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Leptin resistance and diet-induced obesity: central and peripheral actions of leptin



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ABSTRACT

Obesity is a chronic disease that represents one of the most serious global health burdens associated to an excess of body fat resulting from an imbalance between energy intake and expenditure, which is regulated by environmental and genetic interactions. The adipose-derived hormone leptin acts via a specific receptor in the brain to regulate energy balance and body weight, although this protein can also elicit a myriad of actions in peripheral tissues.

Obese individuals, rather than be leptin deficient, have in most cases, high levels of circulating leptin. The failure of these high levels to control body weight suggests the presence of a resistance process to the hormone that could be partly responsible of disturbances on body weight regulation. Furthermore, leptin resistance can impair physiological peripheral functions of leptin such as lipid and carbohydrate metabolism and nutrient intestinal utilization.

The present document summarizes those findings regarding leptin resistance development and the role of this hormone in the development and maintenance of an obese state. Thus, we focused on the effect of the impaired leptin action on adipose tissue, liver, skeletal muscle and intestinal function and the accompanying relationships with diet-induced obesity. The involvement of some inflammatory mediators implicated in the development of obesity and their roles in leptin resistance development are also discussed.

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1. What causes leptin resistance underlying obesity?

Leptin, the product of the *ob* gene, was identified and cloned from rodent adipose tissue in 1994, where a role in body weight control was described [1]. Leptin was pioneeredly

considered as an adipose signal mainly implicated in the regulation of the energy balance, as demonstrated in leptin deficient *ob/ob* mice, in which hyperphagia and obesity are marked features. Many of the actions of leptin are attributable to effects in the brain. Thus, leptin reaches the central nervous system (CNS) by crossing the blood–brain barrier

Abbreviations: AgRP, agouti-related protein; ARC, arcuate nucleus; ASCT2, glutamine/amino acid transporter 2; B⁰AT1, neutral amino acid transporter 1; BBB, blood–brain barrier; CCK, cholecystokinin; CNTF, ciliary neurotrophic factor; DIO, diet-induced obesity; MCT1, monocarboxylate transporter 1; NPY, neuropeptide Y; PEPT1, peptide transporter 1; POMC, pro-opiomelanocortin; PTP1B, protein tyrosine phosphatase 1B; SGLT1, sodium-glucose transporter 1; SOSC3, suppressor of cytokine signaling 3; TLR, toll-like receptor; VAN, vagal afferent neurons; VTA, ventral tegmental area.

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(BBB) through receptor-mediated endocytosis [2]. Long form of leptin receptor (Ob-Rb) is primarily expressed in hypothalamic regions and arcuate nucleus (ARC) is considered the major site for physiological actions of the hormone. Leptin binds OB-Rb of multiple neuronal populations activating the JAK2/STAT3 pathway in an orchestrated scenario to regulate the synthesis of different neuropeptides implicated in the control of food intake and energy balance [3]. For instance, leptin activates pro-opiomelanocortin (POMC) neurons [4] and enhances the levels of the anorectic peptide α -melanocyte-stimulating hormone [5], while inhibiting neuropeptide Y (NPY) neurons [6] in the ARC of rodents.

The finding that after leptin treatment the impaired immune system and fertility associated with leptin deficiency in these mice disappeared suggested that the hormone could have different additional physiological roles in the organism [7]. Peripherally, leptin is implicated in a broad range of physiological processes such as angiogenesis, hematopoiesis, bone formation, wound healing, immunocompetence or lipid and carbohydrate metabolism regulation [8–10], and nutrient intestinal absorption [11,12].

Obesity is a chronic disease whose rates are rapidly increasing around the world and that affects both genders and all ages. Obesity is due to an imbalance in the energy equilibrium caused by a sedentary lifestyle and unbalanced eating patterns that lead to a massive enlargement of the adipose tissue. Given that leptin regulates body weight, impaired leptin synthesis, signaling or sensitivity could lead to disturbances in energy homeostasis and body composition [13]. Indeed, hyperleptinemia is a characteristic manifestation of obesity in humans and rodents, where a resistance to the action of leptin has been suggested [14,15], but high circulating concentrations of leptin may be the necessary levels to maintain the sensitivity to the hormone and the energy homeostasis. Besides, almost 10% of the obese population presents physiological leptin plasma levels and, even in some cases, obesity has been attributed to an impaired leptin production by the adipose tissue [13]. Therefore, the mechanisms that lead to leptin-resistance are still unclear and the concept of it should be defined. Leptin resistance is commonly used to define states of obesity where hyperleptinemia and/or decreased responsiveness to leptin administration is observed. However, different meanings for leptin resistance appear in different frameworks, suggesting the need for a precise definition for this concept [16]. Nonetheless, the lack of response to leptin due to the development of resistance to the hormone may blunt central and peripheral actions of leptin, including food intake, nutrient intestinal absorption, intermediate metabolism and insulin sensitivity, leading to a dysregulation of the energy balance. The actual mechanisms underlying leptin resistance still remain unclear, but several possibilities have been postulated: i) a failure of circulating leptin to cross the BBB and reach its neuron targets in the brain [17], ii) an inhibition of the leptin signaling cascade within neurons in specific brain areas, iii) a “defensive” decrease in the expression of leptin receptors [18], and iv) a desensitization of cellular downstream signaling at central and peripheral level [19,20]. In addition, multiple factors, including inflammation or oxidative stress processes [21], and the type of diet [22,23], may contribute to leptin resistance.

2. Inflammatory mediators in obesity and their effects on leptin sensitivity

Obesity is frequently associated with systemic and local inflammation, as well as with elevated circulating leptin levels [24] (Fig. 1). Various inflammatory cytokines are known to induce oxidative stress; while leptin resistance in obesity has been suggested to be initiated by activation of the inflammatory signaling [21]. Previous *in vitro* and *in vivo* studies have described that several inflammatory mediators such as C-reactive protein (CRP), interleukin-6 and tumor necrosis factor- α (TNF- α) are elevated in obesity [25–27]. More interestingly, TNF- α and other inflammatory factors such as IL-1 α and lipopolysaccharide (LPS), increase circulating leptin concentrations in rodents and humans [28–32], suggesting that these molecules may be involved in hyperleptinemia and also in leptin resistance development. However, *in vitro* studies have evidenced conflicting results. Acute exposure to TNF- α (24 h) stimulates leptin secretion from 3 T3-L1 adipocytes [32] and primary murine adipocytes [28]. Contrarily, no effect on leptin production was detected in primary adipocytes of rats [33], and leptin secretion was inhibited at longer TNF- α exposure times [34]. In agreement with this study, Medina et al. reported a concentration-dependent inhibition of leptin secretion and gene expression by chronic exposure to TNF- α in primary adipocytes of rats [33]. These controversial results suggest that the pro-inflammatory cytokine TNF- α contributes to circulating levels of leptin. However, while a direct inhibitory effect may be concluded by the *in vitro* studies, the increased leptin production induced by TNF- α *in vivo* may be the result of indirect mechanisms. Furthermore, leptin resistance has been partly attributed to an interaction between CRP and leptin, which may worsen the permeability of leptin at the BBB [35]. However, Hsueh et al. have found that CRP increases BBB permeability, but elevated CRP may trigger leptin resistance by inhibiting the binding of leptin to membrane receptors [36]. Other authors support the notion that interaction between leptin and CRP may jeopardize the leptin effect on eNOS activation, suggesting a link between leptin resistance, low-grade inflammation, and endothelial dysfunction [37]. This hypothesis is consistent with the increase of the CRP gene expression [38], TNF- α secretion and ROS production induced by exogenous leptin in endothelial cells [39,40], and also suggest a pro-inflammatory role of leptin which may participate in the leptin resistance development.

Previous studies have reported that leptin stimulates the secretion of anti- and pro-inflammatory cytokines [41], and the level of systemic oxidative stress in non-obese animals [42]. Thereby, a pro-oxidative role of leptin has been noticed. However, it has been also reported that, independently of food intake, leptin administration reduces the oxidative stress occurring in the obese and diabetic *ob/ob* mice [43]. Additionally, the levels of ROS in POMC neurons have been positively correlated with those of leptin in lean and *ob/ob* mice, while central administration of H₂O₂ activated POMC neurons and reduced feeding of obese mice [44]. These reports evidence a controversial relationship between leptin and oxidative stress, but suggest a possible interaction of ROS and leptin in the regulation of energy metabolism in states of obesity.

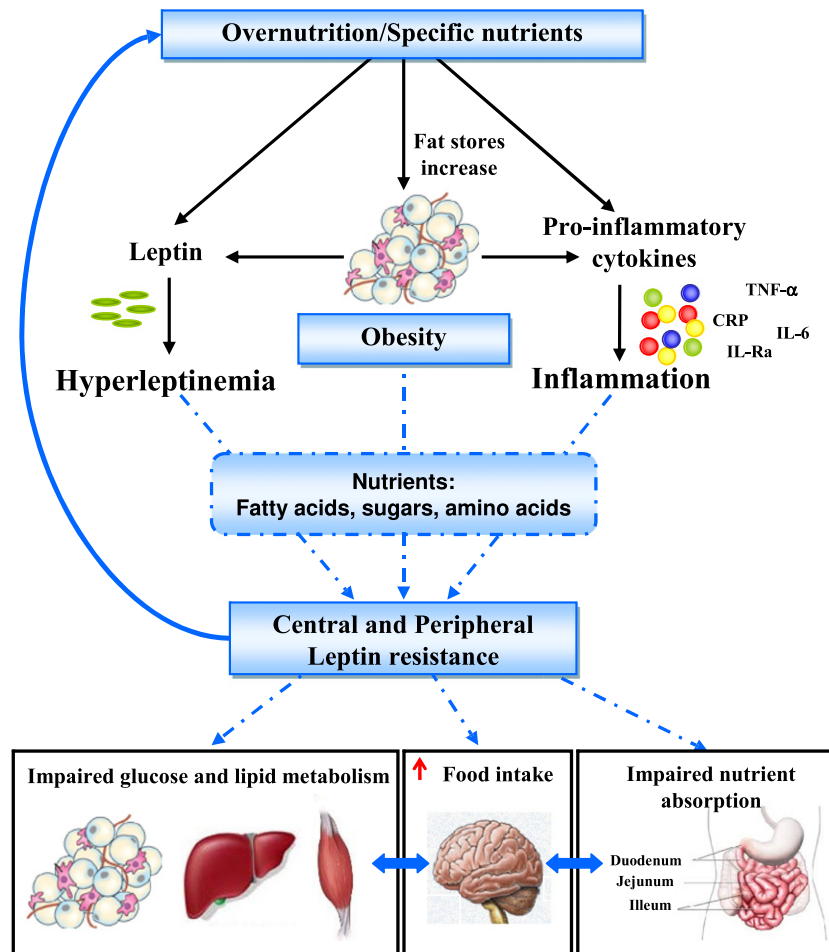


Fig. 1 – Schematic representation of leptin resistance development and the involvement of nutrients.

Another inflammatory factor proposed as a mediator of leptin resistance development is the soluble interleukin receptor antagonist (IL-1Ra), which is a notable regulator of inflammation, but also involved in the control of energy homeostasis [45]. Although soluble IL-1Ra is markedly increased in the serum and white adipose tissue of obese patients [45], IL-1a levels are not elevated in obesity [29,31]. Interestingly, exposure to IL-1a increases leptin circulating levels in mouse [31] and humans [31], while genetic IL-1Ra ablation in mice attenuates high-fat diet-induced caloric hyperphagia [45]. Similarly, lack of TNFR1 protects from obesity induced by high-fat feeding due to increased thermogenesis in mice [46]. On the other hand, some gp130 family cytokines such as the ciliary neurotrophic factor (CNTF) and cardiotrophin-1 have been reported to act both centrally and peripherally mimicking the biological actions of leptin, which have been recently proposed as a potential therapeutic strategy to alleviate obesity-associated complications [47,48]. Administration of CNTF reduces inflammatory signaling cascades associated with lipid accumulation in peripheral tissues [47]. Contrarywise, hypothalamic protein tyrosine phosphatase 1B (PTP1B), as well as activation of Toll-like receptor (TLR) signaling appears to mediate central insulin and leptin resistance and has also been recognized as activators of obesity-induced inflammation

[49,50]. In addition, deletion of the tyrosine phosphatase TCPTP in neuronal cells prevents the development of obesity induced by high-fat feeding in mice, identifying this factor as a critical negative regulator of hypothalamic leptin signaling [51].

On the other hand, inflammation-induced hypophagia is associated with JAK2/STAT3 signaling pathways, as leptin-mediated hypophagia does [52]. In this sense, administration of LPS increases leptin serum levels and tissue expression in mice [29], and a recent research has revealed that the anorectic effects of LPS through the activation of STAT3 in the hypothalamus are abolished by leptin resistance in high-fat fed rats [53]. In addition, LPS increased the expression of the suppressor of cytokine signaling 3 (SOCS3) in cultured vagal afferent neurons (VAN) and inhibited leptin-induced pSTAT3 in high-fat-induced obese rats, which become leptin resistant [54]. Interestingly, high glucose and high free fatty acid concentrations induce inflammatory pathways, leading to the development of leptin resistance [55]. However, *in vitro* studies have shown that saturated fatty acids cause inflammation in different type of cells, but not in cultured hypothalamic neurons [56]. Curiously, a recent study has reported that leptin deficient *ob/ob* mice develop leptin resistance after high-fat diet consumption, independently from hyperleptinemia [57].

Thereby, these studies support that several mediators of inflammation are related with hyperleptinemia and leptin resistance development in states of obesity induced by diet. Furthermore, it seems that the activation of inflammatory signaling initiates the development of leptin resistance; however, different nutrients appear to modulate this relationship (Fig. 1).

3. Peripheral actions of leptin and leptin resistance

Leptin is a pleiotropic hormone with a variety of functions within the organism activity in different tissues [9,10]. Liver and skeletal muscle are the tissues with great metabolic activity and, together with adipose tissue, constitute important targets for leptin regulation of insulin sensitivity as well as glucose and lipid metabolism [10,58]. Thus, several researches have reported that leptin administration improves insulin functions in normal and diabetic rats [59,60] and can reverse the obese and diabetic phenotype of mice [61]. In addition, *ex vivo* and *in vitro* studies have shown that leptin increases both basal and insulin stimulated glucose uptake and oxidation in isolated muscles of rodents [62]. More recently, it has been reported that leptin reduces the gene expression and activity of key negative regulators involved in the translocation and reinternalization of the facilitative glucose transporter GLUT4 in skeletal muscle of *ob/ob* mice [63], reinforcing the role of leptin on glucose homeostasis. However, pathological states as obesity, diabetes and inflammation have been related with peripheral leptin resistance development. In this sense, a loss of leptin actions by desensitization of leptin receptors is a process that specifically occurs in some tissues [18,64]. However, dietary components have been also proposed to modulate leptin actions in peripheral tissues, suggesting that leptin resistance may also result from eating behavior or specific nutrient intake (Fig. 1).

3.1. Adipose tissue

NPY and leptin interact in a homeostatic loop to regulate body-fat mass and energy balance not only at the CNS level, but also directly at the adipocyte level [65]. Different studies have demonstrated the involvement of leptin, among other metabolic and hormonal factors, in the regulation of lipolysis [66,67]. In addition, it has been shown that this regulation is differently exerted in lean or obese *ob/ob* mice, with the latter more sensitive to the action of exogenous leptin than its lean littermates [67]. This study also suggested that leptin resistance may be induced by hyperleptinemia. The lipolytic effect of leptin in adipocytes of *ob/ob* mice was dose-dependent. However, while the maximal lipolytic effect was observed at the physiological concentration of leptin used, the supraphysiological dose of leptin tested yielded the lowest lipolytic response. In addition, the lipolytic effect observed in adipocytes from lean mice was lower than from *ob/ob* animals and not in a dose-dependent manner, maybe suggesting an early saturation in leptin response [67]. In this sense, a damaged regulation of this mechanism, due to leptin

resistance, could lead to the development of more and bigger adipocytes and contribute to the accumulation of the excessive fat mass found in the obese state.

3.2. Liver

Liver plays a major role in regulating homeostasis and is vital for the maintenance of a correct nutrient metabolism. Leptin regulates hepatic gluconeogenesis and insulin sensitivity. Thus, defects in leptin action, as occur in states of leptin resistance, impair the hepatic function leading to hyperglycemia, hyperinsulinemia and hyperlipidemia [9,10]. Recently, it has been shown that leptin resistance, specifically in the liver, modifies the physiological metabolism of lipoproteins and triglycerides. However, this was not a direct effect of the hormone since the leptin replacement in *ob/ob* mice was unable to normalize liver lipase activity [68]. Interestingly, dietary patterns also affect hepatic function [64,69]. High-fat diet intake alters hepatic gene expression, induces liver steatosis [69], and worsens liver regeneration in mice after injury [70]. Furthermore, long-term fructose consumption induces hepatic steatosis and evokes leptin resistance [64]. In this sense, given that leptin administration regulate the lipid and glucose homeostasis in liver, and that liver regeneration is impaired in rodent models of dysfunctional leptin signaling (*ob/ob* and *db/db*) [71,72], a role of leptin in the regulation of this process has been suggested. Recently, an impaired proliferation of non-parenchymal cells has been proposed to be directly involved in the reduced liver regeneration in *db/db* mice [73]. In this context, Leclercq et al. [74] have reported that leptin replacement restores hepatic steatosis, but does not improve liver regeneration after partial hepatectomy in *ob/ob* mice. Thus, given that leptin function recovery does not improve liver regeneration in *ob/ob* mice, data suggest that leptin resistance is not directly implicated in liver regeneration, but it could be involved in the failure of liver regeneration induced by specific nutrients (Fig. 1).

3.3. Skeletal muscle

A role of leptin on fat and carbohydrate metabolism in the skeletal muscle has been described [58,62]. However, beneficial effects of leptin on muscle regeneration have been also proposed. Obesity is associated not only with chronic inflammation and hyperleptinemia [75], but often also with a reduced muscle mass. Various inflammatory cytokines induce muscle loss by decreasing protein synthesis and increasing proteolysis [76]. Furthermore, the regulation of muscle mass depends critically on nutrient intake and anabolic and catabolic factors. In addition, a regulation of muscle proteolysis by dietary fat profile has been reported [77]. Thus, intake of saturated, trans-saturated and monounsaturated fatty acids shows a negative relationship with skeletal muscle mass in humans, while polyunsaturated fatty acids were positively associated [77]. Consistent with this study, exposure to saturated (palmitate) and unsaturated (oleate) fatty acids induces proteolysis of muscle cells [78]. In this context, the *ob/ob* mice showed a lower muscle mass than their wild types littermates, and the intraperitoneal replacement of leptin reverses the inflammatory state and the

muscle loss independently of its inhibitory action on food intake [43,79]. These studies indicate an association of skeletal muscle mass with nutrients and leptin, and given that the recovery of leptin function reverses the loss of muscle mass, it could be hypothesized that leptin resistance may be involved in the atrophy of skeletal muscle related with obesity and inflammation (Fig. 1).

Taking together these investigations, leptin exerts important peripheral actions in adipose tissue, liver and skeletal muscle, playing a key role in the homeostasis of fat and carbohydrate metabolism. However, more interestingly, the studies described above seem to indicate that specific nutrients participate in the impaired hepatic and muscle regeneration. Thereby, given that this hormone can affect glucose and lipid utilization independently of its well-known effects on food intake and energy expenditure, leptin resistance may be a molecular link between obesity and the dysregulation of nutrient metabolism.

4. Gastric leptin action at small intestinal level: obese vs lean subjects

Nowadays, it is known that leptin is secreted not only by adipose tissue, but also by other tissues such as placenta, fetal tissues, muscle, ovary, kidney and stomach [9,80]. In the past years, different actions of leptin on the gastrointestinal tract have been described in the scientific literature supporting the consideration of leptin as a gut peptide within a gut-brain loop [81].

4.1. Gastric leptin

Leptin is secreted into the gastric lumen by pepsinogen-containing secretory granules of the chief cells [82] that also contain the leptin soluble receptor Ob-Re that is released into the gastric lumen together with leptin [83]. Leptin remains stable in the gastric juice because the binding to its soluble receptor protects it from the acidic pH and proteolytic activity of the gastric lumen [81]. The endocrine cells of the gastric mucosa also secrete leptin to the circulation, in particular after meal consumption, contributing to the plasma leptin concentrations [82,84].

The nutritional status of the body regulates exocrine secretion of leptin by the gastric mucosa [85]. Food intake is also a strong stimulus for gastric leptin secretion together with different neurotransmitters and hormones such as acetylcholine release by the vagus nerve, secretin, CCK, insulin, glucocorticoids and trans-retinoic acid [86]. In addition, exogenous leptin administration also increases gastric leptin gene expression in rats [87]. Of interest, Picó et al. also reported that the intake of physiological amounts of leptin during lactation seems to protect animals against leptin-resistance induced by high-fat diet feeding [87]. Additionally, nutrients such as fructose also stimulate leptin production by the gastric mucosa without modifying plasma leptin levels [88,89].

4.2. Leptin in the small intestine

Leptin receptors occur in both the apical and the basolateral membrane of the enterocytes [88]. Once secreted into the

gastric lumen, leptin reaches the intestine, where this adipokine can bind to specific brush border receptors [88] and participate in the short-term regulation of nutrient utilization and assimilation including delay of gastric emptying, intestinal motility, nutrient absorption, and secretion of gastric intestinal and pancreatic hormones as well as in cell proliferation and intestinal barrier function [81,90] (Fig. 2). Circulating leptin can also act as an endocrine hormone on the enterocytes by binding to the receptors of the basolateral membrane and modify nutrient transporters activity [91,92].

Different studies performed in our laboratory have demonstrated (Fig. 3), in rodents' intestine and in the human model of intestinal epithelial cells Caco-2, that leptin inhibited *in vitro* and *in vivo* galactose and glucose uptake mediated by the sodium-glucose transporter SGLT1 [11,12,92–94] through the activation of protein kinase C (PKC) [12,95]. This effect of leptin was not found in receptor-deficient *fa/fa* rats, indicating the requirement of functional leptin receptors for the control of SGLT1 activity. In addition, this rapid inhibition was associated with a parallel decrease in the abundance of SGLT1 in the brush-border membrane of the enterocytes. Further studies also showed that luminal leptin increased the activity of GLUT2 and GLUT5 [89]. Leptin stimulation of fructose uptake in rat was mediated by increase of the phosphorylation (up-regulation) of PKC β II and 5'AMP-activated protein kinase (AMPK), which in turn, enhanced GLUT2 and GLUT5 insertion in the brush border membrane of the enterocytes and reduced the insertion of SGLT1 [89].

In rat intestine and Caco-2 cells, additional luminal or basal leptin inhibited *in vitro* and *in vivo* amino acids uptake [12,96], which was related to a reduced protein expression levels in the brush border membrane of the enterocytes, of the transporters implicated in the amino acid transport process: ASCT2 (Gln, Pro) and B⁰AT1 (Phe) [97] (Fig. 3). In contrast, other studies have shown in Caco-2 cells that the intestinal transport of dipeptides by the peptide transporter 1 (PEPT1) is increased by apical leptin [98]. This effect was associated with an increase on PEPT1 protein expression at the plasma membrane and a decrease on the intracellular PEPT1 content (Fig. 3). In rat jejunum, intraluminal leptin also *in vivo* induced, a rapid 2-fold increase on the intestinal absorption of dipeptides [98].

Furthermore, in Caco-2 cells, leptin, acting from the basolateral membrane side of the monolayer, affected lipid handling by reducing the export of triglycerides to the basolateral medium and decreased the release of *de novo* synthesized apolipoproteins B-100 and B-48, as well as the newly formed chylomicrons and low density lipoproteins [91]. Interestingly, *in vivo* intravenous injection of leptin attenuated apolipoprotein A-IV transcription as elicited by intraduodenal infusion of lipids [99,100]. Luminal leptin also up-regulated butyrate uptake in Caco-2 cells by two different mechanisms: i) the increase of the intracellular pool of the monocarboxylate transporter 1 (MCT1), without affecting the expression of CD14, a protein, which is associated with MCT1 and is required for the butyrate transport activity; ii) the translocation of the CD147/MCT1 to the apical plasma membrane [101].

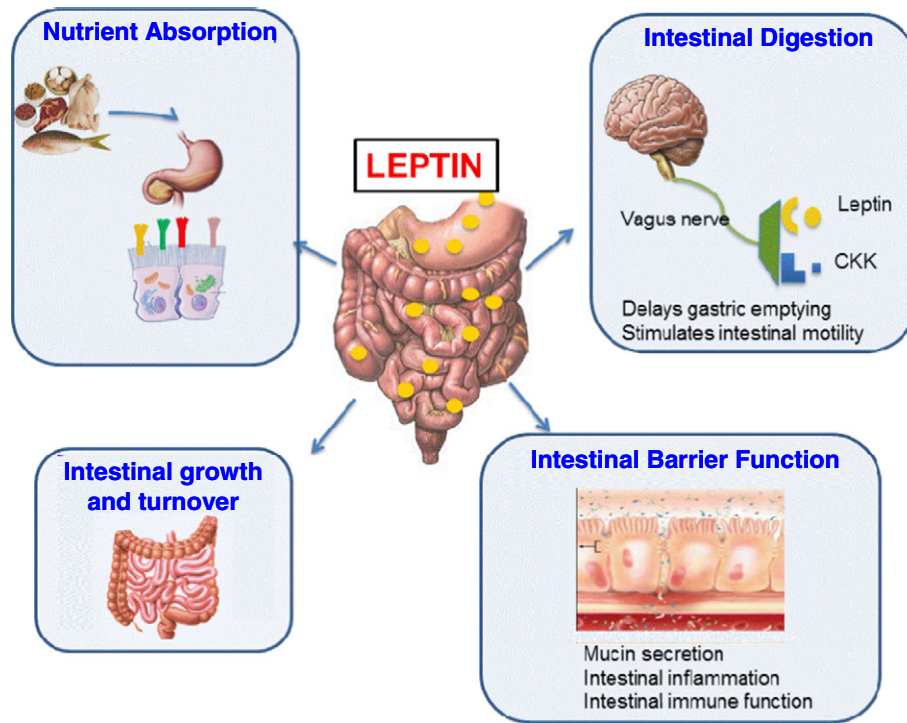


Fig. 2 – Gastric leptin effects at the intestinal level.

4.3. *Leptin regulation of nutrients absorption in obesity*

The effect of exogenous leptin on intestinal galactose absorption in the genetically obese *ob/ob* and *db/db* mice has been also analyzed. Although basal galactose uptake was similar in wild-type mice and the two obese groups, contrarily to what happens in wild-type mice, leptin increased galactose uptake in *db/db* mice. These data evidenced that leptin differently regulates sugar absorption in lean and genetically obese animals. Since the *db/db* strain is leptin resistant due to the lack of the functional long leptin receptor isoform, the

stimulatory effect of leptin on galactose absorption should be mediated by the short receptor isoforms signaling [102]. In contrast, in normal mice, the activation of the long and short receptor isoforms would produce a decrease on galactose uptake [102]. Interestingly, 0.2 nmol/L leptin also increased galactose uptake in *ob/ob* mice. This strain does not secrete leptin suggesting that *ob/ob* mice tissues may have different regulation of leptin receptors expression and, therefore, different sensitivity and response to the hormone [102]. In summary, these data suggest a distinct leptin regulatory mechanism on sugar intestinal absorption in wild-type and

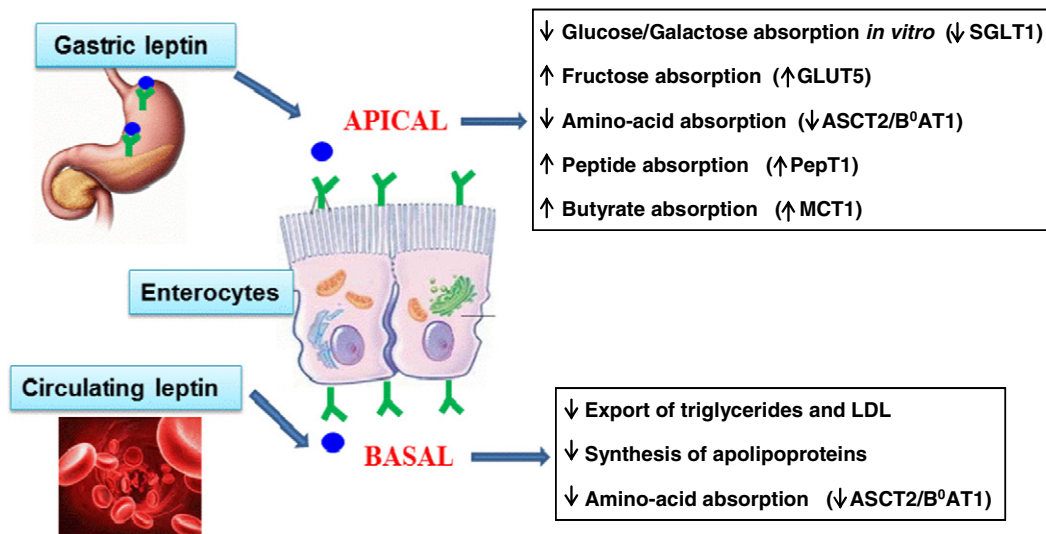


Fig. 3 – Summary of leptin actions on intestinal nutrient absorption.

obese animals, suggesting that high levels of leptin in obesity may lead to modifications of nutrient absorption further contributing to weight gain.

Another study performed in morbidly obese subjects evidenced a different expression pattern of GLUT2 when compared with lean subjects. Thus, GLUT2 was present in the apical membrane of the enterocytes in obese individuals even during fasting state, while in lean subjects the presence of GLUT2 in the brush border membrane is usually limited to the post-prandial state. This feature has been related to clinical manifestations on the obese subjects such as insulin resistance and diabetes [103]. In addition, Osswald et al. demonstrated that genetically modified mice lacking RS1, a peptide implicated in the regulation of SGLT1, developed obesity with high levels of the transporter expression associated with increased sugar absorption in the small intestine [104].

In line with these findings, other authors have published that intestinal peptides absorption mediated by the H⁺-coupled co-transporter PEPT1 was impaired in *ob/ob* mice [105]. While chronic leptin administration increased PEPT1 activity and expression in human and rat intestine, in leptin-deficient *ob/ob* mice the transporter activity and expression were significantly reduced and completely restored after leptin subcutaneous infusion [105]. In addition, it has been shown that a 4-week hypercaloric diet resulted in a 46% reduction in PEPT1-specific transport because of a decrease in PEPT1 protein and mRNA levels in mice. These modifications were supported by a parallel reduction in leptin receptor expression, reflecting possible leptin desensitization associated to the dietary intake [106].

Taken together, these findings indicate that the membrane expression of the nutrients transporters and the leptin levels differ between lean and obese subjects, which in conjunction with the higher leptin levels found in obese subjects could lead to leptin resistance in the gastrointestinal tract impairing the short-term regulation of gastrointestinal digestion and, therefore, contributing to the onset of obesity.

5. Leptin resistance and its relationship with diet-induced obesity (DIO)

Leptin resistance is implicated in the pathogenesis of DIO [107]. High-fat diet consumption triggers central and peripheral leptin resistance as has been extensively demonstrated in rodent models of DIO devoted to study those metabolic disorders related with human obesity. In this sense, hyperleptinemia seems to be a key player in the development of leptin resistance by down-regulating cellular responses to the hormone [108]. However, several controversial outcomes have been reported in this field.

5.1. DIO and leptin resistance at central nervous system

The temporal and spatial dysregulation of the neuronal function linked to leptin under conditions of nutrient excess is little known. However, several groups have suggested that different areas of the brain may be implicated in this process. In this sense, Matheny et al. [109] showed that the

consumption of a high-fat diet induced leptin resistance in the ARC and ventral tegmental area (VTA), while several medial basal hypothalamic regions remained sensitive to the hormone. Consequently, the selective down-regulation of Ob-Rb by lentivirus in ARC promoted DIO in rats [110], evidencing the role of ARC and VTA brain regions on the leptin resistance development in obesity. However, in contrast to the research of Bian et al. [110], selective over-expression of Ob-Rb in POMC neurons also increased the susceptibility to the development of DIO in transgenic mice [111]. Curiously, either the inhibition or induction of leptin in brain regions induced DIO, which may be explained by the different experimental procedures used to regulate the expression of leptin. More importantly, these studies indicate that the anorectic effects of leptin are not specific from a brain region. In this context, short-term central leptin (15 µg, 4 days) suppressed feeding in high-fat/high-sugar diet fed rats despite peripheral and ARC leptin resistance [112], suggesting that the effect on dietary intake of leptin may be regulated by other brain areas, such as VTA [112]. However, other research has shown that the anorectic effects of intra-VTA leptin (150 and 500 ng) were similar in low-fat as compared to high-fat fed rats [113]. Interestingly, long-term leptin adenovirus overexpression in either VTA or the medial basal hypothalamus caused desensitization of leptin signaling in the treated region, but also in the untreated region, evidencing an integrative response to leptin between both brain areas [114]. These controversial studies may be explained by the different doses of leptin used, as well as the method and duration of the treatment. However, these reports also suggest that brain regions appear to work in a coordinated manner and the loss of leptin response in a brain region may be compensated by another one, pointing to VTA and ARC as key areas on leptin responsiveness.

SOCS3 appears to be key in the central leptin resistance development [81]. High-fat feeding in rodents induces the expression of SOCS3 and resistance to STAT3 activation by leptin in POMC [111], ARC [115,116] and agouti-related protein (AgRP)-expressing [117] neurons. In concordance with these studies, silencing the hypothalamic SOCS3 protects against the development of DIO in rodents [118,119]. Furthermore, SOCS3 expression in AgRP neurons was reduced after switching from high to low-fat diet, suggesting that these neurons may be more responsive than POMC neurons to the circulating levels of leptin [117]. Additionally, the onset of leptin resistance in response to high-fat feeding reduces the sensitivity of VAN to the gastrointestinal hormone cholecystokinin (CCK), decreasing its anorectic effects and suggesting that leptin is required for appropriate CCK signaling [120]. Therefore, these studies evidence a coordinated response between the different leptin-sensitive brain regions which can be modulated by different factors and nutrients (Fig. 1).

5.2. Dietary nutrients on the DIO and leptin resistance development

The occurrence of hyperleptinemia is highly correlated with dietary obesity (Fig. 1). However, some studies have observed that hyperphagia-induced obese rats developed hyperleptinemia, but not apparently central or peripheral leptin resistance [121]. Indeed, caloric restriction did not

improve the Ob-Rb deficiency or the impaired leptin downstream signaling in the liver, skeletal muscle, or hypothalamus found in these obese rats [121], suggesting that hyperleptinemia may be an adaptive mechanism to overcome DIO [121]. Another study evidenced that obese animals with permanent low plasma leptin levels remain highly sensitive to exogenous leptin even after long-term exposure to a high-fat diet, indicating that dietary fat alone is not able to block the response to leptin [108]. In this context, it has been revealed that high-fat feeding modifies the methylation pattern of leptin promoter in rats, suggesting that epigenetic mechanisms could be involved in obesity development and leptin resistance [122].

Furthermore, other investigations have reported that the development of leptin resistance may be dependent on the type [22,23,123–125] and the duration of diet [22,23,123]. Recently, the metabolic and biochemical consequences of the high-fructose diets have been examined and it seems that fructose feeding modifies gene expression patterns and satiety factors in the brain, and induces an inflammatory state and leptin resistance development [126]. In addition, Haring et al. [23] have reported that fat and sugars have different effects on leptin response. Thus, rats fed for 39 days on a low-fructose and high-fat diet were leptin resistant, whereas rats fed on a high-fructose and fat diet were sensible to intraperitoneal leptin administration. Importantly, experiments with glucose replicated these results [23], suggesting a possible beneficial effect of sugars within a fat diet. On the contrary, other research has shown that rats fed a high-fructose diet for 6 months developed leptin resistance [123], and another trial reported that removal of fructose from a high-sugar and fat diet prevented leptin resistance despite the high fat content, suggesting a key role of fructose in the induction of leptin resistance [22]. These controversial results cannot be explained by a different diet composition, as the percentage of fructose and fat was the same. However, different methods to analyze the leptin responsiveness were used. Shapiro et al. [22,123] measured the cumulative intake for 24 h after intraperitoneal administration of 0.6 mg/kg of leptin, while Haring et al. [23] measured cumulative intake for less time (14 h) and after a higher dose of the hormone (2 mg/kg, i.p.). The age of the animals may also have influenced in the different results obtained by these studies. Surprisingly, guinea pigs fed with a high fat/low carbohydrate diet or a low fat/high carbohydrate diet did not develop leptin resistance in spite of the weight gained [127], adding more controversy to these experimental trials

Additionally, different nutritional situations and dietary compounds have been demonstrated to improve peripheral leptin sensitivity. Thus, deprivation of the amino acid leucine from the diet promotes leptin signaling in mice fed on a normal diet and restores leptin responses in mice maintained on a high-fat diet [128]. Moreover, dietary combination of fermented red ginseng with levan seems to prevent obesity and leptin and insulin resistance induced by high fat in mice [129]. Furthermore, teasaponin, an extract obtained from tea with anti-inflammatory properties, reduces the leptin resistance in DIO mice through the activation of STAT3 and POMC in the ARC of the hypothalamus [130]. Additionally, studies in high-fat fed rats show that dietary fiber [131], fish oil [132] and

omega-3 [133] supplementation influences circulating levels of leptin, and the release of leptin by adipocytes [133,134].

It is clear that physiological leptin signaling is essential for the maintenance of body weight. However, leptin resistance is a common feature of DIO, in which anorectic responses to leptin are reduced. Different areas of the brain appear to be distinctively implicated in the development of leptin resistance in response to DIO. In addition, isolated nutrients, diet composition, and the duration of the dietary treatment seem to be determinants on the development of leptin resistance.

6. Summary

Nowadays it is clear that leptin is an adiposity signal to the brain and that leptin resistance in some studies is a primary risk factor associated with obesity. At central level, leptin targets multiple neuronal populations (NPY, POMC, AgRP, etc.) in different hypothalamic regions. These neurons connect with other neurons in the brain forming an orchestrated circuit that also integrates other metabolic signals to control energy intake and expenditure. At peripheral level, among other functions, leptin is highly implicated in the regulation of gastrointestinal nutrient absorption, and processes involving lipid and glucose homeostasis in adipose tissue, liver and skeletal muscle. In this context, leptin resistance clearly correlated with the development of obesity, and the desensitization found in obese people can affect the physiological regulation of lipid and glucose handling in the adipose tissue, muscle and liver as well as the gastrointestinal nutrient utilization and, therefore, contribute to the worsening of the obese state. This regulation is differently exerted in lean and obese animals probably due to a different sensitivity to the hormone. Although recent investigations are contributing to understand the mechanisms leading to this impaired response to leptin, desensitization of leptin receptor, down-regulation of its intracellular signaling and inflammation appear as main processes involved.

Thus, the present document reviews those findings regarding the central and peripheral targets of leptin that could lead to the high circulating concentrations of this hormone present in obesity and states of leptin resistance and its relationships with DIO. Overall, as discussed in the present review, although overweight and overeating and also hyperleptinemia and inflammation have been proposed as causative mechanisms of leptin resistance development, increasing relevance is getting the interaction of a large amount of determinants of eating behavior and nutrients. Leptin resistance seems to depend on the type of nutrients consumed from the diet, reinforcing the hypothesis of the importance of a correct distribution in the dietary pattern. Further studies are needed to determine the role of specific nutrients in the leptin resistance development and also their potential benefit to overcome the development of leptin resistance.

Authors' contributions to manuscript

NS, JB, MJMA and JAM designed the manuscript content; NS and JB wrote the initial paper; MJMA and JAM critically revised

the paper; JAM had primary responsibility for final content. All authors read and approved the final manuscript.

Disclosure statement

The authors declare that they have no competing interests.

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