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Neuroendocrine body weight regulation: integration between fat tissue, gastrointestinal tract, and the brain

Neuroendokrynna regulacja masy ciała: integracja tkanki tłuszczowej, układu pokarmowego i mózgu

César Luiz Boguszewski[1], Gilberto Paz-Filho[2], Licio A. Velloso[3]

[1]Department of Internal Medicine, Endocrine Division (SEMPR), Federal University of Paraná, Curitiba, Brazil
[2]The John Curtin School of Medical Research, Australian National University, Canberra, Australia
[3]Laboratory of Cell Signalling, University of Campinas, Campinas, Brazil

Abstract

Human body weight is maintained at a fairly stable level regardless of changes in energy intake and energy expenditure. Compensatory mechanisms within the central nervous system (CNS), which regulate food intake and energy expenditure, are triggered by other central and peripheral signals. Peripherally, the main sources of those signals are the adipose tissue, gastrointestinal tract, and pancreas. The main signal originating from the adipose tissue is leptin, which promotes the activation of anorexigenic pathways in the CNS. Similarly, the central action of insulin also reduces food intake and stimulates catabolic pathways. The gastrointestinal tract contributes with several peptides that influence food intake, such as ghrelin, glucagon-like peptide 1 (GLP-1), peptide YY (PYY), oxyntomodulin (OXM), and cholecystokinin (CCK). Other substances secreted by the pancreas, such as pancreatic polypeptide (PP) and amylin, a hormone co-secreted with insulin, also affect energy balance. More recently, the endocannabinoid system has also been identified as a contributor in the maintenance of energy balance. Better understanding of these mechanistic systems involved in the regulation of energy metabolism will hopefully lead to the development of new therapeutic approaches against obesity, metabolic syndrome, and other nutritional disorders. **(Pol J Endocrinol 2010; 61 (2): 194–206)**

Key words: *central nerwous system, obesity, pancreas*

Streszczenie

Masa ciała człowieka jest utrzymywana na względnie stałym poziomie niezależnie od zmian zarówno w dostarczaniu energii, jak i jej zużywania. Mechanizm kompensacyjny w ośrodkowym układzie nerwowym (CNS, *central nervous system* [OUN]), który reguluje przyjmowanie pokarmu i wydatek energii jest uruchamiany przez inne sygnały ośrodkowe i obwodowe. Obwodowo, źródłem tych sygnałów jest tkanka tłuszczowa, układ pokarmowy i trzustka. Głównym sygnałem pochodzącym z tkanki tuszczowej jest leptyna, która powoduje aktywację anoreksogennych dróg przekazu sygnału w CNS. Podobnie, oddziaływanie ośrodkowe insuliny także zmniejsza przyjmowanie pokarmu i stymuluje kataboliczne drogi przekazu sygnału.

W układzie pokarmowym współdziała kilka peptydów, które wpływają na przyjmowanie pokarmu, takie jak grelina, peptyd podobny do glukagonu (GLP-1, *glucagon-like peptide*), peptyd YY (PYY, *peptide YY*), oksyntomodulina (OXM, *oxyntomodulin*) i cholecystokinina (CCK, *cholecystokinin*), Pozostałe substancje wydzielane przez trzustkę, takie jak polipeptyd trzustkowy (PP, *pancreatic polypeptide*) i amylina, hormon wydzielany jednocześnie z insuliną , także wpływają na równowagę energetyczną. W najnowszych badaniach stwierdzono, że układ endokannabinoidowy przyczynia sie do zachowania równowagi energetycznej. Lepsze zrozumienie mechanizmów układów wpływających na regulacje przemiany energii przyczyni się do rozwoju nowych terapii otyłości, zespołu metabolicznego i innych zaburzeń odżywiania. **(Endokrynol Pol 2010; 61 (2): 194–206)**

Słowa kluczowe: *ośrodkowy układ nerwowy, otyłość, trzustka*

Introduction

The human body is endowed with a complex physiological system that maintains relatively constant body weight and fat stores despite the wide variations in daily energy intake and energy expenditure. With weight loss, compensatory physiological adaptations result in increased hunger and decreased energy expenditure, while opposite responses are triggered when body weight increases. This regulatory system is formed by multiple interactions between the gastrointestinal tract (GIT), adipose tissue, and the central nervous system (CNS) and is influenced by behavioural, sensorial, autonomic, nutritional, and endocrine mechanisms. From

César L. Boguszewski M.D., Ph.D, Endocrinology Department of Internal Medicine, SEMPR, Endocrine Division, Federal University of Paraná, Curitiba, BrazilAv Agostinho Leao Junior, 285 (Alto da Glória), Curitiba, PR, Brazil, CEP: 80030–110, tel.: +55 41 3360 78 76, fax: +55 41 3264 87 21, e-mail: cesarluiz@hc.ufpr.br

an evolutionary point of view, it has been suggested that these control mechanisms are developed primarily to increase survival in times of food shortages. Consequently, they are more active and promote a more robust physiological response during weight loss than weight gain, which is undesirable in the face of the current pandemic of obesity [1–3].

The hypothalamus — particularly the arcuate nucleus (ARC) — and the brain — particularly the nucleus of the solitary tract (NTS) — are the main sites of convergence and integration of the central and peripheral signals that regulate food intake and energy expenditure [4, 5]. There are mechanisms of short-term regulation („satiety signals") which determine the beginning and end of a meal (hunger and satiation) and the interval between meals (satiety) [6], and long-term regulatory factors („signals of adiposity") which help the body to regulate energy depots. Satiety signals from the GIT are transmitted primarily through vagal and spinal nerves to the NTS, while the signals of adiposity reach the median eminence via ARC or by crossing the blood-brain barrier (BBB) through saturable and non-saturable mechanisms. There is, however, a large integration and convergence of these signals by neural connections between the ARC nucleus, NTS, and vagal afferent fibres.

In recent years, the central regulation of energy balance has become even more fascinating and complex with the characterization of new mechanisms of control. The endocannabinoid system participates in energy homeostasis by central and peripheral actions that influence appetite, motivation for consumption of palatable food, production and distribution of fat, energy expenditure, and glucose and insulin homeostasis [7]. These new findings on the neuroendocrine mechanisms controlling energy homeostasis and body weight show the complexity of these integrating systems and the existence of many unravelled mysteries (Fig. 1). Nevertheless, each mystery unveiled opens new opportunities for the development of novel therapies for obesity and nutritional disorders [8].

Leptin, insulin, and neuroendocrine control of energy balance

Leptin

Leptin is a peptide hormone produced by the *ob* gene and secreted mainly by the adipose tissue, playing a key role in energy homeostasis. The production of leptin is higher in subcutaneous fat than in visceral fat, and in the blood, leptin levels correlate directly with the amount of body fat. The secretion of leptin is reduced during periods of fasting and increased after meals, and is influenced by several metabolic and hormonal factors [9]. Congenital leptin deficiency due to genetic defects in the *ob* gene results in early-onset obesity associated with hyperphagia, decreased energy expenditure, and endocrine abnormalities. Similarly, patients with congenital or acquired lipodystrophy also have low leptin levels associated with insulin resistance and dyslipidemia [10]. Administration of recombinant leptin can reverse this phenotype both in animals and humans [10–12]. Low leptin levels are also observed in patients with anorexia nervosa and hypothalamic amenorrhea [13].

Leptin is transported to the brain where it crosses the BBB in a saturable mechanism and binds to its specific receptor (Ob-R, encoded by the *db* gene) in the ARC nucleus of the hypothalamus. Ob-R belongs to the type I cytokine receptor family and exists as five distinct isoforms (splice variants) named Ob-Ra, Ob-Rb, Ob-Rc, Ob-Rd, and Ob-Re. Only the Ob-Rb isoform contains a long intracellular domain essential for biological actions of leptin, while the physiological roles of the short isoforms are related to the transport of leptin in the circulation and through the BBB [14–16]. The Ob-Rb is highly expressed in hypothalamic nuclei — particularly ARC nucleus, ventro-medial, dorso-medial, and lateral hypothalamus. Leptin receptors are also present in several areas of the brainstem including the *nucleus tractus solitarii* (NTS), lateral parabrachial nucleus, and motor and sensory nuclei, which are not normally associated with energy balance. The presence of Ob-Rb in brain areas such as the NTS indicates that leptin effects are due, at least in part, to actions in the brainstem [17]. Peripherally, Ob-Rb is expressed in the lungs, kidneys, liver, pancreas, adrenals, ovaries, stem cells, and hematopoietic skeletal muscle. These peripheral locations of Ob-R evidence the multiplicity of different biological functions exerted by leptin in the body, in addition to its role in energy homeostasis. Mutations in the *db* gene have also been associated with monogenic obesity in both rodents and humans [11, 16], characterized by normal or elevated levels of leptin and the lack of a response to the administration of recombinant leptin.

Leptin stimulates anorexigenic neurons that express pro-opiomelanocortin (POMC) and cocaine- and amphetamine-regulated transcript (CART), and it inhibits orexigenic neurons that express neuropeptide Y (NPY) and Agouti-related peptide (AgRP). These primary targets of leptin action communicate with the second-order neurons in other hypothalamic nuclei (especially paraventricular nucleus, dorso-medial, ventro-medial and lateral hypothalamus), where peripheral information on energy depots is integrated with behavioural, hormonal, and nutritional inputs coming from the periphery and higher cortical centres. Several hypothalamic neurotransmitters produced in these regions, including corticotropin releasing hormone (CRH), thyrotropin releasing hormone (TRH), melanin concentrat-

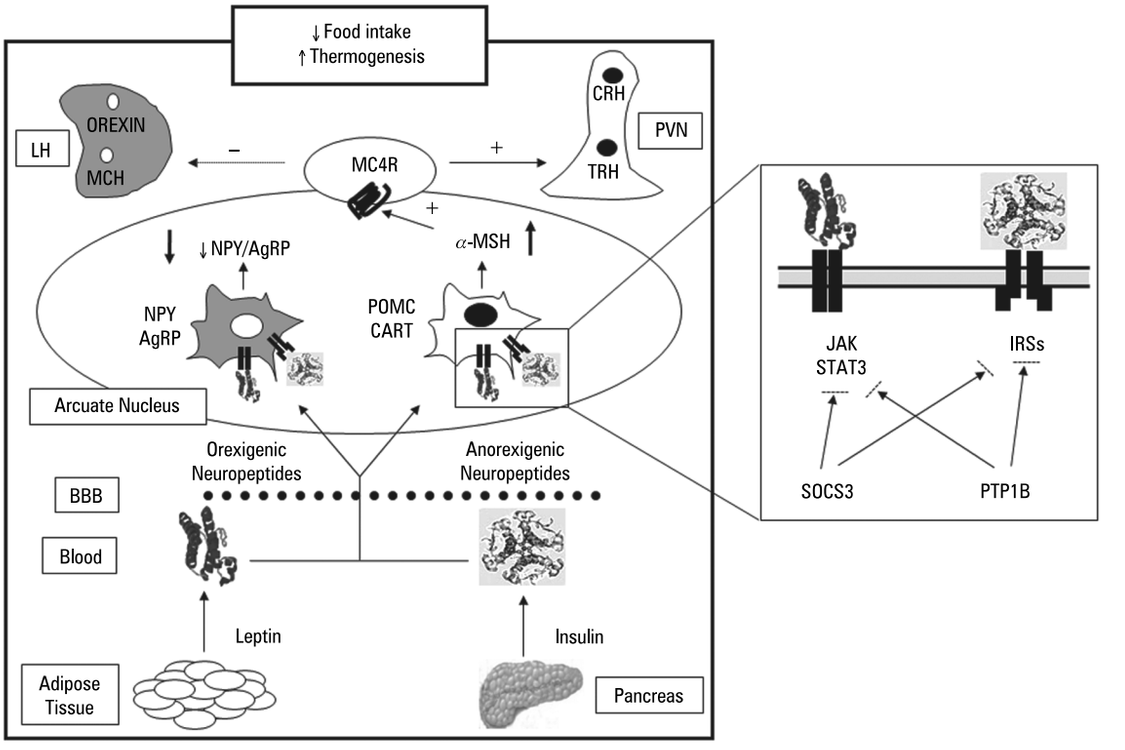

Figure 1. *Central actions of leptin and insulin.* ***Left Panel****. Leptin and insulin are transported to the brain, where they cross the blood–brain barrier (BBB) and bind to their specific receptors in two neuronal populations of the arcuate nucleus (ARC) of the hypothalamus: POMC/CART neurons, where they stimulate α-MSH expression, and NPY/AgRP orexigenic neurons, where they inhibit NPY/AgRP expression. These primary targets of leptin and insulin action communicate with the second-order neurons in other hypothalamic nuclei, especially the paraventricular nucleus (PVN) and lateral hypothalamus (LH), stimulating expression of anorexigenic neurotransmitters (CRH, TRH) and inhibiting orexigenic pathways (orexin, MCH). The final biological actions of central leptin and insulin action are inhibition of food intake and stimulation of thermogenesis.* ***Right Panel.*** *Leptin binding to Ob-Rb promotes activation of JAK and STAT3 proteins, while insulin binding to IR stimulates IR substrates (IRSs), especially IRS-2. Simultaneously, the activation of JAK, STAT, and IRSs proteins leads to the expression of inhibitors of leptin and insulin signalling such as SOCS-3 and PTP1B.*

POMC — pro-opiomelanocortin; CART — cocaine- and amphetamine-regulated transcript; α-MSH — melanocyte-stimulating hormone (melanocortin); NPY — neuropeptide Y; AgRP — agouti-related peptide; CRH — corticotropin-releasing hormone; TRH — thyrotropin-releasing hormone; MCH — melanin-concentrating hormone; Ob-Rb — leptin receptor; JAK — janus kinase; STAT — signal transducer and activator of transcription; SOCS-3 — suppressor of cytokine signaling-3; PTP1B — protein tyrosine phosphatase-1B

Rycina 1. *Działanie ośrodkowe leptyny i insuliny.* ***Strona lewa*** *— leptyna i insulina są transportowane do mózgu, gdzie przekraczają barierę krew–mózg (BBB) i łączą się ze swoistymi receptorami w dwóch populacjach neuronów: w jądrze łukowatym (ARC) podwzgórza: neuronami POMC/CART, gdzie stymulują ekspresję α-MSH i neuronami oreksygenicznymi NPY/AgRP, w których hamują ekspresję NPY/AgRP. Te pierwotne cele działania leptyny i insuliny komunikują się z neuronami drugiego rzędu w innych jadrach podwzgórza, szczególnie w jądrze przykomorowym (PVN) i podwzgórzem bocznym (LH) stymulują ekspresję neurotransmiterów anoreksogennych (CRH, TRH) i hamując oreksygeniczne szlaki przekazywania sygnału (orexin, MCH). Końcową aktywnością biologiczną centralnego działania leptyny i insuliny jest zahamowanie przyjmowania pożywienia i stymulacja termogenezy.* ***Strona prawa*** *— leptyna łącząc się z Ob-Rb, promuje aktywację białek JAK i STAT3, podczas gdy insulina łącząc się z IR, stymuluje substraty IR, czyli IRSs, szczególnie IRS-2. Jednoczesnie aktywacja białek JAK, STAT i IRSs prowadzi do ekspresji inhubitorów przekazywania sygnałów leptyny i insuliny, takich jak SOCS i PTP1B.*

POMC — proopiomelanokortyna; CART — transkrypt regulowany przez kokainę i amfetaminę; α-MSH — hormon stymulujacy melanocyty, melanokortyna; NPY — neuropeptyd Y; AgRP — białko agouti; CRH — hormon uwalniający kortykotropinę; TRH — hormon uwalniający tyreotropinę; MCH — hormon zwiększający stężenie melaniny; Ob-Rb – receptor leptyny; JAK — kinaza janusa; STAT — przekaźnik sygnału i aktywatory transkrypcji; SOCS-3 — supresor przekazu sygnału cytokin — 3; PTP1B — białkowa fosfataza tyrozyny 1B

ing hormone (MCH), brain-derived neurotrophic factor (BDNF), oxytocin, and orexins, are physiologically involved in energy homeostasis. Leptin indirectly inhibits or stimulates these peptides, contributing to the regulation of energy intake and thermogenesis (Fig. 1, left panel). These orexigenic and anorexigenic neuronal

groups are connected at several points in the CNS in such a manner that the activation of one group inhibits the other and vice-versa [1–4].

The binding of leptin to Ob-Rb promotes receptor dimerization, activation of JAK and STAT proteins, and subsequent phosphorylation of cytoplasmic proteins, leading to the signal transmission to the nucleus and modulation of gene transcription. Simultaneously, the activation of JAK and STAT proteins leads to the expression of inhibitors of signalling such as SOCS-3 (suppressor of cytokine signalling 3) and PTP1B (protein tyrosine phosphatase-1B), which modulate the biological response to leptin [18, 19] (Fig. 1, right panel). Leptin is the main catabolic adiposity signal, the actions of which result in reduced food intake, increased energy expenditure, and weight loss.

Serum leptin levels are low in anorexia nervosa and show a progressive increase with the reinitiation of food intake and recovery of body weight. Conversely, human obesity is generally associated with high serum levels of leptin and less efficient transport across the BBB, consistent with a state of central resistance to leptin. This resistance may be secondary to obesity or vice-versa, and genetic factors, age, diet, sedentary lifestyle, and stress might contribute to the development of defects in the transport of leptin across the BBB or might lead to abnormalities in leptin signalling [2, 9]. The latter might potentially involve increased intracellular expression of SOCS-3, as genetically modified animals that do not express neuronal SOCS-3 are resistant to diet-induced obesity. Moreover, it has been shown that obese subjects show increased neuronal SOCS-3 activity [2, 8]. Other factors that alter leptin signalling, leading to a decrease in its effects, are serum leptin-interacting proteins (such as C-reactive protein), PTP1B, and P-STAT [19, 20].

When obese subjects are subjected to diet-induced weight loss, there is deterioration in the central transport of leptin and subsequent worsening of leptin resistance. This is one of the mechanisms that explain the well-known problem in maintaining negative energy balance during weight loss. In a clinical trial, we showed that a catecholaminergic drug, licensed in some countries as an anti-obesity agent, increased the cerebro-spinal fluid (CSF)/serum leptin ratio after weight loss, suggesting an improvement of leptin transportation to the CNS [21]. This catecholaminergic effect was greater than that seen with sibutramine (a drug with serotoninergic and catecholaminergic combined effects), and it was not observed after weight loss induced by orlistat (a drug with no central effect), indicating that the anorexigenic effect of catecholamine is partly due to an increased hypothalamic sensitivity to leptin [21]. In agreement with our clinical findings, Banks demonstrated in rats that leptin transport across the BBB is enhanced only by adrenergic agents [22]. On the other hand, serotoninergic drugs, such as fenfluramine, dexfenfluramine, and sibutramine can promote anorexia by binding to the serotonergic 5-HT2C receptors expressed in POMC neurons, activating the same anorexigenic pathways that are critical for the biological effects of leptin. There is evidence that the concomitant activation of serotonergic 5-HT1B receptors, which are expressed in NPY/AgRP neurons, could produce an even greater catabolic effect and be a target for the development of new anorexigenic drugs [8]. Apart from resistance to leptin, lower production of leptin by the adipose tissue, which has been observed in individuals heterozygous for inactivating mutations in the *ob* gene, is associated with an increased prevalence of overweight and obesity in this population, suggesting that partial or relative leptin deficiency may be also an aetiological mechanism in human obesity [23]. Accordingly, we have found that leptin production by the adipose tissue is decreased in obese subjects with three or more risk factors for metabolic syndrome, suggesting a state of relative leptin deficiency in obesity associated with advanced stages of metabolic syndrome [24].

Despite its action on decreasing food intake, leptin replacement has not been shown to be an effective treatment for common obesity. Clinical trials in an obese population led to very modest, if any, weight loss after leptin replacement [25–27]. However, patients with relatively low leptin levels showed better therapeutic responses, and future studies need to show whether leptin replacement can be useful in treating selected obese patients. In addition, co-administration of recombinant human leptin with pramlintide — an amylin agonist — may yield significantly greater weight loss than either agent alone [28].

Insulin

The central action of insulin promotes the same biological effects as leptin, characterizing insulin as an adiposity signal [29] (Fig. 1, left panel). Serum levels of insulin are proportional to the amount of body fat and are influenced by peripheral insulin sensitivity, which is chiefly determined by the amount of visceral fat. Insulin crosses the BBB and binds to the insulin receptor (IR) present in high concentrations in neurons POMC/CART and NPY/AgRP in the ARC nucleus. Several studies have shown that the central action of insulin promotes anorexia, increases energy expenditure, and reduces body weight. In animals, reduced expression or deletion of the neuronal IR results in hyperphagia, obesity, and dyslipidemia, with high peripheral levels of insulin [30, 31]. In humans, the central anorexigenic effects of insulin are potentially important for the future development of insulin analogues with higher and fast-

er hypothalamic signalling compared to their peripheral actions, in order to avoid the weight gain commonly observed in the treatment of diabetes [32].

The IR is composed of an extracellular α sub-unit that binds to insulin and an intracellular β sub-unit that carries the signal and has intrinsic tyrosine kinase activity. Among various IR substrates (IRS), IRS-1 and IRS-2 are identified in neuronal cells with high expression of messenger RNA (mRNA) of IRS-2 in the ARC nucleus. Neuronal IRS-2 knock-out animals show increased food intake, and increased adiposity and infertility, suggesting that the central effects of insulin are mediated by this substrate [33]. Ob-Rb and IR share intracellular signalling pathways by PTP1B, JAK 2, and IRS [2, 29, 34] (Fig. 1, right panel). PTP1B inhibition seems to increase insulin and leptin sensitivity as PTP1B knock-out animals are lean, insulin sensitive, and resistant to diet-induced obesity [35].

In addition to regulating energy intake, both insulin and leptin have direct effects on adipose tissue: leptin stimulates lipolysis and insulin increases lipogenesis. Within the so-called adipoinsular axis, leptin exerts an inhibitory feedback on pancreatic insulin secretion in order to decrease lipogenesis. Insulin, in turn, also plays a role in stimulating leptin production and secretion in the adipose tissue [36].

Integration of peripheral signals in the CNS: neuropeptide Y (NPY) and the melanocortin system

NPY is the most abundant neurotransmitter in the brain and is expressed in the hypothalamus mainly by neurons of the ARC nucleus. As expected for an orexigenic factor, hypothalamic NPY levels increase during fasting and decrease after feeding [3]. NPY integrates a large family of peptides that includes peptide YY (PYY) and pancreatic polypeptide (PP), the effects of which are mediated via six G-protein-coupled receptors named Y1 to Y6. It is likely that the orexigenic effects of NPY are mediated by a combination of these receptors and not by only one [37].

The obese phenotype of *ob/ob* mice is attenuated by the loss of NPY [29]. In contrast, mice genetically deficient in NPY have normal food intake and body weight, suggesting the presence of alternative orexigenic mechanisms to compensate for the absence of NPY [38]. The redundancy of orexigenic pathways may be linked to evolutionary mechanisms for protection against starvation. Today, in the presence of epidemic obesity, these redundant pathways might partly explain the easy recovery of weight observed in obese individuals undergoing treatment, since weight reduction leads to a decrease in leptin, activation of NPY, and, consequently, hyperphagia and reduced metabolic expenditure. The melanocortin system refers to a group of peptides that originate from the cleavage of POMC — including adrenocorticotropic hormone (ACTH) and melanocyte-stimulating hormones (MSHs) — five different subtypes of G-protein-coupled receptors, and the endogenous antagonists AgRP and Agouti. This system has many important physiological roles in pigmentation, adrenocortical steroidogenesis, natriuresis, erection, and exocrine secretion. Moreover, various components of the melanocortin system play a role in energy balance [39].

In the ARC nucleus, the cleavage of POMC results in the production of α-MSH, which, by binding to its MC4 receptor (MC4R) situated in other hypothalamic nuclei, produces anorexia. There is evidence demonstrating the physiological role of the melanocortin pathway on the regulation of food intake and body weight [3, 39]. Fasting animals and *ob/ob* mice have low expression of POMC mRNA, which is reversed by leptin administration. MC4R agonists suppress food intake, whereas selective antagonists promote the opposite effect. The absence of MC4R in genetically modified animals causes hyperphagia and obesity, which is also observed in the Agouti mouse. In this model, the production of the Agouti protein inhibits the action of α-MSH on the MC1R in the skin, resulting in discoloration of hair, and on the MC4R in the hypothalamus, leading to hyperphagia and obesity. AgRP (Agouti homologous human protein) is another orexigenic factor synthesized in the same neurons of the ARC nucleus that express NPY, which acts as an endogenous antagonist to MC3R and MC4R, inhibiting the anorexigenic effects of α-MSH [3, 39].

Besides MC4R, a possible role of MC3R in energy homeostasis has been investigated, but the results are not conclusive yet [39]. It has been suggested that MC3R acts as a self-inhibitory receptor in POMC neurons, and its stimulation causes increased food intake. In contrast, its absence produces an unusual syndrome in MC3R knock-out animals, characterized by moderate obesity, mild or absent hyperphagia, mild insulin resistance, mild steatosis, no increment in lean mass, and increased fat in both sexes, but with weight gain seen only in females [40, 41].

In humans, mutations in the POMC gene and abnormalities in its processing result in obesity associated with early-onset adrenal insufficiency and red hair [42]. In some populations, functional abnormalities of the MC4R have been implicated as a monogenic cause of obesity in up to 6% of individuals, with recent indications that its true prevalence varies from 1 to 2.5% in subjects with body mass index (BMI) > 30 kg/m^2. These percentages confirm MC4R mutations as one of the most prevalent genetic defects in human obesity, at least in some populations [11].

Table I. *Effects of the main adipocytokines on energy homeostasis*

Tabela I. *Wpływ głównych adipocytokinin na zachowanie energii*

Adipocytokine	Source	Function
Leptin	Mainly Adipose tissue	↓ food intake ↑ satiety ↑ energy expenditure ↓ insulin ↓ insulin resistance ↓ lipogenesis ↑ lipolysis
Adiponectin	Adipose tissue	↓ food intake ↑ energy expenditure ↓ insulin resistance ↑ lipolysis
Resistin	Macrophages	?
Tumor necrosis factor α (TNF-α)	Adipose tissue and immune cells	↓ food intake ↑ satiety ↑ insulin resistance
Interleukin 6 (IL-6)	Adipose tissue, immune cells and muscle	↓ food intake ↑ satiety ↑ energy expenditure ↑ insulin resistance

Other adipocytokines involved in energy balance

The adipose tissue produces numerous other factors that may directly or indirectly influence the energy balance and body weight [43, 44] (Table I). Adiponectin is one of these factors. Adiponectin-deficient mice develop insulin resistance, glucose intolerance, dyslipidemia, and susceptibility to vascular injury and atherosclerosis. However, the absence of adiponectin does not seem to alter energy intake or thermogenesis [45, 46]. Central injection in rats increases energy expenditure and decreases fat mass and body weight without affecting food intake. In turn, peripheral administration of adiponectin results in less weight gain and improvements in insulin sensitivity and dyslipidemia. Those effects are possibly mediated by sympathetic activation. In humans, serum adiponectin levels are inversely related to adiposity and insulin resistance, increasing after weight loss induced by diet or bariatric surgery. Thus, the physiological effect of adiponectin is to increase energy expenditure and to protect against insulin resistance and atherosclerosis [3, 43, 44]. In fact, recent studies have shown that adiponectin activates signal transduction pathways similar to leptin and insulin in the hypothalamus, reinforcing its role in the central control of energy homeostasis [47].

In contrast, resistin is a peptide produced by adipocytes that increases insulin resistance via paracrine actions. Serum levels of resistin are increased in obesity, and although it has been suggested as a link between obesity and diabetes, its pathophysiological relevance is still not clarified [43, 44, 48]. Tumor necrosis factor-α is correlated with the amount of body fat, and inhibits feeding, increases metabolic rate, and induces cachexia [49].

Another potential adipocytokine involved with energy homeostasis is interleukin-6 (IL-6), which exerts both paracrine and endocrine effects. Intracerebral administration of IL-6 results in increased energy expenditure, while IL-6 concentrations in CSF are negatively correlated with the amount of fat mass. Moreover, the absence of IL-6 in knock-out animal models has been associated with obesity in adulthood. Taken together, these data suggest a potential protective role of IL-6 against the development of obesity [50]. However, the data are not consistent between different research groups, and the actual involvement of IL-6 in controlling energy requires additional studies [44].

Gastrointestinal peptides in the control of energy balance

Cholecystokinin

Studies from the 1970s were the first to correlate gastrointestinal peptides with the regulation of energy balance. At that time, cholecystokinin (CCK) — a peptide predominantly produced in the duodenum and jejunum — was shown to be involved in the control of appetite by acting on the vagus nerve or directly into hypothalamic nuclei [5, 6]. Cholecystokinin reduces food intake, meal size, and meal duration when administered to both rodents and humans, by binding to CCK_A receptors. However, the interest in CCK as a therapeutic target for obesity progressively decreased with the demonstration that animals compensate the reduction in food intake by increasing the number of meals, with no change in body weight. Similarly, studies in obese subjects with CCK1R agonists have failed to show any effect in weight loss or cardiovascular risk factors. Nevertheless, the initial findings with CCK paved the way to investigate a system that recognizes the presence of food in the GIT, signalling to the brain via neural and endocrine mechanisms to regulate appetite and satiety (Table II). Since then, several gastrointestinal peptides have been discovered and their therapeutic potential in physiological or pharmacological concentrations has been tested in both animal and humans studies [51].

Ghrelin

Ghrelin is a 28-amino acid peptide produced predominantly by oxyntic cells of the stomach, and in the intestine, pancreas, and other tissues in smaller quantities.

Table II. *Gastrointestinal and pancreatic peptides involved in the appetite regulation*

Tabela II. *Peptydy żołądkowo-jelitowe i trzustkowe wpływające na regulację apetytu*

Peptide	Site of production		Site of Action			Effects on food intake
		Receptor	Hypothalamus	Brainstem	Vagal nerve	
Ghrelin	Gastric X/A-like cells	GHS-R	X	X	X	Increase
CCK	Intestinal I cells: Duodenum Jejunum	CCK1R	X	X	X	Decrease
GLP-1	Intestinal L cells: Ileum Colon	GLP1R	X?	X?	X	Decrease
OXM	Intestinal L cells: Ileum Colon	GLP1R Others ?	X?			Decrease
$PYY_{3–36}$	Intestinal L cells: Ileum Colon	Y2R	X	X	X	Decrease*
PP	Pancreatic F Cells	Y4R, Y5R		X	X	Decrease*

*central administration yields opposite results; GHS — growth hormone secretagogues; CCK — cholecystokinin; GLP-1 — glucagon-like peptide-1; OXM — oxyntomodulin; PYY — peptide YY; PP — pancreatic polypeptide; X? — unknown effect

Discovered in 1999 as the natural ligand of GHS-R (growth hormone secretagogues receptor) type 1a, ghrelin stimulates GH secretion in the pituitary gland and exerts other neuroendocrine activities. In the energy balance, ghrelin plays an important role as the only known peripheral hormone with orexigenic properties. In healthy volunteers, intravenous and subcutaneous administration of ghrelin promotes a 30% increase in food intake [52, 53].

Ghrelin has two forms: 1) acylated ghrelin contains an n-octanoic acid at the serine-3 position, which is essential for activation of GHS-R1 and modulation of neuroendocrine and orexigenic effects; and 2) nonacylated ghrelin, which is unable to activate GHS-R1 and is devoid of any endocrine effects; however, nonacylated ghrelin is the most abundant form in the circulation and exerts some cardiovascular and antiproliferative actions, probably by binding different GHS-R subtypes or as yet unidentified receptor families. GHS-R1a is widely distributed in the body, with high expression levels in the hypothalamus and pituitary, and low expression in other brain areas and in peripheral tissues, particularly in the endocrine pancreas [52, 53]. The gene encoding ghrelin also encodes another peptide, called obestatin. The administration of obestatin reduces food intake and weight gain in rats via activation of GPR3, an orphan G-protein coupled receptor [54]. Therefore, one gene produces two products with opposing metabolic effects, which are exercised through different receptors.

Serum ghrelin concentrations vary widely throughout the day, with higher values during sleep, pre-prandial elevations, and falls after meals. This pattern of secretion was the origin of the concept of ghrelin as a "hunger hormone", responsible for meal initiation. However, it is more likely that the physiological role of ghrelin is to prepare the body for an influx of metabolic energy [55–57]. Ghrelin secretion differs between lean and obese, which might have some relevance for the pathogenesis of obesity. The postprandial falls of serum ghrelin concentrations are proportional to energy intake in lean subjects, but not in obese subjects. Moreover, obesity is associated with a much lower reduction of postprandial ghrelin levels and an absence of nocturnal elevations as seen in subjects of normal weight [5, 6, 58].

Gastric secretion of ghrelin may be influenced by numerous factors, such as administration of glucose and insulin, activation of somatostatin receptors, the cholinergic system, GLP-1 (glucagon-like peptide 1), PYY (peptide YY), oxyntomodulin, thyroid hormones, and testosterone. Ghrelin levels are inversely related to BMI, with higher values observed in anorexia nervosa and cachexia, and lower levels in obesity. An exception to this rule is Prader-Willi Syndrome, in which obesity is associated with increased ghrelin concentrations in serum. Changes in body weight are accompanied by changes in ghrelin levels, which increase with weight loss and decrease with weight gain [52, 53].

Ghrelin stimulates food intake by acting in the ARC nucleus, in a pattern representing a functional antagonism to leptin. The orexigenic action of ghrelin occurs independently of its effects on GH secretion. Ghrelin reaches the hypothalamus through the circulation, and the brain stem through vagal innervation. The integrity of the vagus nerve is crucial for ghrelin effects since vagotomy prevents its orexigenic effect in animal

models and humans [52, 53]. In addition, ghrelin can be produced in the brain by a group of neurons adjacent to the third ventricle. These neurons form connections with other orexigenic and anorexigenic pathways, giving rise to a central circuit with potential involvement in energy homeostasis.

Ghrelin and GHS-R knock-out animals have no significant changes in food intake and body weight [59, 60]. However, the lack of ghrelin and GHS-R make the animals resistant to diet-induced obesity and favours the use of fat as an energy substrate when these animals are subjected to a fat-rich diet [60, 61]. In addition, the absence of ghrelin in ob/ob mice attenuates the diabetic phenotype but not obesity in these animals, supporting the role of ghrelin in the glucose metabolism [62]. Taken together, these data show a potential for future use of ghrelin antagonists and agonists in the treatment of anorexia nervosa and cachexia as well as in the prevention of weight recovery in obesity [5, 8, 51].

Pancreatic polypeptide, enterostatin, and amylin

Pancreatic polypeptide (PP) is a 36-amino acid peptide produced in the endocrine pancreas and released into the circulation after meals in proportion to their caloric content. PP blood levels are lower in the early hours of the morning and highest at night, with postprandial elevations that last up to six hours after the meal. Peripheral administration of PP promotes reduced food intake in rodents and humans, but this is not observed with central injection. Obese animals are less sensitive to the effects of PP than those of normal weight. The mechanism of PP action is not fully established, but possibly involves activation of Y4 and Y5 receptors in the area postrema of the ARC nucleus. PP sends anorexigenic signals via the brainstem and hypothalamic neuropeptides, and modulates the expression of other peripheral peptides such as ghrelin. This latter mechanism may explain the efficacy of PP infusion in promoting a 12% reduction of food intake in patients with Prader-Willi Syndrome. Another potential mechanism of action could be a delay in gastric emptying, but this seems to occur only in animal models and not in humans [3, 5].

The exocrine pancreas is also responsible for the production of enterostatin, a peptide secreted in response to fat intake to facilitate its digestion. Although the administration of enterostatin in animals reduces fat intake, no significant effects have been observed in humans [6]. Amylin, a peptide co-secreted with insulin, inhibits gastric emptying, gastric acid secretion, and glucagon secretion, and reduces food intake and meal sizes in animals. The synthetic analogue of amylin, pramlintide, has been shown to cause a modest and gradual reduction in weight over 26 weeks in diabetic patients [6].

Peptide YY

Peptide YY is structurally related to PP and NPY. This peptide is produced throughout the gut, with tissue concentrations that increase distally, reaching higher levels in the colon and rectum. The predominant form of PYY stored in intestinal cells (together with GLP-1) and released into the circulation is $PYY_{3–36}$, with the N-terminal truncated by the enzymatic action of dipeptidyl peptidase (DPP-IV). Peptide $YY_{3–36}$ is secreted in proportion to the amount of calories in the meal, with serum concentrations increasing rapidly in the first two hours and remaining high for up to six hours after the meal. The secretion is mediated by neural reflex and by direct contact of nutrients. Fat intake promotes higher secretion of $PYY_{3–36}$ than carbohydrates and proteins [3, 5].

The peripheral administration of physiological doses of $PYY_{3–36}$ leads to significant reduction of food intake in rodents. However, the use, adaptation, and creation of an appropriate environment are essential to observe the effects of $PYY_{3–36}$ on appetite [5, 8]. In contrast, the central administration of $PYY_{3–36}$ increases the appetite in animals. In normal weight volunteers, intravenous $PYY_{3–36}$ reduces energy intake by 30%. In obesity, circulating levels of $PYY_{3–36}$ are relatively low and its postprandial secretion is deficient in comparison with lean individuals. However, administration of $PYY_{3–36}$ in obese individuals may also result in reduced appetite and caloric intake. Nonetheless, it is possible that the reduction in food intake promoted by $PYY_{3–36}$ in humans might be secondary to side effects such as nausea and aversion to food [3, 5, 8, 51].

The most likely mechanism of action of $PYY_{3–36}$ is through its binding to the Y2-pre-synaptic inhibitory receptors expressed in the NPY/AgRP neurons. This action inhibits the NPY production and results in increased activity of POMC/CART neurons. However, the $PYY_{3–36}$ acts even in the absence of the melanocortin system, as observed in genetically modified animals that do not express POMC or MC4R [63]. Additionally, administration of $PYY_{3–36}$ reduces ghrelin levels, and this may be an additional factor that contributes to its anorexigenic effect [3].

Glucagon-like peptide 1 (GLP-1)

The pro-glucagon gene is cleaved in different products by the enzymes convertase 1 and convertase 2, and this process varies among the tissues. In the pancreas, the main product of this cleavage is glucagon, whereas in the intestine the main products are GLP-1 and GLP-2. GLP-1 is released into the circulation after meals, physiologically acting as an incretin that promotes increased pancreatic insulin secretion and, consequently, influences glucose homeostasis. Some studies show that circulating levels of GLP-1 are lower in obese patients and

increase with weight loss. The half-life of GLP-1 is very short, being rapidly degraded by the DPP-IV [3, 5].

Central and peripheral actions of GLP-1 are important in the regulation of energy balance. GLP-1 receptors are present in hypothalamic nuclei and brain stem areas known to be involved with energy balance. When centrally injected in rodents, GLP-1 produces anorexia, induces satiety, and increases energy expenditure, leading to reduced body weight if given over a long period. In lean and obese subjects, peripheral infusion of GLP-1 causes a dose-dependent reduction of caloric intake. Similarly, the use of exenatide and DPP-IV inhibitors — drugs currently available for the treatment of type 2 diabetes — is associated with modest but progressive weight loss and reductions in both fasting and postprandial glycaemia in diabetic patients [64]. However, the use of these agents as anti-obesity drugs in non-diabetic individuals is limited due to hypoglycaemic events.

Oxyntomodulin

Oxyntomodulin (OXM) is another product of the proglucagon gene, which is released from intestinal cells into the circulation in proportion to caloric intake. OXM inhibits gastric acid secretion, reduces food intake when administered centrally to rodents or peripherally in rodents and humans, and reduces body weight and fat mass when injected chronically. The weight loss observed is greater than it would be expected by a reduction in food intake, indicating that OXM promotes increased energy expenditure. In humans, OXM administration also reduces the levels of circulating ghrelin, which may contribute to its effects on appetite [3, 5].

The most likely mechanism of action of OXM on energy homeostasis is through its binding to the GLP-1 receptor. Although OXM binds the GLP-1 receptor with lower affinity than GLP-1, they are equally effective in causing anorexia. Thus, differences in the biological effects of OXM and GLP-1 may be due to variations in tissue penetration, degradation, or intracellular signalling pathways [5]. However, the existence of a specific receptor for OXM cannot be completely ruled out. Preliminary results of short-term studies indicate that OXM could be the first treatment for obesity that combines appetite suppression without tachyphylaxis with increased energy expenditure [51, 65].

The endocannabinoid system

The anti-emetic and appetite-stimulating properties of *Cannabis sativa* have been known for centuries, but the isolation and characterization of its active compound D^9-tetrahydrocannabinol (THC) was only performed in the sixties. THC and its synthetic analogue nabilone have been prescribed to treat nausea and vomiting associated with chemotherapy and weight loss associated with acquired immunodeficiency syndrome [66]. In the last two decades, new insights into the mechanisms of action of THC have been acquired with the cloning of two cannabinoid receptors (CB1 and CB2) and the discovery of their main endogenous ligands (endocannabinoids): N-arachidonoyl-ethanolamine (anandamide) and 2-arachidonoyl glycerol (2-AG). Endocannabinoids are synthesized from arachidonic acid and are rapidly hydrolyzed to inactive compounds by the action of specific catalytic enzymes. Endocannabinoids, CB1 and CB2 receptors, and enzymes responsible for biosynthesis and degradation constitute the endocannabinoid system, which is present in the brain and in various peripheral tissues [7, 66, 67].

Endocannabinoids modulate neuronal activity through the process of retrograde suppression of neurotransmitter release. In this process, the actions of neurotransmitters in postsynaptic neurons stimulate rapid, transitory, and on-demand production of endocannabinoids from phospholipid precursors located on the cell membrane. Anandamide and 2-AG are released and pass through the synapse in a retrograde pathway, interacting with CB1 receptors on presynaptic axons, which result in a variety of intracellular events that modulate the activity of these neurons. The final result of the endocannabinoid action depends on the excitatory or inhibitory nature of the synapse, resulting in repression or release of neuronal transmission [7, 66, 67].

While CB1 is mainly involved with anabolic functions of the endocannabinoids, CB2 is more related with the immune system. CB1 is widely and abundantly distributed in tissues involved with energy homeostasis, including brain areas such as the hypothalamus, brainstem, and mesolimbic region, and peripheral tissues such as the GIT, fat, liver, muscle, thyroid, and pancreas. The binding of endocannabinoids to CB1 receptors results in increased appetite, weight gain, lipogenesis, and lower insulin sensitivity. In the hypothalamus, endocannabinoids increase the production of orexigenic neurotransmitters and reduce the anorexigenic signals [68]. In the reward centre of the mesolimbic region, they promote motivation to seek and consume palatable food, while in the brainstem they block the signals of nausea and satiety transmitted by the vagus nerve. Peripherally, endocannabinoids facilitate the absorption of nutrients in the GIT, stimulate lipogenesis and impair glucose uptake in muscles [7]. Accordingly, CB1 knock-out mice are hypophagic, lean, insulin sensitive, and resistant to diet-induced obesity [69].

The adipose tissue and the GIT are closely integrated, and the peripheral signals are able to determine changes within the activity of the central endocannabinoid receptor. Central effects of leptin result in reduced

levels of endocannabinoids due to interference with 2-AG synthesis and increased anandamide degradation [67, 68]. In contrast, increased circulating levels of ghrelin in situations of food deprivation are associated with higher endocannabinoid activity, suggesting that the orexigenic effect of ghrelin occurs, at least in part, by activation of the endocannabinoid system [7, 67, 68].

It has been hypothesized that human obesity could be caused by an overactive endocannabinoid system [69]. Transient activation of this system, as seen after fasting and/or exposure to palatable foods, induces increased appetite, smaller satiety, increased lipogenesis, and reduced energy expenditure. Thus, sustained hyperactivity could lead to hyperphagia with progressive and excessive accumulation of fat and subsequent development of obesity and metabolic syndrome (Fig. 2). Excessive endocannabinoid activity, in turn, could be caused by a high-fat diet that would provide more substrate for the synthesis of anandamide and 2-AG, and would be perpetuated with the development of leptin resistance that often occurs in obesity.

In recent years, the development of CB1 antagonists has appeared to be a promising therapy for obesity and metabolic syndrome [7, 8]. CB1 antagonists are able to reduce appetite and body weight in genetically obese animals such as *ob/ob, db/db* mice and Zucker rats, as well as in animal models of diet-induced obesity. In addition to weight loss, other benefits have been experimentally observed including improvements in insulin sensitivity, lipid profile and hepatic steatosis, and increased adiponectin levels. In humans, clinical protocols with the CB1 antagonist rimonabant (Rimonabant In Obesity — RIO Studies), lasting up to two years and including more than 6600 overweight and obese participants with or without diabetes and dyslipidemia, were carried out some years ago [70]. In brief, these studies showed that 20 mg of rimonabant significantly reduced body weight (a mean loss of 7.4 kg after 2 years), with one third of participants showing a reduction of 10% or more in body weight. Metabolic improvements were reported, including elevation of HDL-cholesterol, reduced triglycerides, increased adiponectin, decreased C-reactive protein, improvement in HOMA-IR index, and reduction in glycosylated haemoglobin in diabetic patients. However, depression and anxiety, varying from mild to severe, developed in a substantial number of patients leading to discontinuation of the drug [71].

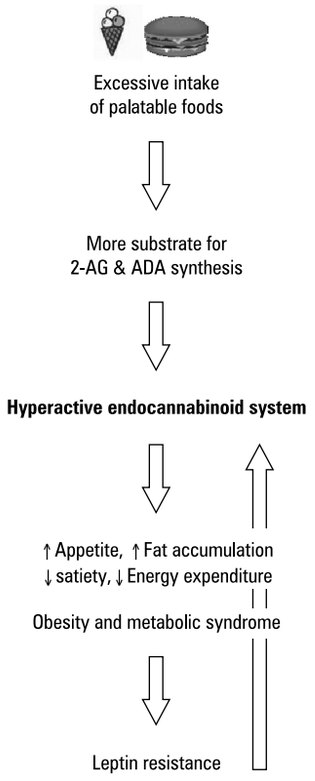

Figure 2. *Overactive endocannabinoid system as a potential cause of human obesity. Transient activation of the endocannabinoid system induces increased appetite, smaller satiety, increased lipogenesis, and reduced energy expenditure. The ingestion of highly palatable foods, especially those associated with high-fat diet, would provide greater quantities of substrates for the synthesis of anandamide (ADA) and 2 arachidonoyl glycerol (2-AG), causing hyperactivity of the endocannabinoid system and consequent hyperphagia, fat accumulation, obesity, and metabolic syndrome. The development of obesity-associated leptin-resistance would perpetuate the endocannabinoid hyperactivity*

Rycina 2. *Nadaktywny układ endokanabinoidowy jako potencjalna przyczyna otyłości u ludzi. Przejściowa aktywacja układu endokanabinoidowego powoduje wzrost apetytu, zmniejsza sytość, wzmaga lipogenezę i zmniejsza wydatek energetyczny. Spożywanie smacznego jedzenia, szczególnie sprzyjającemu diecie wysokotłuszczowej, dostarczyłoby więcej produktów do syntezy anandaminy (ADA) i 2 arachidonolu glicerolu (2-AG), powodując nadaktywność układu endokanabinoidowego, a następnie hyperfagię, gromadzenie tłuszczu, otyłość i zespół metaboliczny. Rozwój oporności na leptynę związanej z otyłością będzie powodowało wzrost nadaktywność układu endokanabinoidowego*

Conclusions

Several signals produced by the adipose tissue, GIT, and pancreas are involved with energy homeostasis and body weight regulation. The hypothalamus, particularly the ARC, is responsible for integrating these peripheral signals with other information that arises from the endocannabinoid system and also from the internal and external milieu (Fig. 3). The analysis of all these input signals triggers several compensatory responses to maintain a balance between energy intake and expenditure. The malfunctioning of one or more component

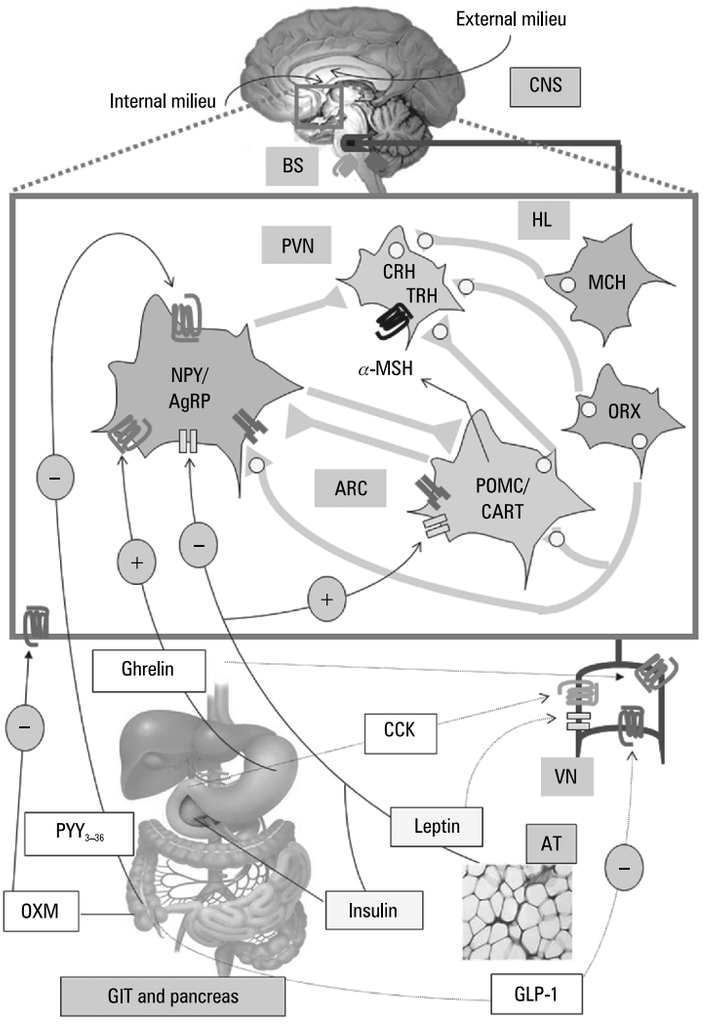

Figure 3. *Energy balance regulation through interactions between the central nervous system (CNS), gastrointestinal tract (GIT), pancreas and adipose tissue (AT). The elements inside the large box represent the hypothalamic nuclei (PVN, paraventricular nucleus; HL, lateral hypothalamus, ARC, arcuate nucleus) with orexigenic neurons (NPY/AgRP, MCH and ORX), anorexigenic neurons (POMC/ /CART and CRH/TRH) and their connections. The open circles indicate the locations of the CB1 receptors of the endocannabinoid system. Stimulatory or inhibitory effect of a peptide on the neuronal population is indicated by the positive or negative signs, respectively. The actions carried out through the vagus nerve (VN) are indicated by dotted arrows. Receptors are represented in hypothalamic neurons and VN: Ob-Rb (leptin) in NPY/AgRP and POMC/CART neurons and in the stomach; IR (insulin) in NPY/AgRP and POMC/CART neurons; GHS-R (ghrelin) in NPY/AgRP neurons and in the stomach; Y2R [peptide YY (PYY3-36)] in NPY/AgRP neurons; MC4R (melanocortin) in CRH/TRH neurons; CCKR1 [cholecystokinin (CCK)] in the stomach and in the brain stem (BS); GLP-1R [glucagon-like peptide (GLP-1) and oxyntomodulin (OXM)] in the stomach and in the BS. CCKR1 and GLP-1R receptors in the BS indicate a direct action on this area. Leptin and insulin are „adiposity signals" that help in regulating longterm body stores energy, while the GIT peptides (ghrelin, PPY, OXM, CCK and GLP-1) act mainly as „satiety signals", determining the start and end of the meal and the interval between meals.*
NPY — neuropeptide Y; AgRP — agouti-related peptide; POMC — pro-opiomelanocortin; CART — cocaine- and amphetamine-regulated transcript; α-MSH — melanocyte-stimulating hormone (melanocortin); CRH — corticotropinreleasing hormone; TRH — thyrotropin-releasing hormone; MCH, melanin-concentrating hormone; ORX — orexins

Rycina 3. *Regulacja równowagi energetycznej przez interakcje między ośrodkowym układem nerwowym (OUN), układem pokarmowym (GIT), trzustką i tkanką tłuszczową (AT). Elementy w dużym kwadracie odpowiadają jądrom podwzgórza (PVN, jądro przykomorowe; HL, podwzgórze boczne; ARC, jądro łukowate) z neuronami oreksygenicznymi, anoreksygenicznymi, i ich połączeniami. Kółka wskazują na lokalizację receptorów CBI układu endokannabinoidowego. Efekt stymulujący lub hamujący białek na populację neuronów jest wskazany przez znaki dodatnie lub ujemne, odpowiednio. Aktywności przewodzone przez nerw błędny (VN) wskazują kropkowane strzałki. Receptory w neuronach podwzgórza i nerwu błędnego: Ob-Rb (leptyna) w neuronach NPY/AgRP i POMC/CART i w żołądku; IR (insulina) w neuronach NPY/AgRP i POMC/CART, GHS-R (grelina) w neuronach NPY/AgRP i w żołądku, Y2R[peptyd YY (PYY3-36)] neuronach NPY/AgRP; MC4R (melanokortyna) w neuronach CRT/TRH; CCKR1 [cholecystokinina] w żołądku i pniu mózgu , GLP-1R [(peptyd podobny do glukagonu (GLP-1) i oksyntomodulina (OXM)] w żołądku i pniu mózgu. CCKR1 i GLP-1R receptory w pniu mózgu (BS) wskazują na bezpośrednią aktywność tego obszaru. Leptyna i insulina są „sygnałami tłuszczowymi" które pomagają w regulacji tworzenia długoterminowych zapasów energetycznych, podczas gdy peptydy GIT(grelina, PPY, OXM, CCK i GLP-1) działają głównie jako „sygnały sytości" określając początek i koniec posiłku oraz przerwy między posiłkami.*
NPY — neuropeptyd Y, AgRP — białko aguti; POMC — proopiomelanokortyna; CART — transkrypt regulowany przez kokainę i amfetaminę; α-MSH — hormon stymulujący melanocyty (melanokortyna), CRH — hormon uwalniający kortykotropinę; TRH – hormon uwalniający tyreotropinę; MCH — hormon zwiększający stężenie melaniny; ORX — oreksyny

of this complex machinery might result in an energy imbalance and significant changes in body weight. The outstanding scientific advances regarding the knowledge of this machinery, particularly in the last two decades, encourage the future development of safer and more effective therapeutic approaches against obesity and other nutritional and metabolic disorders.

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