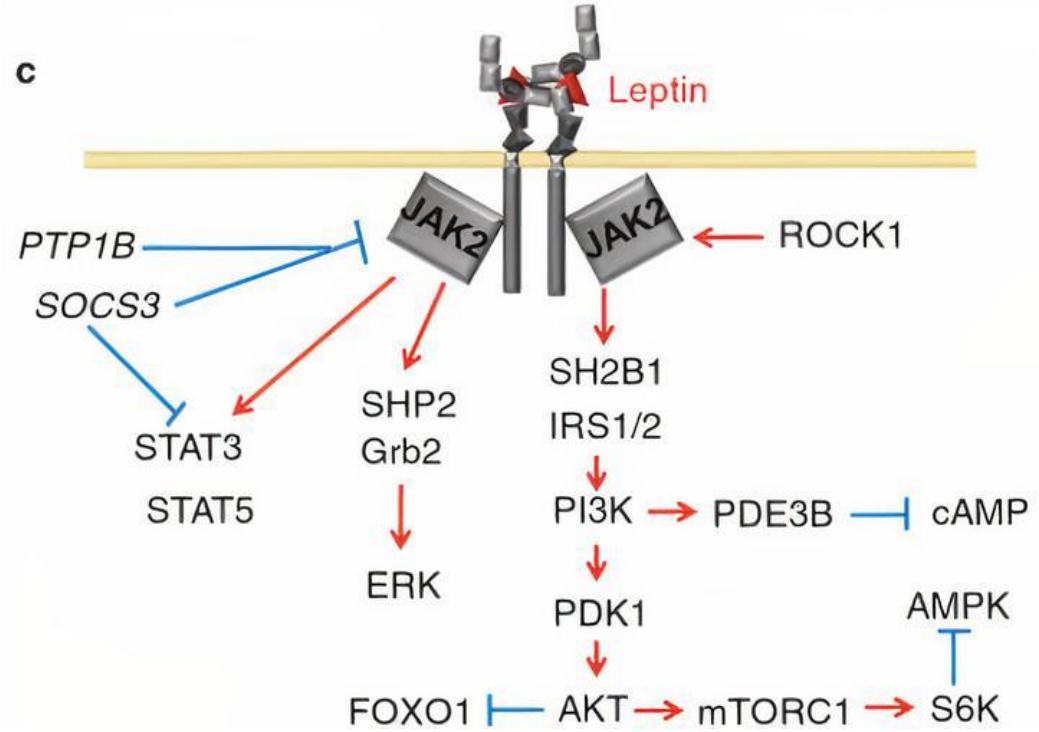

With respect to leptin activation, its activation results when one of the binding sites (designated as site II) of LEP interacts with the cytokine receptor CRH2. An interesting point to note is that there are two other LEP connection sites: I and III.

Another item to be pointed out regarding the mechanism of action of LEPR is its signaling system, which occurs after its activation with leptin. Since LEPR lacks intrinsic enzyme activity, its signaling depends on a tyrosine kinase titled JAK2. This compound, activated by RhoA-associated protein kinase 1 (ROCK1), is responsible for phosphorylating tyrosine residues in the cytoplasm, which results in a recruitment of signaling and transcription-activating proteins, called STATs.

Essential attention is given to the signaling pathway performed by STAT3, since it directly influences the effects of leptin on satiety and body weight. In view of this, as soon as STAT3 is recruited, it undergoes phosphorylation by JAK2, which causes dimerization and translocation of the nucleus to mediate gene transcription. Additionally, other signaling pathways detached by LEPR include, in the intracellular medium, the protein tyrosine phosphatase 2 (SHP2)/extracellular regulated kinase (ERK) pathway and the insulin receptor substrate (IRS)/phosphoinositide 3-kinase (PI3K) pathway. This entire process is certified in Figure 3.

When the intention is to interrupt signaling, the responsibility lies with the cytokine signaling suppressor 3 (SOCS3), which inhibits this activity through a negative *feedback loop*. Other inhibitory compounds are protein tyrosine phosphatase-1B (PTP1B) and T-cell protein tyrosine phosphatase (TCPTP).

Figure 3: LEPR signaling pathways. Leptin binds to the LEPR-b isoform and activates ROCK1 and JAK2. This phosphorylates LEPR-b, resulting in an interaction with signaling molecules, activating several pathways. The inhibition of this signaling is effected by the negative feedback of SOCS3 and PTP1B (DAGOGO-JACK, 2015, p. 18)




Continuing the course of this chain, Dagogo-Jack (2015) prints that the subsequent stage is consummated through neural pathway, which encompasses peripheral sensory and autonomic stimuli, acting on the CNS, which cross the blood-brain barrier. It deals with an important process of body regulation, since obesity, by way of example, can result from this homeostatic poor regulation in the circuits of the central nervous system.

The largest hypothesis pertinent to the regulation of body weight by the CNS is based on the idea of adiposity signaling, which sends constant information to the brain about its relative amount. The most significant signs of adiposity for the brain, if glimpsed over a long period of time, are leptin and insulin. In this same tone, Machado, Monteiro and Pinto (2015), complement indicating that the primary function of leptin is to take to the central nervous system a signal of the energy stores in the body (adipose tissue) so that the brain makes necessary adjustments to balance the expenditure and energy consumption.

The aforementioned authors also review that although leptin physiologically is in dispersed concentrations in the brain, some factors that influence its increase are insulin, glucocorticoids and pro-inflammatory cytokines, in turn, the factors that influence its decrease are testosterone, exposure to cold and catecholamines.

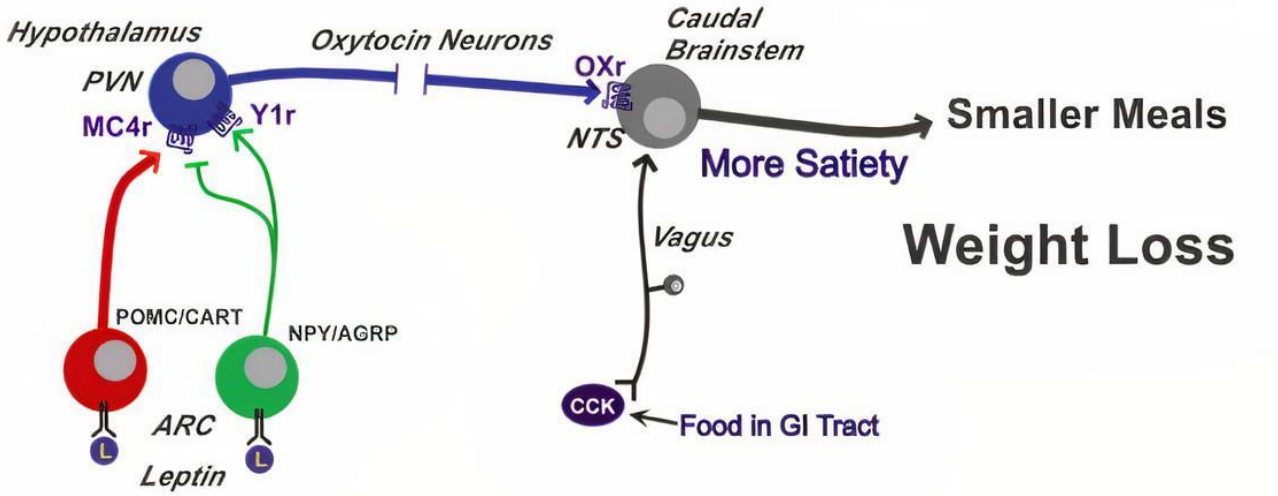
Moreover, regarding the general characteristics of LEP, important to the context, it is elucidated that its manufacture is effective through white adipose tissue cells, in addition to placenta, bone

marrow, stomach, muscle and brain. Moreover, it is noteworthy that its peak release occurs at night and its plasma half-life is approximately thirty minutes (Machado, Monteiro, Pinto, 2015)

Given this conjuncture of facts, Negrão and Licínio (2000) observe that subsequently the production of leptin by adipose tissue, the hormone is directed to the bloodstream, crossing the blood-brain barrier, and in the brain binds to specific receptors. Dagogo-Jack (2015) reasoning continues by adding that the effects of leptin on neural cells are mediated by transmembrane leptin receptors, encoded by the LEPR gene. In this same perspective, it is worth noting that the Ob-Rb isoform, widely distributed in the brain region, is the only functional variant responsible for the anorectic effect. In turn, the receptors of this isoform can be captured in the hypothalamus, especially in the arcuate nucleus, in addition to the ventromedial nucleus, dorsomedial nucleus, paraventricular nucleus and perifornical areas. Negrão and Licínio (2000) record that the region of the arcuate nucleus is the one with the highest uptake, given that leptin acts on four peptides produced by neurons in this region, they are: neuropeptide Y (NPY), peptide related to the agouti strain (AGRP), pro-opiomelanocorticotropin (POMC) and cocaine-amphetamine-dependent transcription factor (CART).

Thus, Dagogo-Jack (2015) finds that the anorectic effect in the brain opens when LEP acts on LEPR in neuropeptides named ARC / AGPR and NPY / CART, which causes the stimulation of POMC / CART neurons (which are anorexigenic) and a discouragement of NPY / AGPR neurons (which are orexigenic, that is, have the function of inciting hunger). That said, a substance symbolized by aMSH is released by POMC/CART neurons, activating the MC4r melanocortin receptors, which aims to elevate the oxytocin signal in NTS neurons, responsible for satiety signals. This course can be contemplated as shown in Figure 4:

Figure 4: Diagram on the leptin pathway in the brain, modeling the level of satiety. (DAGOGO-JACK, 2015, p. 30)

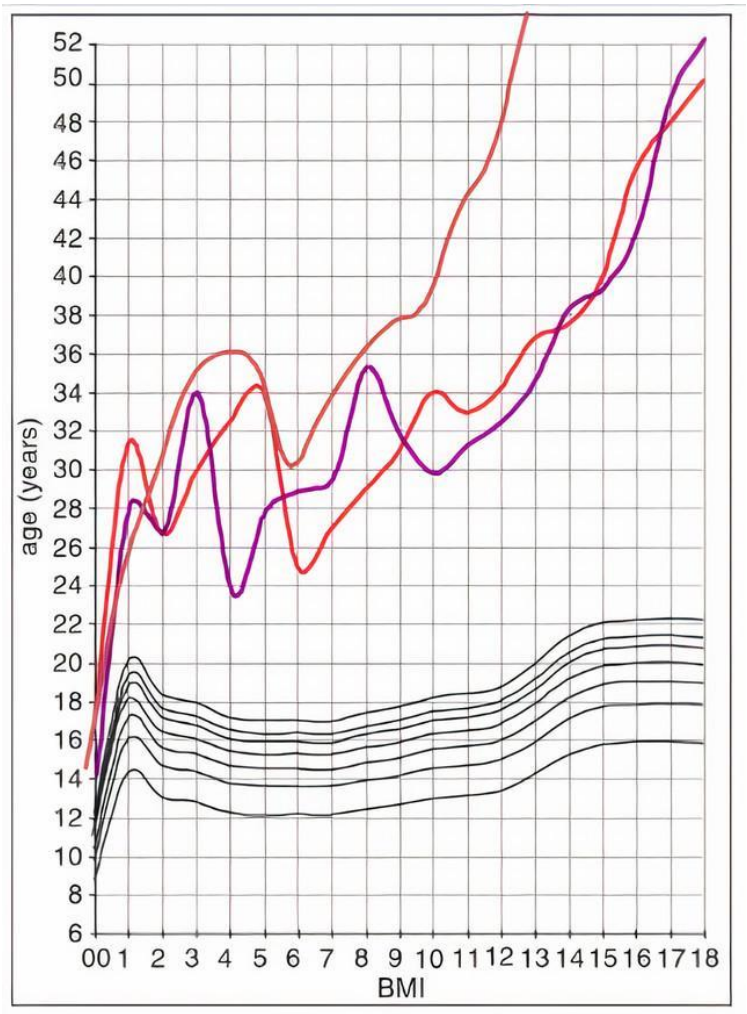



Pertinent to the anorectic effect, Negrão and Licinium (200) add that their result is fundamentally justified by the fact that leptin promotes the oxidation of fatty acids and triglycerides,

possibly through an inhibitory effect on the activity of acetyl-CoA carboxylase, one of the enzymes that regulate the synthesis of fatty acids.

The pathophysiological history of LEPR correlated with obesity is ascertained by Dagogo-Jack (2015), when he stated that the first mutation of this gene was described in 1998, announcing that the affected individuals were homozygous for the mutation resulting from an *abnormal "splicing*" in the transcription of LEPR, which generated a receptor without domains (CRH1, CRH2 and Ig) and, consequently, the obese phenotype. It is pertinent to repair the graph of the research, corresponding to Figure 5, which presents a relationship between BMI and age in people with the mutated gene.

Figure 5: Graph referring to the first research describing the association between LEPR mutation and obesity. Illustrates severe weight gain as age advances. The subjects with the mutation are demonstrated by the traces in color (DAGOGO-JACK, 2015, p. 20)




Since then, several LEPR mutations have been detected in extremely obese people, and in 2007 the gene was sequenced in 300 patients with hyperphagia and obesity, accounting for a high prevalence of pathological mutation.

In this direction Dagogo-Jack (2015) clarifies that, mostly, the main obesogenic pathophysiological mechanism involved in the LEP/LEPR gene is the so-called leptin resistance.

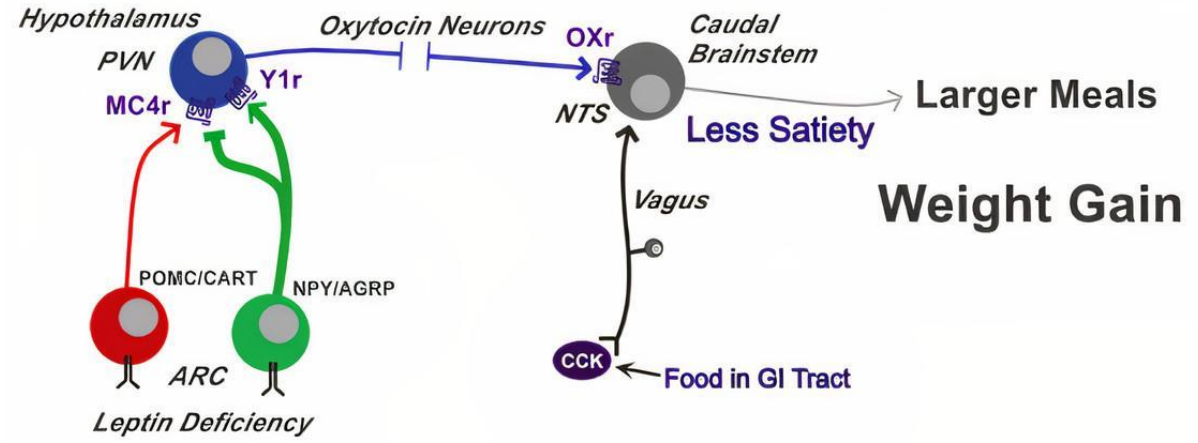
When this mechanism is ruled out, other less common mechanisms are insufficient leptin production or a partial or total sensitivity of leptin at its binding sites. Whatever the situation, the motivation derives either from a polymorphism and / or from some embarrassment in the neural pathway, causing a deficit in leptin signaling and, therefore, the ability to regulate appetite is reduced.

This clarified, Machado, Monteiro and Pinto (2015) see that leptin resistance occurs when high plasma concentrations of LEPR are unable to act effectively in the control of energy balance. The main motivator that triggers this mechanism comes from the increase in the serum concentration of triglycerides, which can act by binding directly to leptin in the bloodstream or in the leptin receptor located at the blood-brain barrier. This prevents the connection between leptin and its receptor. Other drivers of this dysfunction may be hyperleptinemia, which decreases receptor expression in skeletal muscle, deficiency in leptin transport to the brain, disturbances in the signaling mechanism in specific receptors and cytokines, as well as failure in the hypothalamic circuit that regulates energy homeostasis

The partial or total sensitivity of leptin at its binding sites arises in the activation of the LEP gene through LEPR. Therefore, if there is a mutation in binding sites II and III, but the I is intact, the leptin and its receptor will still bind, but activation of the compound will not be possible.

Another adversity, which is predominant in comparison to the previous case, can be identified in the neural pathway, when there is a mutation in leptin, causing its functional production to be insufficient. In this case, POMC/CART signals are minute and NPY/AGPR signals are stimulated. Thus, the orexigenic effects are activated and the anorectic effects are blocked, since aMSH does not receive activation incentive and, therefore, MC4r receptors are not incited to signal to oxytocin. Below, the succession of events is printed in Figure 6:

Figure 6: Diagram on the mutated leptin pathway in the brain, modeling the level of satiety. (DAGOGO-JACK, 2015, p. 30)




Machado, Monteiro and Pinto (2015) review that, once the mutation is identified, attention should be paid to the possibility of the most predicted polymorphisms in obesogenic syndrome by LEP

/ LEPR. In this sense, the most expected SNPs are: Gln223Arg (rs1137101), Lys109Arg (rs1137100) and Lys656sn (rs8179183). The first of these is the most commonly found, and its provenance comes from the replacement of adenine by guanine, resulting in the exchange of the amino acid glutamine (Gln) for arginine (Arg) at position 223. It deals with a mutation intrinsically associated with increased BMI, hyperleptinemia, increased fasting glucose, and predispositions to metabolic syndrome. The Lys109Arg SNP is strongly related to increased cholesterol and triglyceride concentrations, changes in weight; BMI and waist and hip circumference. Finally, the Lys656sn SNP has interaction with phenotypic obesity and low-fat dietary habits.

Alusive to the obesogenic clinical associations by LEP/LEPR, the first valuable mention concerns its relationship with the sleep period. As well supported by Negrão and Licínio (2000), leptin is secreted in pulses throughout the day, and its peak secretion is during the night. It is recommended by ABESO (2016) that sleep deprivation causes decreased secretion of leptin and TSH, increased levels of ghrelin ("hunger hormone") and decreased glucose tolerance. This is concatenated to that prescribed by Carneiro, *et. al.* (2007), when clarifying that patients with Obstructive Sleep Apnea Syndrome (OSAS) have increased leptin levels, considering that the pathology causes resistance to LEP and its concentration in the environment rises, without, however, making connections.

Another clinical association of genetic obesity intrinsically associated with the genes of the case in question is breast cancer. Cleveland, *et. al.* (2010) elucidate that, although there is no direct relationship with mortality, there is a modest increase in the risk of developing this pathology when the LEP gene is mutated (2548AA and 2548GG), especially in obese postmenopausal women.

Psychopathologies are added to the list of obesogenic comorbidities linked to LEP/LEPR. Pereira and Brandão (2014, p. 152) argue that "changes in inflammatory cascades, appetite-regulating hormones (leptin, adiponectin and resistin) and the hypothalamic-pituitary-adrenal axis may constitute correlation mechanisms between psychiatric pathologies and obesity." The authors note that, due to the insufficiency of the hormone and/or its resistance, there is a strong possibility that leptin has a role in the pathophysiology of depression. This occurs because depression, being considered a pro-inflammatory state, LEP tends to alter the immune system, increasing the response mediated by lymphocytes and Th1 and suppressing the Th2 response, which increases the production of pro-inflammatory drugs such as TNFα and IL-6.

An intrinsic relationship is also seen between leptin and Polycystic Ovary Syndrome (PCOS). In this step, Lecke (2010) leads reasoning when declaring that the gene expression of leptin in the subcutaneous adipose tissue in obese women with PCOS presents levels higher than those of normal weight. Melo (2001) adds that commonly these women are also insulin-resistant, hyperinsulinemic and/or infertile.

Regarding intervention resources, it is important to clarify that, as stated by ABESO (2016), treatment with recombinant leptin is effective in rare cases of leptin deficiency. In cases of polygenic obesity, most of the clinical pictures, the treatment has no effect. Its indication is pertinent only in children and adolescents with monogenic obesity in cases of production deficiency. Negrão and Licínio (2000) add this understanding when they discuss the inefficiency of recombinant leptin in cases of leptin resistance, since the level of the hormone is already high. In addition, these highlights complement Dias, *et. al.* (2012), when adducing that there is an importance in identifying cases of obesogenic mutations in younger age groups, having that this provides the possibility of changing the course of treatment, choosing the most appropriate therapy and allowing genetic counseling for affected families.

Another intervention model indicated by Negrão and Licínio (2000) was leptin injected directly into the cerebral ventricles, which led to the expression of the transcription factor STAT3 and neuronal activation in hypothalamus nuclei. However, more studies are needed to complete appropriate treatment.

## 3 CONCLUSION

The effects of genetic mutations on obesity can be reflected from the loss of the perception of satiety, reduction of the ability to control the balance of energy expenditure, as well as a greater tendency to comorbidities, which, in turn, can also influence even more weight gain and its consequences. It is also worth mentioning that the alteration in the leptin genes or its receptor can hinder the loss of weight and fat, requiring a more detailed and individual intervention so that there is an adequate response to treatment.

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