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Trends in
Molecular Medicine

Review

Obesity wars: hypothalamic sEVs a new hope

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There are currently several pharmacological therapies available for the treatment of obesity, targeting both the central nervous system (CNS) and peripheral tissues. In recent years, small extracellular vesicles (sEVs) have been shown to be involved in many pathophysiological conditions. Because of their special nano-sized structure and contents, sEVs can activate receptors and trigger intracellular pathways in recipient cells. Notably, in addition to transferring molecules between cells, sEVs can also alter their phenotypic characteristics. The purpose of this review is to discuss how sEVs can be used as a CNS-targeted strategy for treating obesity. Furthermore, we will evaluate current findings, such as the sEV-mediated targeting of hypothalamic AMP-activated protein kinase (AMPK), and discuss how they can be translated into clinical application.

Highlights

There are three main weaknesses in the available antiobesity treatments: (i) lack of specificity and therefore harmful side effects, (ii) unknown mechanism of action in many cases, and (iii) they mainly target either intestinal absorption or feeding/appetite and not energy expenditure.

Small extracellular vesicle (sEV)-based therapeutics are already undergoing clinical trials, although as yet no sEV-based therapy has been approved by the FDA.

Targeting of specific sets of hypothalamic neurons with engineered sEVs would provide: (i) increased specificity and therefore reduced side effects, (ii) knowledge of the mechanism of action, and (iii) an integral targeting of energy balance, modulating both feeding and energy expenditure.

Current preclinical evidence has demonstrated that specific targeting of hypothalamic cell populations using sEVs ameliorates diet-induced and genetic obesity.

Obesity treatment: an unmet clinical need

Environmental and genetic factors interact with global food system drivers to create wide variations in the prevalence of **obesity** (see Glossary) among populations [1–5]. Future predictions are alarming, with overweight – **body mass index (BMI)** ≥25 – and obesity (BMI ≥30) estimated to reach pandemic proportions [1–5]. Cancer, cardiovascular diseases, and type 2 diabetes (T2D) are among the direct and indirect comorbidities linked with obesity [1–5]. In terms of treating morbid obesity, bariatric surgery is undoubtedly the most effective intervention [6,7]. However, this surgery is highly invasive and not without risk for adverse effects, so it cannot be recommended for obesity-induced metabolic complications in the broader obese population, and it is currently applicable only for those who have BMI >40 or BMI >35 if comorbidities are present. Furthermore, surgery effectiveness varies from patient to patient, with many experiencing poor long-term results, such as reoccurrence of weight gain [6,7]. Pharmacological treatment represents an alternative strategy to surgery, and a growing number of innovative antiobesity drugs are being developed [2–5]. However, the main weaknesses of the currently available antiobesity treatments in the clinic are three: (i) low specificity and therefore undesired harmful side effects, (ii) in many cases unknown mechanism of action, and (iii) in most instances they exclusively target either intestinal absorption (with unpleasant side effects) or feeding/appetite, and therefore elicit a nonintegral approach to energy homeostasis [2–5] (Figure 1). Furthermore, it is well established that obesity is a heterogeneous condition regarding etiology, phenotype, and response to treatment [1–5]. Consequently, there is a need for personalized adaptions in obesity treatment. In this review we will discuss the advantage of **nanotechnology**-mediated drug delivery and assess the effectiveness of sEVs as nanocarriers to modulate specific hypothalamic neurons to combat obesity (Figure 1).

Pharmacotherapy as a treatment for obesity

For many people who live with obesity, lifestyle changes such as diets and physical exercise are not very effective in losing weight, and in recent years there has been an increased focus on pharmacotherapy options [2–5]. Several drugs have been developed to treat this disease, including phentermine-topiramate (a noradrenergic sympathomimetic amine), orlistat (a triacylglycerol lipase inhibitor), liraglutide [a **glucagon-like peptide 1 (GLP-1)** receptor (GLP-1R) agonist],

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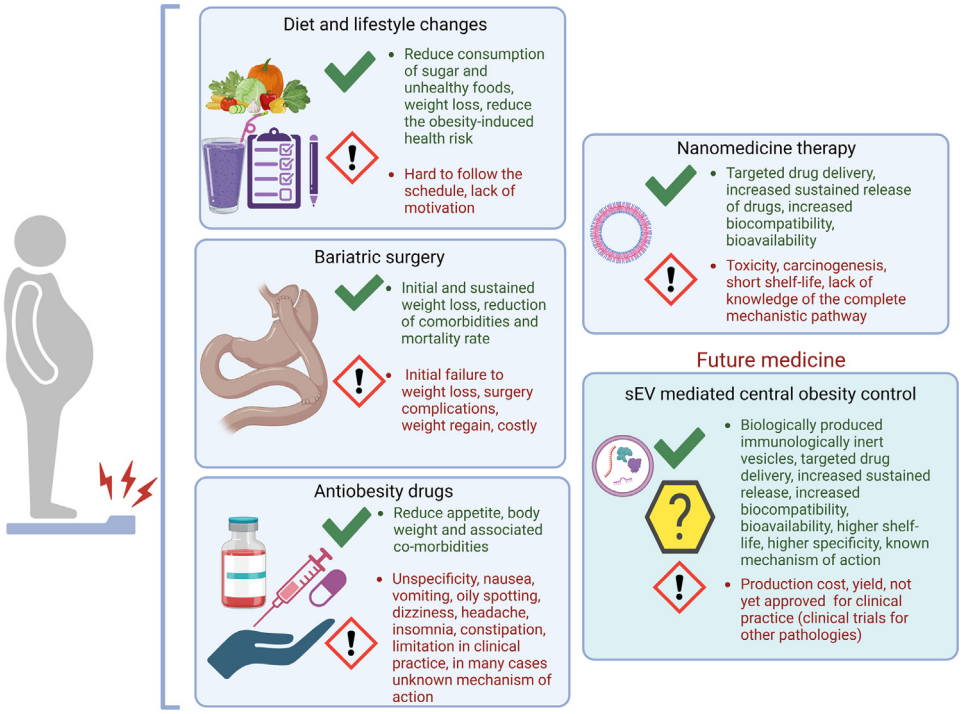

Figure 1. Pros and cons of available (and possible) antiobesity treatments. To control obesity, the first choice is normally lifestyle and dietary changes. Most antiobesity drugs are not specific and have a variety of side effects, including nausea, vomiting, headache, insomnia, and constipation, and some have limitations in clinical practice. So far, bariatric surgery has proved to be the most effective way to combat morbid obesity. This surgery, however, is very invasive and is associated with several risks. It is also very costly, and each patient's results are different, with recurrence of obesity and obesity-related comorbidities representing a major problem. Recent studies have shown that nanotechnology may improve the delivery of antiobesity drugs. There is, however, a risk of toxicity and carcinogenesis associated with this method, as most of the nanoparticle (NP) compounds are synthetic. They have a short half-life, and there is a lack of knowledge in their complete mechanistic pathway. A majority of these NP-mediated antiobesity therapies have not yet been translated into clinical trials. The use of small extracellular vesicles (sEVs) may overcome many of the problems associated with the synthetic NPs, and it is highly possible that they could serve as an antiobesity treatment in the future. The sEVs have more immunological inertness and lack of toxicity, as well as highly specific targeting and known mechanism of action. However, until this technology is clinically available, the yield, production cost and isolation technique should be evaluated comprehensively. Figure created with BioRender.

lorcaserin (a selective serotonergic 2C receptor agonist), and naltrexone-bupropion (a noradrenaline and dopamine reuptake inhibitor/opioid receptor antagonist) [2–5]. The scope of this review is not to summarize the actions of these drugs, which has been reviewed elsewhere recently [2–5], but to emphasize that new treatment options are still warranted.

The incretin GLP-1 has received enormous attention during the past decades as a therapeutic target for the treatment of obesity and T2D. Continuous improvement of the pharmacokinetic profile of GLP-1R agonists – starting from the natural hormone with a half-life of approximately 2–3 min, to the development of twice daily, and even once-weekly drugs – highlights the pharmaceutical evolution of GLP-1-based drugs [3,5]. In peripheral organs, and in the brain as well, the agonism of the GLP-1R affects both energy and glucose homeostasis [3,5,8]. These drugs were originally developed for the treatment of T2D, and it is therefore noteworthy that the recently approved GLP-1R agonist semaglutide decreases body weight in obese or overweight subjects without diabetes by 14.9% in comparison to 2.4% for placebo-treated individuals [9]. In contrast to GLP-1, the incretin hormone

Glossary

Body mass index (BMI): the measure of body fat based on a person's height and weight. BMI equals a person's weight in kilograms divided by the square of their height in meters.
Brown adipose tissue (BAT): also named brown fat, BAT is a specialized adipose tissue comprising several small lipid droplets (multilocular) and many mitochondria that express uncoupling protein 1 (UCP1). BAT regulates energy expenditure (e.g., by thermogenesis).
Browning: the inducible process of differentiation of white adipocytes to brown-like/beige adipocytes.
Glucagon-like peptide 1 (GLP-1): a peptide hormone derived from the tissue-specific post-translational processing of the proglucagon peptide. It is produced and secreted by intestinal enteroendocrine L-cells and certain neurons within the nucleus of the solitary tract in the brainstem upon food consumption. The initial product GLP-1-(1–37) is susceptible to amidation and proteolytic cleavage which gives rise to the two truncated and equipotent biologically active forms: GLP-1-(7–36) amide and GLP-1-(7–37). Alongside glucose-dependent insulinotropic peptide (GIP), GLP-1 is an incretin; thus, it can decrease blood sugar levels in a glucose-dependent manner by enhancing the secretion of insulin.
Nanotechnology: the manipulation of matter on a near-atomic scale to produce new structures, materials, and devices. This technology promises scientific advancement in many sectors, medicine among them. Nanotechnology refers to engineered structures, devices, and systems. Nanomaterials have a length scale between 1 and 100 nm; 1 nm = a billionth (10^{-9}) of a meter. On this scale, materials begin to exhibit unique properties that may affect their physical, chemical, and biological behavior.
Obesity: abnormal or excessive fat accumulation that presents a risk to health. A body mass index (BMI) >25 is considered overweight, and >30 is obese.
Polyagonist: a unimolecular drug that combines the activities of two of more hormones acting on their receptors with balanced (equipotent) or unbalanced (not equipotent) activities.
Thermogenesis: the process of heat production in organisms, including mechanisms of shivering (via contractile activity of the skeletal muscle) and

glucose-dependent insulinotropic polypeptide (GIP) initially attracted limited interest as a pharmacological target, because of conflicting observations that argued whether activation or inhibition of the GIP receptor (GIPR) provides beneficial effects on systemic metabolism [3,5]. The significance of GIPR agonism in the treatment of obesity and diabetes recently re-emerged with the clinical success of unimolecular dual-agonists (targeting both GIPR and GLP-1R) with significantly improved body weight and glucose control in patients with obesity and T2D [9–11]. A 72-week clinical study with 5 mg, 10 mg, and 15 mg once weekly of tirzepatide – a unimolecular coagonist with imbalanced (not equipotent) activities at GLP-1R and GIPR – was shown to sustain weight loss among patients with obesity [10]. In another 2-week study in patients with T2D, treatment with the GLP-1/GIP coagonist RG7697 displayed a dose-dependent decrease in pre- and postprandial plasma glucose concentration, without causing hypoglycemia [12]. Moreover, the combination of peptides and nuclear hormones, such as steroids and thyroid hormone, has also shown promising data as antiobesity therapeutics [13–15]. Finally, very recent results showed encouraging evidence based on unimolecular peptide triagonist, such as SAR441255. This unimolecular balanced (equipotent) triple incretin, which combines the activities of GLP-1, GIP, and glucagon, decreases body weight and improves glycemic control in rodents and monkeys [16]. Importantly, SAR441255 also improved glycemic control in healthy subjects [16]. Results from other triagonists are needed to better understand the multiorgan and molecular mechanisms by which triple receptor agonism drives weight loss. The kind of therapeutic response obtained by these **polyagonists** is astonishing, being in a range only previously reached by bariatric surgery. As could be expected, the response to treatment varied considerably among the patients, with approximately 10% of patients not reaching the treatment goals in the latest clinical trial with tirzepatide [10]. Nevertheless, the results are still very promising, and the efficacy numbers of the drugs, when used in routine clinical practice, is eagerly awaited.

nonshivering (mostly dependent on the uncoupling process in BAT).
White adipose tissue (WAT): the main adipose tissue and a critical endocrine and immune organ that stores energy in the form of triglycerides.

Hypothalamic neuronal circuitry and metabolic homeostasis

A widespread belief is that hormone action in peripheral tissues is responsible for nutrient handling. However, a large body of evidence suggests that the CNS, and particularly the hypothalamus, contributes more to metabolic homeostasis throughout the body [17–19] (Box 1). A key

Box 1. Hypothalamic nucleus specificity

The hypothalamus is the brain region located below the thalamus, comprising the major portion of the ventral diencephalon [17–19]. The role of the hypothalamus in the regulation of homeostasis is essential for survival and reproduction of the individual [17–19]. One of the most remarkable features of the hypothalamus is its cytoarchitecture. Opposite to other brain areas, which are organized in layers (e.g., the cortex or the cerebellum, among others), the hypothalamus is organized in anatomically defined neuronal clusters called nuclei, forming interconnected neuronal circuits via axonal projections [17–19]. Notably, the cellular specificity of the hypothalamus does not end with its organization in nuclei [17–19]. Within each nucleus there are populations of neurons (and possibly glial cells) with genetic profiles and, what is more important, with different functions [17–19,64]. Even more, molecular profiling at single-cell resolution has shown that there are different subsets of neurons within those specific populations [18,19,65–67]. Taking advantage of this neuronal specificity has allowed modulation of the expression, activity, and function of discrete populations of hypothalamic neurons. For example, Cre-mediated recombination under the control of specific promoters has provided genetic access to overexpress and delete genes, and to modulate neuronal function with designer receptors exclusively activated by designer drugs (DREADD)-based chemogenetics and optogenetics [18,19,68,69]. Several hypothalamic specific promoters are known. Agouti-related peptide (AgRP) – an orexigenic (feeding-promoting) neuropeptide encoded by the *Agrp* gene – and proopiomelanocortin (POMC) – an anorexigenic (feeding-inhibiting) neuropeptide encoded by the *Pomc* gene – in the arcuate nucleus (ARC), as well as steroidogenic factor 1 (SF1; an orphan nuclear receptor, although phospholipids can be its ligands [70], encoded by the gene *Nr5a1*) in the ventromedial nucleus (VMH) are likely the most widely used [18,19,69]. Extensive evidence showed that manipulation of several genes in transgenic mice and optogenetic/chemogenetic approaches regulating the activity of these neurons elicits major changes in feeding, energy expenditure, and peripheral lipid and glucose metabolism [17–19,69]. Moreover, recent data have allowed the specific genetic modulation of protein activities using peripheral injections of small extracellular vesicles (sEVs) (Box 2) as nanocarriers of expression plasmids under the control of the SF1 promoter [53,54]. All this evidence suggest that specific targeting of hypothalamic cell populations might be a feasible strategy for obesity treatment.

component of the hypothalamus is the arcuate nucleus (ARC), which regulates metabolism and feeding [17–19]. ARC neurons are located near the median eminence, a circumventricular organ whose fenestrated capillaries leave a 'leaky' blood–brain barrier (BBB) [18,19]. Consequently, the ARC receives hormonal, nutritional, and neuronal signals from the periphery and processes them in a coordinated manner to provide feedback. Two major neuronal clusters have been identified in the ARC: orexigenic neurons expressing neuropeptide Y (NPY) and agouti-related peptide (AgRP), and anorexigenic neurons expressing proopiomelanocortin (POMC) and cocaine- and amphetamine-regulated transcript (CART) [17–19] (Box 1). These first-order neurons sense peripheral metabolic hormones and nutrients, and contact second-order neurons in other hypothalamic sites, including the paraventricular (PVH) and ventromedial (VMH) nuclei, the lateral hypothalamic area (LHA), as well as autonomic preganglionic nerve cells in the brainstem and spinal cord [17–19] (Box 1).

The specific targeting of these cell populations implies three major constraints: (i) the search for a clinically suitable way of administration to reach them, (ii) the crossing of the BBB, and (iii) the redundance of hypothalamic mechanisms, which is an obvious evolutionary advantage, but a major challenge in terms of treatment that makes difficult to select an 'ideal' target. In this regard, many of the currently available antiobesity drugs are designed mainly for targeting the effectors (hormones, neuropeptides, receptors, signaling pathways, etc.) [2–5]. However, this approach disregards the fact that obesity is a chronic state of positive energy balance, and consequently, targeting the mechanisms of hypothalamic energy sensing could be a more relevant treatment strategy.

Hypothalamic regulation of energy balance: a matter of energy sensing

AMPK is a highly conserved heterotrimeric serine/threonine kinase composed of a catalytic (α) and two regulatory (β and γ) subunits. AMPK functions as an energy sensor, being activated: (i) upon a decrease in the cellular energy status (reduced ATP levels) as reflected by an increased AMP/ATP and ADP/ATP ratios, (ii) by the action of upstream kinases/phosphatases, and (iii) also during conditions of glucose starvation without change in the adenine nucleotide ratio [17,20,21]. By modulating hypothalamic fatty acid and complex lipid metabolism, AMPK regulates the whole-body energy balance [17,22]. Thus, genetic ablation of AMPKα2 in POMC and AgRP neurons can activate hyperphagic and hypophagic characteristics, respectively [23]. Decreased food intake could also be reached by genetic inhibition of AMPKα2 function within the VMH [24,25]. Notably, pharmacological/adenovirus-mediated stimulation of hypothalamic AMPK has been shown to impact homeostatic and hedonic food intake by modulating NPY and AgRP in the ARC [25–27] and corticotropin-releasing hormone (CRH) in the PVH [28], respectively. A particular function of AMPK in steroidogenic factor 1 (SF1) neurons of the VMH is to regulate energy expenditure based on its actions on **brown adipose tissue (BAT) thermogenesis** and **white adipose tissue (WAT) browning** via the sympathetic nervous system (SNS) [17,29], as well as lipogenesis via the parasympathetic nervous system (PSNS). The modulation of AMPK in the context of BAT thermogenesis has been analyzed in different experimental models. For example, 3,3′,5-triiodothyronine (T3) specifically in the VMH triggered BAT thermogenesis, linked with a significant decline in phosphorylated AMPKα (pAMPKα) levels in the VMH and increased SNS firing on BAT [29,30]. This integrative VMH AMPK–SNS–BAT axis is valid for other hormones as well. Estradiol (E2) [31], leptin [32], bone morphogenetic protein 8B (BMP8B) [33,34], and, importantly, pharmacological agents with well-known antiobesity action (such as liraglutide [8] and nicotine [35]), all acting (among other mechanisms) by inhibiting hypothalamic AMPK signaling in the VMH to elicit a BAT and, in some cases, WAT thermogenic activation. Notably, SF1 AMPKα1 knockout (KO) mice on a high-fat diet (HFD) exhibit lower body weight, with increased energy expenditure, higher BAT activity, and thermogenesis than

littermate controls, which is independent of food consumption [24]. Furthermore, we have found that administration of adenoviruses encoding a dominant negative (DN) AMPKα1 isoform (AMPKα1-DN) into the VMH induces weight loss through increased thermogenesis in HFD-obese [24] and in ovariectomized (and therefore obese) female rats [31]. Notably, in none of these studies were undesired side effects related to increased SNS tone or elevated BAT thermogenesis reported [24,31].

A one-sided approach to treat obesity is challenging due to the homeostatic nature and multifaceted regulation of energy balance mechanisms [1–5]. Interestingly, this problem has been partially overcome with the development of incretin-related drugs, such as liraglutide and tirzepatide, which likely target BAT thermogenesis, in addition to their effects on feeding [8,36]. However, the importance of BAT thermogenic activation by these drugs is still controversial, with studies showing opposite results or lack of effects of liraglutide and GLP-1R polyagonism in preclinical models and humans [13,37]. Targeting several signaling mechanisms simultaneously, for example, GLP-1Rs and GIPRs, may also have limitations because of a number of possible side effects (such as nausea, vomiting, and diarrhea) [3,5]. The development of triagonists might increase this issue [16]. However, the targeting of AMPK may offer an interesting and perhaps a much more global alternative for obesity treatment. As mentioned above, in addition to affecting thermogenesis, hypothalamic AMPK plays a major role in the regulation of homeostatic feeding [23,25–27], food preference (selection of carbohydrates over fat) [28], and glucose homeostasis. All those actions result from the specific action of this enzyme, not only in discrete hypothalamic nuclei – such as the VMH, ARC, and PVH – but even in very precise hypothalamic neurons inside those areas, such as SF1 (VMH), POMC, and AgRP (ARC), and CRH (PVH) cells [17,22]. However, the crucial question is whether hypothalamic AMPK is a realistic target using a clinically suitable way for administration, and sEVS may provide answers here.

Nanotechnology in the treatment of obesity

The use of nanotechnology in medical treatment has so far proved promising. Nanotechnology may provide extraordinary pharmacological properties, such as cell-specific targeting, preservation of drugs from physiological/enzymatic decomposition, and stable release [38]. As a result, nanotechnology represents a groundbreaking direction in medicine, with the potential of reducing side effects and increasing patient adherence, in addition to efficacy [39,40]. In this regard, there are numerous advantages of nanoparticles (NPs), including their large surface area-to-volume ratio, encapsulation properties, and chemically tunable surfaces, making them an excellent delivery system to specifically target particular organs or cell types, as well as making possible the oral or transdermal administration of existing drugs [41]. By modifying surfaces with targeting ligands, specificity can be achieved *in vivo* [42]. Moreover, due to the small size of NPs, tissues can also be targeted passively, and novel formulations can deliver drugs in a controlled manner [43]. Numerous categories of NP therapeutics exist, including inorganics, lipids, and polymers. It is important to consider the unique properties of each category of NP when deciding how to apply them. In this sense, several types of nanotherapies have been studied for the treatment of obesity and metabolic diseases (Table 1) including NP injections, nanogels, nanopatches, and liposomes.

Despite this, there are still some limitations in NP-mediated drug delivery. As the surface area increases during the transformation from microparticles to NPs, problems – such as increased penetration in the cells, reactivity, and sticking – occur, leading to cytotoxicity and DNA damage [38,44]. Moreover, because of their small size, NPs usually have a large clearance rate from the body, which may make them ineffective for drug delivery. Furthermore, most drugs are loaded on the outer surface of the nanocarriers, and they are sometimes degradable, or they have shorter circulation half-life [38,45]. Aside from these challenges, nanomedicine is hindered by

Table 1. Current nanomaterial technologies in obesity and metabolic disease[a]

Type of delivery	Materials	Advantages	Disadvantages	Disease	Effects
Nanoparticle (NP) injection	Dextran, YSK05, PEG, cholesterol, egg yolk, oligopeptide, lipid, phosphatidylcholine Zein nanoparticle	Controlled and sustained drug release [75] Tunable physicochemical properties [75] High level of penetration across biological barriers [76]	There is a greater risk of toxicity from NPs compared to large particles of the same dose and liposomes [77,78]	Diabetes	Specific targeting, reduces inflammation in adipose tissue of obese mice [79], gene silencing action in obese mice [80], reduces inflammation [81], controlled release of insulin [82] Zein-based NPs improve glycemic controls in rats [83]; also currently in a clinical trial (NCT05560412)
NP injection	PLGA, PVA, PEG, egg yolk, phosphatidylcholine, cholesterol, peptide conjugated PEG-lipid, MSN, PCL			Obesity-lipid accumulation	Browning of adipocytes inhibition of Notch signaling in adipocytes of obese mice [84], nontoxic to liver in HFD-fed mice [85,86]
NP oral delivery	GLP-1 analog, chitosan, γ-PGA			Diabetes	Oral absorption, sustained release and improved T2D [87]; increased oral bioavailability of insulin in diabetic rats [88]
Nanogel	CHC	Biologically controlled degradation [89]	As it is diffusion-based, the rate of release can be too fast [90]	Diabetes	Balanced insulin release in diabetic mouse model [89]
Microneedle patch	PVA, dextran, CMC, silk	Administration by self, compact size, painless application [91] Increased drug delivery efficacy, and increased drug stability [91]	Delivery may be affected by skin types (hydration) [92]	Diabetes	Efficient metformin and insulin delivery in rodent models [93,94]
Microneedle patch	Dextran, HA, PVP/PVA, alginate			Diabetes	Efficient delivery of insulin and liraglutide in rodent model [95,96]
Microneedle patch	HA			Obesity	HA-microneedle patch assists in improving lipolysis [97], ensures consistent drug levels in the blood, and helps in adipose tissue browning [98]
Liposomes	Egg yolk, phosphatidylcholine, PEG, LITA	Controlled and sustained release of drugs [78] Improved biocompatibility and biodegradability [78] Low level of toxicity and antigenicity when compared with other nanoparticles [78]	Possibility of oxidation and hydrolysis of phospholipids [99]	Obesity	Liposome-coated peptide shows effective control of obesity in obese mice [100]

[a]Current NP-mediated drug delivery strategies for the treatment of obesity and diabetes. The following chemicals are being used to encapsulate the antiobesity drugs: dextran, YSK05, cholesterol, egg yolk, oligopeptide (ATS-9R), phosphatidylcholine, poly(dl-lactic-co-glycolic acid) (PLGA), polyvinyl alcohol (PVA), cholesterol, peptide-conjugated PEG–lipid, mesoporous silica nanoparticles (MSNs), polycaprolactone (PCL), liposome acetate NP (LITA), chitosan, γ-polyglutamic acid (γ-PGA), carboxymethylcellulose (CMC), hyaluronic acid (HA), and polyvinylpyrrolidone (PVP). NP-assisted delivery has the advantage of being efficient, specific, and reducing side effects for unspecific targets, reducing inflammation, controlling adipose tissue activity and obesity, and reducing type 2 diabetes (T2D).

the fact that NPs do not share any universal characteristics other than their size. Therefore, it is necessary to evaluate the suitability of each type of particle separately. As they are made up of synthetic materials, there may be a risk of toxicity. Furthermore, in most cases they have only been developed for use in preclinical settings. In this sense, it is then more appropriate to use immunologically inert vesicles derived from biological sources, such as sEVs.

Modulation of sEVs for targeted drug delivery

sEVs are small (30–200 nm in diameter) lipid-bilayer-bound vesicles released from living cells into the extracellular environment by the fusion of multivesicular bodies (MVBs) with the plasma membrane. sEVs lack functional nuclei and cannot replicate. They can be part of the intercellular communication systems and can carry distinct cargos such as miRNAs, mRNAs, noncoding RNAs, lipids, and proteins (Box 2 and Figure 2) [46–50]. This property of sEVs can be useful in different ways. sEV cargos can be used as biomarkers for different diseases such as cancer, cardiovascular disorders, rheumatoid arthritis, T2D, and obesity [46–50]. In addition, sEVs can be biochemically engineered to deliver specific molecules to target cells to change their mode of action. Due to their special structures, modifications of their surface can be done via genetic/biological/chemical manipulation [51–54]. By chemically altering lipids or membrane-bound proteins, different moieties – such as lipids, proteins, peptides, polymers, aptamers, or small molecules – can be added [51–54].

One major challenge when delivering pharmacological compounds to manipulate energy balance in the CNS is the crossing of the BBB. Bioengineered sEVs carrying the rabies virus glycoprotein (RVG) peptide bound to lysosome-associated membrane protein 2b (Lamp2b; a protein highly expressed in sEV membranes) at their surfaces have been reported to allow precise neuronal targeting [52–54]. Another challenge is to focus on discrete neuronal subsets, but here several options exist. One example is to use specific promoters expressed in discrete hypothalamic cell populations [53,54] (Box 1). Hypothalamic AMPK offers a very interesting option in this sense, with the following nucleus specificity of its actions: (i) AMPK in AgRP and POMC neurons is involved mainly in homeostatic feeding control [23,25–27], (ii) AMPK in SF1 neurons is involved mainly in the central control of BAT thermogenesis, browning, and glucose homeostasis [24,31,33,34], and (iii) AMPK in CRH neurons of the PVH mediates the preference for carbohydrate intake [28]. Remarkably, as mentioned earlier, the selective deletion of AMPKα1 in SF1 neurons has been shown to lead to resistance towards diet-induced obesity in mice [24,30]. Thus, targeting this subunit of AMPK in those hypothalamic populations could serve as an interesting target to fight obesity via both sides of the energy balance equation, including feeding (by targeting the ARC and PVH) and energy expenditure and peripheral metabolism (by targeting the VMH). This strategy, however, requires a considerable level of hypothalamic specificity, because any side effects associated with inhibiting peripheral signals of AMPK may cause a detrimental effect on metabolic status [17,22]. Therefore, considering this issue, Lamp2b RVG-sEVs from immature dendritic cells were loaded with an AMPKα1-DN plasmid which is under

Box 2. sEV composition and function

sEVs are membrane-bound extracellular vesicles 30–200 nm in diameter released by exocytosis following the fusion of intracellular multivesicular bodies (MVBs) [46–50]. The membranes of sEVs are composed mainly of lysosome-associated membrane protein 2b (Lamp2b), glucose phosphate isomerase (GPI), and four transmembrane proteins (CD9, CD63, CD47, and CD81) which can bind to receptors on corresponding target cells, affecting their specific uptake [46–50]. For instance, CD47 has the function of protecting cells from phagocytosis [71]. CD47–signal–regulatory protein (SIRP) binding usually extends sEVs' half-life in circulation by emitting a 'do not eat me' sign [50]. The presence of sEVs is widespread in all bodily fluids and tissues [46–50]. In cells, sEVs are responsible for mediating intercellular communication [46–50]. They can transfer exogenous substances to recipient cells, such as proteins, miRNAs, and mRNAs [46–50]. Due to their naturally equipped properties, these nanocarriers have been shown to be employable as drug delivery agents [51,53,54,61]. As compared with synthetic drug nanocarriers, sEVs isolated from patients' own cells are more biocompatible and less toxic [72]. Moreover, sEVs have been demonstrated to penetrate cellular structures, move through the bloodstream, and can easily cross the blood–brain barrier (BBB) [52–54]. Notably, sEVs are also highly engineerable [51–54]. In this regard, besides being cell- and tissue-specific, sEV surface proteins can be engineered to confer specificity [51–54]. Compared with lipid nanoparticles (LNPs), sEVs provide better safeguards for drugs during delivery, as drugs are encapsulated within the bilayer sEV membrane, while they are usually attached outside in case of the LNPs, making them easily degradable [73]. Apart from these properties, sEVs can transport both hydrophobic and hydrophilic compounds, and possess strong homing properties because they display a wide range of cell-derived surface moieties [74].

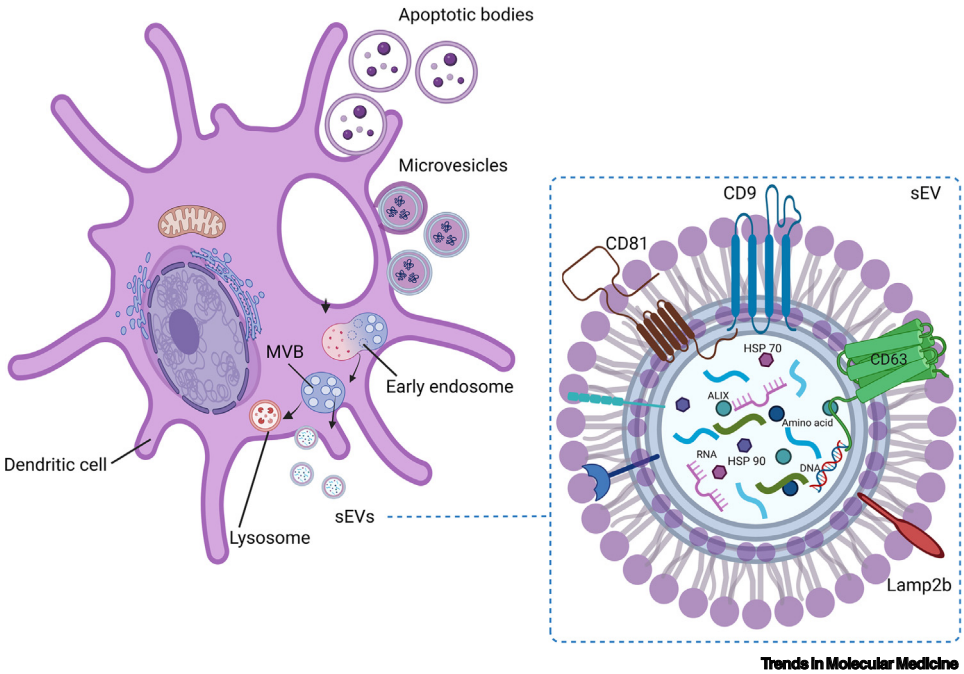

Figure 2. Structure, biogenesis, and secretion of small extracellular vesicles (sEVs). The figure shows how dendritic cells release extracellular vesicles (EVs) and the structure of a sEV. A donor dendritic cell can release three types of EVs. Apoptotic bodies are the largest EVs, followed by microvesicles and sEVs. Microvesicles are formed when plasma membranes bud outward and fuse. A sEV develops as an intraluminal vesicle and then incorporates itself into an early endosome. There are two main pathways for multivesicular bodies (MVBs): either fusion with the lysosomes, or plasma membrane fusion, allowing the release of their cargo into the extracellular space. sEVs have numerous markers, including proteins, lipids, and nucleic acids. The figure shows the cargo of a sEV consisting of bilayer lipids, surface markers lysosome-associated membrane protein 2b (Lamp2b), CD81, CD9, CD63, ALIX, heat shock protein 90 (HSP90), HSP70, DNA, lipids, amino acids, and RNA. Figure created with BioRender.

Clinician's corner

Despite all the efforts, obesity is still a major health challenge in need of new therapies.

Until quite recently, most available weight-loss medications have exhibited unwanted side effects, limited effectiveness, and in many cases an unclear mechanism of action.

Recent incretin-based therapies – particularly GLP-1 agonists and inhibitors or GLP-1 degradation, acting mostly at central level – have shown a good degree of efficacy in patients with obesity.

A new generation of unimolecular polyagonists targeting GLP-1 and GIP receptors have shown to be almost as effective as bariatric surgery.

New nanodelivery sEV-based therapies, acting in specific neuronal populations at the CNS, are being developed and are expected to exert benefits similar to those of current therapies and with fewer side effects.

the control of the SF1 promoter, and these sEVs were peripherally administered into the tail vein of mice [53,54]. Indeed, the SF1–AMPKα1–DN-containing sEVs triggered significant reduction in body mass and adiposity without affecting the food intake in obese mice. Moreover, the weight-reducing effect was primarily due to the increase in SNS-mediated UCP1-dependent BAT thermogenesis and the subsequent increase in energy expenditure [53,54]. Notably, no signs of peripheral and systemic inflammation were found with this treatment [53]. Importantly, the weight-reducing and metabolic improving effect of SF1–AMPKα1–DN-loaded sEVs is achieved in models of diet-induced obesity and in leptin-receptor-deficient *db/db* mice, in both cases through increased SNS-driven BAT thermogenesis [53,54].

This novel research demonstrates that the specific control of neural activities in given hypothalamic areas may provide a highly efficient strategy for drug development [17–19,22]. Furthermore, these data demonstrate that sEVs can be employed as neuron-specific organic nanovehicles that act as a suitable alternative to common delivery systems to treat obesity and/or related metabolic comorbidities. Future strategies using sEVs that contain targeted, promoter-driven plasmids encoding short hairpin RNAs (shRNAs), as well as DN or constitutively active (CA) isoforms against the AMPKα1 or AMPKα2 subunits or alternative targets (Figure 3), would allow a global modulation of energy expenditure and feeding to control whole-body energy homeostasis [22]. As a result, a more effective treatment of obesity will be possible.

Clinical translation of sEV-mediated drug delivery in obesity treatment

sEVs are not without challenges when they are used as delivery vehicles [55,56]. While sEVs have several advantages over traditional drug delivery systems (DDSs), they are mostly challenged by complicated large-scale production and poor loading methods, preventing effective and reproducible release [57]. It is also important to note that any change in the physiological environment may alter functional charges present in sEVs, and they may initiate undesirable side effects, including immunosuppression and tumorigenesis [58]. sEVs are also characterized by their cellular source, which determines many of their properties. As a result, it is imperative to identify the safest and most reliable methods for isolating sEVs. It is possible to functionalize sEVs with exogenous materials – such as ligands, peptides, and proteins – to achieve target-specific DDSs [51–54]. In order to optimize the packaging of therapeutic cargos into sEVs, different methods have been employed to improve the loading efficiency [59]. Even though many studies have explored sEV-based DDSs, there are still many challenges, including isolation, characterization, and finally translating the findings into clinical practice [60,61]. There is also a need to enhance the ability to extract sEVs from cell-conditioned medium; clinical needs largely surpass what can be generated experimentally in the laboratory [61]. There is no doubt that although this issue can be overcome, sEVs must be produced at a sufficiently low cost to make DDSs based on sEVs affordable, and this is another real challenge [61]. Taken together, if the current shortcomings of sEVs as DDSs can be overcome, these molecules can have great potential as nanocarriers also in the clinical setting.

With respect to the treatment of obesity, the primary findings of sEV-based delivery systems are encouraging. However, some questions need to be validated before clinical use can begin with the SF1–AMPKα1–DN-loaded sEVs (see Outstanding questions). Clinical trials involving these strategies will raise some questions about their translatability. It is possible to precisely target hypothalamic AMPK, but there are other issues involved. One of the biggest concerns is the potential long-lasting effects of targeting AMPK in the brain. In numerous studies, it has been demonstrated that hypothalamic AMPK plays a major role in several processes, such as glucose and lipid metabolism [17,20,21]. Another question is whether chronic AMPK modulation will influence hypothalamic neuronal health. Although reported evidence did not show alteration in neuronal or glial cells in mice after up to 4 weeks of treatment with sEVs harboring SF1–AMPKα1–DN [53], further long-term studies of neuronal metabolism, toxicity, and integrity will be needed. Similarly, while no hormonal impairment was detected in those settings [53], the possible chronic side effects on endocrine axes should be further investigated. An explicit understanding of the neuronal and molecular downstream AMPK signaling in specific neurons (which remains mostly unknown) and all their interconnected mechanisms will be necessary to address these questions in the years to come to translate these preclinical findings into clinical practice.

Concluding remarks

During the past few decades we have observed a global obesity epidemic which is imposing a health as well as a social and economic burden [1–5]. The higher incidence of obesity increases the risk of cardiovascular disease, cancer, diabetes, inflammatory disease, and many other comorbidities [1–5]. The intense research activity fostered by this global obesity pandemic has also led to a growing understanding of how the CNS contributes to energy homeostasis and metabolism, and has unequivocally demonstrated that the brain must be a primary target for its treatment [1–5,17,22]. In addition to changes in lifestyle, there are two major types of obesity treatment currently available on the market: pharmacotherapy and bariatric surgery [2–5]. However, still waiting for the long-term actions of the last generation of polyagonists, and the exact knowledge of their mechanisms of action, classical antiobesity drugs and bariatric surgery are both associated with significant risks and side effects [2–7]. Thus, the development of novel

Outstanding questions

sEVs have a limited loading efficiency, which sometimes makes them unsuitable for drug delivery. In mice, the modified Lamp2-b RVG-sEVs have worked well so far. However, is it predictable whether the same dose of sEVs will produce the same results in rats or higher primates, or finally in humans?

In terms of body weight maintenance in mice, sEVs have shown to exert an antiobesity effect through an increase in energy expenditure. Will this strategy provide a better therapeutic approach than other pharmacologically available antiobesity drugs in humans?

The formulation of sEVs involves highly complicated protocols. Could this, and issues related to the scaling up of production, hamper the translation of these sEVs into clinical practice?

Considering tumorigenesis as a potential side effect induced by native sEVs, would this affect their possible translation into already susceptible (obese) individuals?

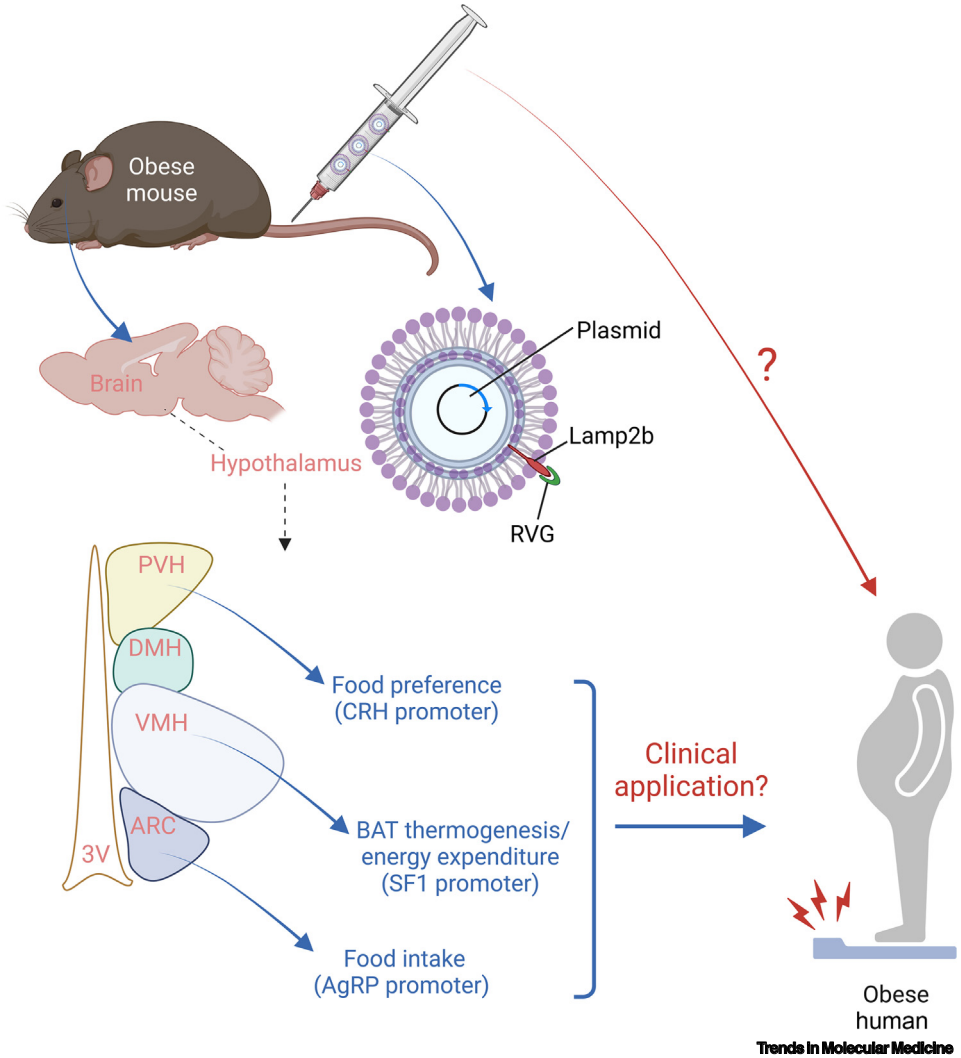

Figure 3. Hypothalamic small extracellular vesicle (sEV)-mediated targeting using cell-specific promoters. A schematic diagram showing the possibilities of modifying lysosome-associated membrane protein 2b–rabies virus glycoprotein (Lamp2b–RVG) sEVs with different plasmids under various neuronal promoters to control obesity centrally through the modification of energy expenditure – through brown adipose tissue (BAT) thermogenesis – and appetite (food preference and homeostatic food intake). Consequently, this method may have a high impact on reducing obesity in humans. Abbreviations: AgRP, agouti-related peptide; ARC, arcuate nucleus of the hypothalamus; CRH, corticotropin-releasing hormone; DMH, dorsomedial nucleus of the hypothalamus; PVH, paraventricular nucleus of the hypothalamus; SF1, steroidogenic factor 1; 3V, third ventricle; VMH, ventromedial nucleus of the hypothalamus. Figure created with BioRender.

complementary methods that will effectively reduce obesity, without undesired secondary effects, is therefore an unmet clinical need [1–5] (see Clinician's corner). The list of NP-based drug delivery strategies for obesity and obesity-related metabolic disorders is given in Table 1. However, these therapies have two major drawbacks: (i) most of them have been tested only in preclinical settings, with the exception, for example, of Zein NPs for glycemic control (GLUCOCAPS) which are undergoing a randomized, double-blind, crossover clinical trial currently in Phase 1 (NIH: NCT05560412[i]), and (ii) they are synthetic and may have unspecific off-target effects during obesity treatments that are still unexplored; however there is evidence that

most of the NP compounds are metabolizable polymers that discharge the drugs following degradation, and the therapeutic effectiveness of NPs can be reduced by interactions with macrophages in the reticuloendothelial system (RES) or mononuclear phagocytic system [62]. For example, in the liver and spleen, nanomaterials are trapped by resident macrophages – such as Kupffer cells, B cells, and sinusoidal cells – leading to inflammation and toxicity [63]. This evidence indicates the need to decrease NP-induced biotoxicity and unwanted immune responses. Hence, it is imperative to develop therapeutic approaches with greater efficacy and a safer profile to reach the clinical setting.

In this review we propose sEV-mediated targeting as a novel, highly specific, and promising approach to treat obesity. Indeed, successful effects of sEV-targeted modulation of AMPKα1 in SF1 neurons to control both diet and genetic-induced obesity have been demonstrated in mice [53,54]. Of course, the targeting of hypothalamic AMPK may not be exempt from side effects, and this needs to be carefully evaluated and addressed. However, this technology could be used for the targeting of other central mechanism, by modulating energy homeostasis in very defined cellular populations using specific promoters. Moreover, the use of Lamp2b RVG-sEVs as nanocarriers for proteins or other pharmacological agents could be another option to explore. It can therefore be seen as an innovative nanobiomedical approach that increases specificity of delivery, while reducing unwanted side effects. Further work will be needed to translate these findings into clinical practice and maybe other metabolic (anabolic and catabolic) diseases.

Acknowledgments

This work was funded by the Ministerio de Ciencia e Innovación and cofunded by the FEDER Program of EU (PID2021-128145NB-I00 and PDC2022-133958-I00).

Declaration of interests

M.L. declares holding an International Patent Application entitled 'Populations of small extracellular vesicles for use in the treatment of obesity', PCT/EP2022/071463. The other authors declare no competing interests.

Resources

[i]https://clinicaltrials.gov/ct2/show/NCT05560412

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