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Drug Treatment of Obesity: From Bench to Bedside

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Abstract

Obesity is a complex metabolic and behavioural disorder associated with increased health risk, including coronary artery disease, congestive heart failure and sudden cardiac death. Effective prevention and treatment strategies for obesity are needed. This unmet need for efficient and safe antiobesity medication resulted in many new therapies at various stages of development. Obesity has become one of the most intensively studied diseases because of the availability of suitable animal and cell culture models of adipocyte differentiation and appetite regulation.

Keywords: obesity, novel therapeutic targets, animal models of obesity, cell models of obesity

1. Introduction

1.1. Obesity: a global health issue

Obesity is a complex metabolic and behavioural disorder defined as an excess of body fat with a body mass index (BMI) greater than or equal to 30 kg/m². A combination of Western diet habits (increase in consumption of animal products, refined grains and added sugar) and sedentary lifestyle results in the excessive nutrient intake, leading to the hypertrophy and hyperplasia of the adipose tissue [1]. Obesity is associated with increased health risk, and the most perturbing danger is the increased predisposition for coronary artery disease [2], congestive heart failure and sudden cardiac death. Beside this, obesity is a major risk factor for a wide range of diseases, including type 2 diabetes, dyslipidaemia, obstructive sleep apnea, asthma, musculoskeletal disorders and certain types of cancer.

Since 1980, the worldwide prevalence of obesity has more than doubled [3]. The predictions for the course of obesity rates in the future are not optimistic because it has reached epidemic proportions in many countries, affecting more than one-third of adults regardless of socioeconomic status [4]. Also, the economic cost of obesity-associated diseases has been estimated at \$147 billion annually [5]. The health and economic consequences carry a significant burden on the global population. Given the fact that cardiovascular disease (CVD) is still the leading cause of death globally [6], the harmful impact obesity has on cardiovascular health makes this disease one of the main global health problems.

1.2. Physiology and pathophysiology of adipose tissue

Adipose tissue is connective tissue which forms a layer under the skin with multiple functions: helps to regulate body temperature, attaches the skin to the underlying tissue and protects body parts. It is divided into the central and peripheral compartment, the former including the subcutaneous upper abdominal and visceral fat masses and the latter consisting of hip and gluteal-femoral fat. The forming of adipose tissue takes place when adipocytes accumulate in large numbers and become the predominant cell type. With the increase in their number and size, the adipose tissue expands. The most important enzymes involved in adipocyte metabolism are endothelial-derived and hormone-sensitive lipoprotein lipase, crucial to lipid storage and release, and acyl-coenzyme A synthetases essential for fatty acid synthesis. A cascade of enzymes is further included in beta-oxidation and fatty acid metabolism.

Adipocytes are complex and metabolically active cells, increasingly perceived as an endocrine gland that produces several metabolites and peptides relevant to the body weight control [7]. Some of the adipocytokines secreted by adipocytes play a role in inflammation (tumour necrosis factor, interleukin 6) or blood coagulation (prostaglandins) and others are engaged in appetite regulation (leptin) and insulin sensitivity (adiponectin). Adipogenesis is highly controlled process during which fibroblast-like preadipocytes differentiate into mature lipid-laden, insulin-responsive adipocytes [8]. This process of changes in morphology and gene expression of preadipocytes depends on the communication between the cells and their surrounded environment and between the cells themselves. Adipogenic stimulators are peroxisome proliferator-activated receptor γ (PPAR γ), single transducers and activators of transcription (STATs), enhancer binding protein α , β and δ (C/EBP α , C/EBP β and C/EBP δ), fatty acids, prostaglandins and glucocorticoids. Also, there are recently found activators such as Wingless and INT-1 proteins (Wnts), clock proteins (Bmal1 and Rev-erb α), interferon regulatory factors (IRFs; IRF3 and IRF4) and B-cell factor 1 (EBf1). Inhibitors of adipogenesis are growth hormone, transforming growth factor- β (TGF- β), glycoproteins and inflammatory cytokines.

The nucleus arcuatus of the hypothalamus is a key nucleus in the regulation of appetite which integrates many peripheral signals controlling food intake [9]. Two major neuronal populations in the ARC are involved in the feeding regulation. One of them increases food intake by coexpressing neuropeptide Y (NPY) and agouti-related protein (AgRP), and the other inhibits food intake through coexpression of cocaine- and amphetamine-related transcript (CART) and pro-opiomelanocortin (POMC) [10]. Their neuronal projections communicate with other hypothalamic areas for appetite regulation [11], including areas involved in the reward system. Adipokines represent peripheral signals that influence the hypothalamic network [12].

After crossing the blood-brain barrier, leptin binds to receptors in the hypothalamus [13]. Leptin receptors are a class I cytokine receptor which act through Janus kinases (JAKs) and signal transducers and activators of transcription (STATs). Leptin activates anorexigenic POMC neurons and inhibits orexigenic AgRP/NPY neurons, leading to an overall reduction in food intake [14].

The GI tract releases a plethora of regulatory peptide hormones, which mediate short-term feelings of hunger and satiety by changes in their concentrations. Cholecystokinin is released postprandially and inhibits food intake [15]. The preproglucagon gene is expressed in the intestine, pancreas and brainstem. It is cleaved by prohormone convertases 1 and 2 to produce glucagon and glucagon-like peptide (GLP)-1 in the CNS and intestine. GLP-1 is released into the circulation after the meal in proportion to the calories consumed and inhibits food intake via the vagus nerve [16]. Ghrelin is produced by the stomach and binds to the growth hormone secretagogue (GHS) receptor. Ghrelin initiates hunger before a meal and increases food intake by increasing hypothalamic NPY mRNA expression [17]. Peptide YY (PYY) is released into the circulation following a meal and cleaved by dipeptidyl peptidase IV (DPP-IV) to create the truncated form PYY3-36, which exerts its anorexigenic properties via Y2 receptors.

The adipose tissue becomes dysfunctional in obesity, with an overproduction of proinflammatory and reduced production of anti-inflammatory adipokines [18]. During obesity, several cells of the innate and adaptive immunity are infiltrating the expanding white adipose tissue as a result of local microenvironment stimuli (fatty acids, adipocyte cell death products, increased hypoxia). The presence of such inflammatory conditions in the obese adipose tissue influences other organ systems and contributes to the development of cardiovascular disease and metabolic dysfunction [19]. When adipose tissue ceases to store energy efficiently, the lipid flux is directed towards non-adipose organs. This ectopic accumulation of lipids promotes lipotoxic insults in cells, leading to insulin resistance, apoptosis and inflammation [20].

2. Current approaches for drug treatment of obesity

Effective prevention and treatment strategies for obesity are needed. Diet, exercise and behaviour modification are the starting point. However, with the constant failure of lifestyle modifications to be successful, the use of antiobesity drugs is vital. Pharmacological treatment of obesity is available for patients with a BMI ≥ 30 kg/m² or a BMI ≥ 27 kg/m² with comorbidities. Pharmacotherapy is successful in improving obesity-related health risks and quality-of-life and preventing the development of comorbidities [21]. If a clinically significant effect (<5% weight loss in patients without diabetes and <3% in patients with diabetes) is not achieved after 3 months, the pharmacological treatment should be stopped [22]. Options for the medical management are still limited because of the paucity of drugs approved by the Food and Drug Administration (FDA).

2.1. Centrally acting medications

Phentermine is a sympathomimetic amine that reduces the appetite and increases the resting energy expenditure. As a norepinephrine-releasing agent [23], it augments the adrenergic signalling in the brain and peripheral tissues. It was primarily approved in 1959 for short-term

treatment of obesity. When taken in the morning, the patient can take advantage of the increase in energy during the day and avoid insomnia during the night. It is well tolerated with the most common side effect being dry mouth and constipation [24], although heart rate and blood pressure can potentially rise.

Topiramate was initially approved as an anticonvulsant in 1996, but during epilepsy trials, it has also shown effects on the weight loss [25]. It is a drug with multiple mechanisms of action although the mechanism responsible for weight loss is largely unknown. It is thought to be related to appetite suppression and enhanced satiety via blockade of sodium and L-type calcium channels, inhibition of AMPA/kainate receptors, facilitation of GABA-mediated chloride fluxes and inhibition of carbonic anhydrase [26].

The phentermine/topiramate (PHEN/TPM) combination product was approved in 2012 as a combination of a lower dose of phentermine and extended release formulation of topiramate [27]. So far, it has produced the highest weight loss effect. The exact mechanism of action may be related to a reduction in compulsive food craving via antagonism of AMPA/kainate receptors, decreased lipogenesis, increased energy expenditure due to GABA-receptors activation and modification of food taste by inhibiting carbonic anhydrase isoenzymes [25]. PHEN/TPM is taken once a day without regard to meals. The most common side effects are dysgeusia, constipation, dry mouth, paresthesia, headache, upper respiratory tract infection and nasopharyngitis [28].

Lorcaserin is a selective serotonin 5-HT_{2C} agonist approved for long-term weight loss in June 2012. It has a high affinity for the 5-HT_{2c} receptor in the POMC cell region of the hypothalamus. The activation of POMC neurons results in decreased food intake and increased satiety. Recommended dosing is 10 mg tablet twice a day with or without food. Lorcaserin is metabolised by multiple hepatic pathways to inactive metabolites, which are excreted in the urine and can accumulate in patients with severe renal impairment. It is usually well tolerated with a headache, nausea, dizziness, dry mouth, back pain and upper respiratory infections being the most frequent adverse effects [29]. Activation of 5-HT_{2A} can cause hallucinations [30].

The combination of naltrexone and bupropion was approved in September 2014. The idea for this combination therapy emerged when bupropion was shown to promote weight loss in patients with obesity [31] who were treated for depression. Naltrexone, an opioid receptor antagonist, demonstrated a reduction in food intake in animals but failed to produce significant weight loss in obese humans [32]. However, animal experiments suggested that addition of an opioid antagonist to serotonergic, noradrenergic, or dopaminergic drugs could help activate POMC neurons in the CNS and thereby promote satiety. This combination is available as fixed-dose 8 mg naltrexone/90 mg bupropion tablet. The most common adverse effects are gastrointestinal (dry mouth, nausea, vomiting, constipation) and nervous system related (insomnia, anxiety, headache, dizziness). This therapy is contraindicated in patients with uncontrolled hypertension, seizure disorders and eating disorders.

Liraglutide is the representative of glucagon-like peptide one receptor (GLP-1R) agonists initially approved in 2010 for the treatment of type 2 diabetes. Weight loss and improved glycaemic control were often observed during clinical trials of liraglutide for the treatment of diabetes, which led to the approval of liraglutide for the treatment of obesity in December 2014. GLP-1 is a gut hormone secreted by the endocrine L-cells after food intake and presented

in the brain where it modulates CNS pathways involved in energy homeostasis. It suppresses glucagon production and stimulates pancreatic insulin secretion [16]. Liraglutide delays gastric emptying and increases satiety through stimulations of POMC neurons [33]. The recommended dose of liraglutide for weight management is 3 mg daily with the starting dose of 0.6 mg injected subcutaneously in the abdomen, thigh, or upper arm. It is well tolerated with gastrointestinal side effects, specifically nausea, which occurs in almost 40% of patients; vomiting and diarrhoea are also frequent. If weight loss is less than 4% after 4 months, the therapy should be discontinued.

2.2. Modulators of dietary absorption

One of the important factors contributing to the ongoing obesity epidemic is a Western diet rich in animal fat, where dietary fat intake provides >40% of the caloric content of daily food consumption. Although the energy is obtained through metabolism of protein and carbohydrates as well, the amount of energy gained from fat is almost twice that of other compounds, and subsequently, a fat-rich diet promotes obesity more rapidly [34]. Most of the dietary intake of fat is in the form of triacylglycerols, and their absorption is more than 95%. The triglycerides are composed of saturated or unsaturated long fatty acid chains. After consuming triacylglycerols, they undergo emulsification through the gastrointestinal tract and subsequently hydrolysis into diacylglycerol, monoacylglycerol and free fatty acids by pancreatic lipase. Incorporated into bile acid phospholipid micelles, they enter the circulation as chylomicrons. When they reach the membranes of hepatocytes, adipocytes, or muscle fibres, they can either be stored or oxidised for energy. An effective weight loss could be achieved by targeting the molecules participating in the absorption and digestion of fat. Pancreatic lipase is the key enzyme in this process, responsible for the catabolism of triacylglycerols to free fatty acids and monoacylglycerols. Inhibition of pancreatic lipase decreases the number of absorbed triglycerides by inhibition of the free fatty acids and monoglycerides production in the intestinal lumen [35, 36].

Orlistat is the tetrahydro derivative of lipstatin, a natural product of *Streptomyces toxytricini* [36]. It has been widely used for weight management in combination with reduced caloric diet since 1999. Orlistat works via the reduction in dietary fat hydrolysis and absorption in the gut by inhibition of gastric and pancreatic lipases. It prevents absorption of approximately 30% of fat, and it becomes effective when the fat content of patient's diet is at least 30% of their total dietary intake. Recommended dosing starts at 60 mg increasing to the full dose of 120 mg three times daily, administered during or up to 1 hour after the meal. The main side effect is steatorrhoea, followed by defecation urgency, diarrhoea, flatulence and abdominal pain. With the use of lipase inhibitors, there is a risk of inadequate absorption of the fat-soluble vitamins. For that reason, multivitamin tablets containing those vitamins should be taken 2 hours before or after the administration of orlistat.

2.3. Medications that increase energy expenditure

Inhibition of the sodium/glucose cotransporter (SGLT) 2 in renal tubules increases urinary glucose excretion [37]. SGLT2 inhibitors were initially designed to reduce hyperglycaemia in people with diabetes by competitive inhibition of SGLT2 transport system in the kidney. Since SGLT2 is essential for the glucose reabsorption, its inhibition led to reduced glucose reabsorption by the proximal tubule and increased glucose loss through the urine. However, glucose

reabsorption requires good glomerular filtration rate (GFR) and individuals with reduced GFR will probably experience little benefit from this class of drugs [38]. In obese patients with type 2 diabetes, 300 mg canagliflozin daily for 26 weeks resulted in weight loss of 3.3%, improved glycaemic control and lower blood pressure compared to placebo. However, there was a high incidence of genital and urinary tract infections [39]. In contrast, empagliflozin for type 2 diabetes showed impressive results concerning reduction of cardiovascular mortality along with kidney protective effects [40].

3. Translational research of obesity treatment

Translational research is needed to transfer basic science findings into novel therapeutical interventions. Obesity has become one of the most intensively studied diseases because of the increasing prevalence and the availability of suitable animal and cell culture models of adipocyte differentiation and appetite regulation that have permitted detailed studies impossible to conduct in other models.

3.1. Data derived from animal models of obesity

With the increased incidence of obesity, it is imperative that animal models sharing characteristics of human obesity serve in the quest for finding novel prevention and treatment approaches. Human obesity is considered to be polygenetic in addition to environmental influences. Animal models of obesity are therefore divided into different categories based on manipulations of individual genes, but genetically intact animals exposed to obesigenic environments were also used. Most animal models of obesity are small rodents (rats or mice), commonly the leptin-deficient *ob/ob* mouse, the leptin receptor-deficient *db/db* mouse and its rat counterparts.

Animals with a defect in the leptin-signalling pathway, including lack of leptin production and leptin resistance, develop a morbidly obese phenotype characterised by hyperphagia, reduced energy expenditure and hypothermia. A single-base spontaneous mutation of the *ob* gene terminates leptin synthesis prematurely, thus preventing the secretion of bioactive leptin. The transcription factor STAT3 is a key component of the signalling pathway that mediates leptin's effects on energy homeostasis. The amino acid tyrosine at position Ty 1138 plays a critical role in the activation of this pathway [41]. The specific replacement of the gene encoding the leptin receptor in homozygous *s/s* mice disrupts the transcription factor STAT3 [42]. The diabetic characteristics of *db/db* mice derive from a single autosomal recessive mutation, a Gly to Thr mutation in the leptin receptor gene on chromosome 4 (*Lepr^{db}*). The abnormal mRNA splicing leads to the subsequent production of a nonfunctional Ob-Rb protein and defective leptin receptor with the result of overproduction of extracellular leptin [43]. Homozygous mice are hyperphagic and obese, but they are fertile and less hyperglycaemic compared to the *db/db* mouse.

POMC is the precursor of the α -melanocyte-stimulating hormone (α MSH), a potent anorexiogenic neuropeptide that reduces eating and increases energy expenditure by activating MC3-R and MC4-R receptors. Transgenic mice lacking POMC (*POMC^{-/-}*) overeat and develop marked obesity that can be exaggerated by a high-fat diet [44], while heterozygous mutants develop

an intermediate phenotype. Treatment with α MSH or other agonists of the MC4 receptor can reduce obesity in POMC^{-/-} mice. The MC4 receptor subtype is involved in the control of food intake by mediating α MSH and AgRP influence on energy homeostasis. Specific inactivation of the MC4 receptor causes hyperphagia and morbid obesity [45]. MC4^{-/-} mice also have high levels of insulin, glucose and leptin and do not respond to AgRP or α MSH. The lethal yellow (Ay) mutation of the agouti gene leads to ectopic agouti expression. The homozygous expression is lethal while heterozygous offsprings are viable, but develop obesity within the first few months of life [46]. The obesity results from the ectopic AgRP expression and α -MSH antagonism at MC3 and MC4 receptors. The mice are prone to developing type II diabetes and are infertile.

Cholecystokinin (CCK) plays an important role in satiation as a stimulator of fat digestion, an effect mediated by CCK-1 receptors. The Otsuka-Long-Evans-Tokushima Fatty (OLETF) rat is a spontaneous CCK-1 receptor knockout model for studying dysregulated control of eating and obesity [47]. The obesity phenotype is relatively mild, and as a result of obesity, they develop diabetes with hyperglycaemia, polyuria and polydipsia by the end of 5 months. OLETF rats respond less to CCK-induced stimulation of pancreatic secretions due to the lack of functional CCK1 receptors in the exocrine pancreas. However, these rats can prevent obesity if they have access to a running wheel.

Many Sprague-Dawley rats become obese (drug-induced obesity, DIO) when exposed to a high-energy diet, whereas others have a body weight similar to that of control rats on a low-energy diet (diet resistant, DR). The exposure of animals to high-fat (HF) diet often results in the development of obesity due to reducing the central actions of insulin and leptin by a post-receptor effect [48]. Additionally, HF diet directly affects intracellular signalling pathways in hypothalamic target neurons resulting in changes in neuropeptide expression. Offspring of DIO dams are heavier and more obese than offspring of DR dams [49] and that obesity extends into adult life.

The GLUT4 glucose transporter is vital for glucose transport in adipose tissue stimulated by insulin. Transgenic mice overexpressing GLUT4 develop early-onset obesity with a marked increase in the number but not the size of fat cells. Therefore, the mice have been used to study fat cell replication and differentiation during the development of obesity [44]. Mice deficient in β 3-receptors are moderately obese [50] as a result of decreased activity of the sympathetic nervous system. Similarly, mice lacking functional serotonin 5-HT_{2C} receptors develop hyperphagia [51], which results in marked body weight gain and adiposity. NPY1R-deficient mice are suitable for the study of obesity in the absence of overeating because they develop obesity independently of an increase in eating, which seems to be caused by decreased energy expenditure [52] due to a reduced expression of the uncoupling protein type 2 (UCP2) in white fat tissue.

Receptor-interacting protein-140 (RIP140) is a nuclear hormone co-repressor, which regulates fat accumulation by interacting with oestrogen, thyroid hormone and retinoic acid receptors through 2C-terminal receptor-interacting domains (RIDs). Mice with global RIP140 knockout are lean and resistant to HF diet-induced obesity. Silencing RIP 140 in animal models results in a long-lasting weight loss and enhanced metabolic rate [53]. SMRT is another nuclear hormone receptor co-repressor. In genetically engineered mice, the disruption of the molecular interaction between SMRT and nuclear hormone receptors causes increased adiposity and a decreased metabolic rate [54].

The synthesis of triacylglycerols occurs in two steps, both catalysed by enzymes of the endoplasmic reticulum. The synthesis ends with the conversion of diacylglycerol to triacylglycerol, which is catalysed by the enzyme diglyceride acyltransferase (DGAT) [55]. DGAT has two isoforms, DGAT1 and DGAT2, highly expressed in liver and white adipose tissue. DGAT1-deficient mice demonstrated significantly reduced adipose mass and increased insulin sensitivity [56], indicating that pharmacological inhibition of DGAT1 may be a useful strategy for treating human obesity and type 2 diabetes. The DGAT1 inhibitor XP620 reduced apolipoprotein B secretion, triacylglycerol synthesis and dietary fat absorption in mice. Another DGAT1 inhibitor, compound 4a, caused weight loss and a reduction in liver triglycerides [57]. Acyl-CoA carboxylase-2 (ACC-2) has a regulatory role in fatty acid oxidation. An increased fat oxidation by ACC-2 inhibitors could be a potential future approach to maintain weight loss [58]. On the other hand, stearoyl-CoA desaturase-1 (SCD-1) is an important enzyme in the synthesis of monounsaturated fatty acids. Spontaneous and targeted deletion of SCD-1 reduces triacylglycerol and cholesterol esters in the liver and increases insulin sensitivity and energy expenditure, as well as diet-induced obesity in animals [59].

3.2. Data derived from cell models of obesity

The process of adipogenesis has been extensively studied since the 1970s [60]. The ability to study the transformation of fibroblasts into preadipocytes in a tissue culture has enabled the exploration of general cellular mechanisms. Different cell culture models and protocols have become available to study adipocyte biology and to illustrate the transcriptional cascade that promotes fat cell differentiation [61]. Mature adipocytes, mesenchymal stem cells and preadipocytes can be easily isolated from adipose tissue homogenates and used for research purposes. The advantages when using this model is a homogenous population of cells that are all in the same stage of differentiation and the ability to passage the cells. On the other hand, the molecular events representing adipogenesis in a cell line are not necessarily transferrable in a human preadipocyte, and the ability of a preadipocyte cell line to differentiate often falls with increasing passage number [62].

3.2.1. Animal cell models

Studies in animal models of obesity offer valuable insights, but their applicability to humans is limited by the existing differences in the physiology and metabolism [63]. The most commonly used animal cell models are murine preadipocytes. The advantage of animal cell models is that they can be derived from various locations and from animals of different ages, which give valuable information about depot- or age-dependent adipogenic or secretory mechanism [64]. However, these cell lines have a considerable triacylglycerol store that interferes with biochemical and microscopy analyses, and they depend on the genetics and conditions of the animals from which they are isolated.

The 3T3-L1 cell line is a well-established preadipose cell line derived from disaggregated 17- to 19-day-old Swiss 3T3 mouse embryos, which display a fibroblast-like morphology. Under appropriate conditions, such as treatment with adipogenic agents insulin, dexamethasone (DEX) and 3-isobutyl-1-methylxanthine (IBMX), they acquire an adipocyte-like phenotype.

These cells provide an equal response following treatments because they are homogeneous regarding the cell population [65]. Moreover, they are easier to culture, less costly and can tolerate an increased number of passages. The main goal of the research on 3T3-L1 cell lines is to establish the underlying molecular mechanisms of adipogenesis and to evaluate the effects of compounds or nutrients on adipogenesis to find the potential treatment of obesity [66]. It was found that compounds such as quercetin and resveratrol [67] inhibit adipogenesis in 3T3-L1 adipocytes. Additionally, these cells have been used to describe the effect of reactive oxygen species, antioxidants or melatonin on adipogenic differentiation [68]. Furthermore, different gene silencing techniques have been applied to study the function of various genes associated with adipogenesis in 3T3-L1 cells, particularly inflammatory pathways, adipokine synthesis and enzyme's function [69]. However, the adipogenic differentiation of 3T3-L1 cell line needs at least 2 weeks, and they require careful observation because when they become confluent, the differentiation into adipocytes cannot proceed.

The 3T3-F442A cell line is another important cell line derived from murine Swiss 3T3 cells and isolated from the third selection of clones that converts into bigger fat cell clusters capable of accumulating more fat than the 3T3-L1 cells and resistant to an early exposure to glucocorticoids in the matter of adipogenic differentiation [70]. Although significantly less than 3T3-L1, these cells have also been used to study the effects of different compounds and drugs on adipocyte differentiation. Additionally, gene silencing through siRNA has been carried out to study the role of alkaline phosphatase in lipid metabolism and gene expression and the secretion of adipokines.

OP9 cells are bone marrow-derived stromal cells that accumulate large triacylglycerol filled droplets only 3 days after adipogenic stimuli [71]. OP9 cells can be passaged for long periods of time and can differentiate into adipocytes even after reaching confluence. Furthermore, their rapid differentiation enables to detect protein expressed from transiently transfected DNA in fully differentiated adipocytes. This cell line has been used to evaluate the effects of different compounds on the adipogenesis process, especially the antiadipogenic activity of quercetin, and its effects on lipolysis [72]. Other studies investigated the inhibitory effects of *Pericarpium zanthoxyli* extract on the adipogenic differentiation of OP9 cells and the inhibition of adipogenesis in the OP9 cell line by ascorbic acid [73]. These cells are widely used to study the role of oxidative stress in the adipogenesis process, where it was shown that lipid uptake causes reactive oxygen species generation in OP9 preadipocytes.

The C3H10T1/2 cell line is extracted from 14- to 17-day-old C3H mouse embryonic stem cell precursors with the capacity to differentiate into mesodermal cell types such as adipocytes. The main research in this cell line is focused on investigating the molecular mechanisms related to adipogenic differentiation associated with obesity [74]. Additionally, a study of food contaminants was carried out on this cell line, finding that tributyltin, an endocrine disrupting compound, promotes adipogenic differentiation in vitro [75].

Primary mouse embryonic fibroblasts (MEFs) are derived from totipotent cells of early mouse mammalian embryos. What makes them different is their capability to differentiate into adipocytes with no need for pro-adipogenic transcription factors such as PPAR or C/EBP [76]. Their advantages are easy establishment and maintenance, rapid proliferation and producing

in large numbers. Nevertheless, because of the cellular heterogeneity of embryonic tissue, it is often difficult to culture them, and they reach biological ageing at passage 12. This cell line has been used to study mechanisms related to obesity such as genes or transcription factors, signalling pathways and obesity-associated (FTO) gene. In this sense, MEFs derived from FTO overexpressing mice had an increased potential for adipogenic differentiation, while MEFs derived from FTO knockout mice showed a reduced adipogenesis [77].

Porcine preadipocytes are highly similar to human cells and therefore a much better model for the study of adipogenesis and obesity-related diseases. The adipogenesis in porcine cell culture is shown in two steps: the recruitment of lipid-free preadipocytes and the stimulation of lipid growth in the recruited preadipocytes. Studies carried out on this cell line showed that phloretin enhances the lipid accumulation in a time-dependent manner [78], retinol-binding protein 4 (RBP-4) suppresses differentiation in porcine preadipocytes by decreasing the activation of insulin and that miR-125a promotes the differentiation of porcine preadipocytes upon inhibition [79]. In contrast, miR-199a promotes cell proliferation while attenuating the lipid deposition in porcine adipocytes. Furthermore, miR-181a overexpression represses the tumour necrosis factor in porcine preadipocytes [80].

3.2.2. Human cell models

Human cells have been rapidly developed and are gaining more importance as candidates for in vitro studies. They are derived from the human stromal vascular fraction, a mixture of stem cells, endothelial cells, preadipocytes and immunological cells. That is the reason why the obtained results are more reliable than those from animal models and find better applicability towards human diseases such as obesity.

Stromal vascular fraction (SVF) of adipose tissue contains adipose-derived stem cells (ADSCs), the multipotent cell population firstly discovered in the last century and remained a focus of many studies to characterise their nature, including their potential to differentiate into numerous cell types [81]. There are many reasons why ADSCs are suitable for conducting studies—multipotency, a high number of passages and high expansion capacity, the reflection of donor- and depot-specific characteristics and the possibility of being cryopreserved for long periods of time. Once differentiated into adipocytes, ADSCs display phenotypic characteristics of genuine adipocytes, the most important being responsive to hormones including insulin and adrenergic agonists. During last 5 years, ADSCs have been used to study the effect of different compounds on adipogenesis for the characterisation of molecules and cellular processes involved in adipogenesis [19] and to investigate the role of different genes associated with adipocyte metabolism. Finally, because of their capability to convert from white to brown adipocytes, this cell line has been used in the study of the differential effect of specific molecules, such as p53, in white and brown adipogenesis.

Preadipocytes emerged as an excellent model for the study of adipogenesis and fat cell biology. They can be easily obtained from adipose tissue and differentiate into mature adipocytes under appropriate conditions. Unlike ADSCs, which retain multilineage differentiation capacity, preadipocytes from the SVF are already committed to adipogenic differentiation. Human primary preadipocytes are an excellent model for the study of obesity-related alterations because they

reflect a situation close to that of adipose tissue due to the presence of depot-specific properties. Their reflection of donor characteristics makes them useful in studies assessing differences between individuals [82]. Moreover, human preadipocytes do not require extensive proliferation *in vitro* to differentiate and can successfully differentiate in serum-free conditions, which rules out possible effects of serum components on inhibition of adipogenesis. Preadipocyte adipogenic differentiation protocols are divided into an induction period characterised by the presence of insulin, IBMX, a PPAR-agonist or indomethacin, and a maintenance period. The induction period can be prolonged from three to 7 days, resulting in a significantly higher proportion of cells with adipocyte morphology and higher adipogenic marker expression. In the last years, human preadipocytes have been widely used for the characterisation of regulatory molecules during the adipogenic differentiation process [83]. Finally, ADSCs have been used to validate findings from animal adipocyte models.

4. Antiobesity drugs in the pipeline

Current therapeutic approaches often fail to achieve clinical efficacy or have adverse effect profiles that limit their use. This unmet need for efficient and safe antiobesity medication resulted in the investigation of some new monotherapies or combination therapies (**Table 1**) [84]. Targeting of endogenous endocrine circuits regulating energy homeostasis is a mechanism shared by many antiobesity drugs under development. Most of these endocrine circuits are anorexigenic, starting with the postprandial release of peripheral peptide hormones. The receptors for peptide hormones are expressed in the dorsal vagal complex in the medulla and nucleus arcuatus of the hypothalamus. Some of the medications act through direct binding in CNS, other affect peripheral tissues, and some therapies act both centrally and peripherally.

The history of antiobesity drugs has seen the fall of many pharmaceutical agents that were highly effective yet ultimately dangerous. This is why the research has given up to find a magic pill and turned to the goal of providing a safe and effective drug regimen that will achieve a sustainable reduction in body weight in combination with exercise and improved diet habits.

4.1. Central targets

Melanocortins regulate energy balance through the MCR3 and MCR4 receptors and mediate the effects of leptin in the central nervous system. That is why some MCR4 agonists have been under development. The first generation failed because of increased blood pressure or lack of efficacy [85]. In contrast, the injectable MCR4 reached phase 1/2 trials in obese humans (US), demonstrating increased resting energy expenditure and a weight loss ranging from 2.5 to 4.8% after 12 weeks without any adverse cardiovascular effects.

Neuropeptide Y participates in weight determination by stimulating the creation of new fat cells. It stimulates food intake and reduces energy expenditure by activating NPY receptors Y1 and Y5 present in the hypothalamus. The idea for some of the new therapies was to target the NPY pathway in the hypothalamus, but it has shown less promising results. One of those

| Name | Type of agent | Current status |
|----------------------|--|---------------------|
| Velneperit | NPY antagonist | Phase II, abandoned |
| Tesofensine | 5-HT/DA/NA reuptake blocker | Phase III |
| Bupropion/zonisamide | Antidepressant/anticonvulsant | Phase II |
| Cetilistat | Lipase inhibitor | Phase III |
| PAZ-320 | Carbohydrate hydrolysing inhibitor | Phase III |
| Beloranib | Methionine aminopeptidase-2 | Phase II, abandoned |
| ALSL1023 | matrix metalloproteinase inhibitors | Phase II |
| IONIS-FGFR4Rx | Fibroblast growth factor receptor 4 inhibitors | Phase II |
| MB-11055 | AMP-activated protein kinase activators | Phase II |
| JNJ-16269110 | MTP inhibitor | Phase II |
| Resveratrol | SIRT1 activator | Phase II |
| Langlenatide | GLP1 agonist | Phase II |
| Semaglutide | | Phase III |

Table 1. Current status of antiobesity drugs under development.

medications was Velneperit that prevented the binding of NPY to the receptors, decreasing hunger and controlling energy balance. In one study on 656 patients, 800 mg Velneperit showed an average loss of 3.8 kg compared to the placebo group and higher than 5% loss of body weight in 35% of all patients. Despite preliminary reports, Velneperit was discontinued in 2013 after phase 2 data demonstrated no clinically significant benefit over placebo [86].

Modulators of monoamine neurotransmitters (dopamine, serotonin and norepinephrine) can suppress appetite efficiently by enhancing POMC neuronal activity [87]. However, a major concern for the use of this therapy is the risk of adverse cardiovascular events and psychiatric morbidity. Tesofensine is the noradrenalin/serotonin/dopamine reuptake inhibitor initially developed for Alzheimer’s and Parkinson’s disease and currently under development for therapy of obesity. It primarily not only acts by suppressing appetite, but possibly also works by increasing thermogenesis. Tesofensine has completed phase 1 and showed promising levels of weight loss after 24 weeks compared with placebo, in addition to improved glucose and lipid metabolism with reduced waist circumference. It is currently in advanced phase 3 testing and is yet to enter confirmatory phase. It was well tolerated with side effects such as dry mouth, dizziness, insomnia and gastrointestinal disorders, but has recently been under inspection for serious side effects such as elevated heart rate and blood pressure [88].

Bupropion/zonisamide SR is a combination of the antidepressant bupropion and the anti-convulsant zonisamide. Bupropion reduces weight by the dopamine/norepinephrine reuptake inhibition. Increase in the levels of dopamine decreases appetite. Zonisamide is the anticonvulsant GABA-receptor agonist, and it is believed that the mechanism for inducing weight loss includes modulation of the sodium channel, carbonic anhydrase inhibition and

enhancement of dopamine and serotonin transmission. This medication completed phase 2 in approximately 1500 participants with the result of weight loss up to 15% after 48 weeks, along with the improved cardiometabolic risk markers such as triglycerides levels, blood pressure, waist circumference and insulin sensitivity [57]. The most common side effects were nausea, headache and insomnia. The drug is now in the process of initiating phase 3 clinical trial with the fixed-dose combination.

4.2. Peripheral targets

Pancreatic lipase is a key enzyme that causes breakdown of triglycerides into free fatty acids. Inhibition of pancreatic lipase reduces this conversion and the absorption of free fatty acids in the intestine, resulting in increased excretion of triglycerides in the urine. Cetilistat is a pancreatic lipase inhibitor with the similar mechanism of action to orlistat. During phase 2, it was as effective as orlistat but showed superior safety profile with less gastrointestinal side effects [89]. A significant reduction in body weight, LDL and haemoglobin A1c (HbA1c) levels was also noted. Cetilistat has completed phase 1 in Europe and in the USA and has now undergone phase 3 clinical trial.

Carbohydrate hydrolysing inhibitor PAZ-320 works by blocking the enzymatic breakdown of complex carbohydrates into simple sugars, resulting in the reduction in the intestinal absorption of glucose, fructose and other monosaccharides. It is now undergoing phase 3 clinical trial for type 2 diabetes and obesity.

Expansion of adipose tissue requires continuous remodelling of capillary networks. Since obesity involves the pathological formation of angiogenetic vessels, inhibiting angiogenesis could be a novel treatment target [90]. Methionine aminopeptidase-2 (MetAP2) inhibitors hold antiangiogenetic properties and were primarily used as a therapy for solid tumours. During these trials, they have been demonstrated to effectively lower bodyweight, not just by inhibiting angiogenesis, but via converting stored fats into useful energy and reducing the production of new fatty acid molecules. Beloranib caused dramatic weight loss compared with placebo, along with the improved cardiometabolic risk markers, including circulating lipids, waist circumference and glycaemic control. The development of this drug was terminated during phase 2 due to increased incidence of thromboembolic events.

Matrix metalloproteinases (MMP) are endoproteinases that break down extracellular matrix, thus participating in tissue remodelling and angiogenesis. High levels of MMP are associated with obesity and cardiovascular diseases. The target of the oral angiogenesis and MMP inhibitor ALSL1023 is visceral fat, which is closely linked to the metabolic risks of obesity [91]. In a phase 2 study on 126 patients, ALSL1023 demonstrated 15% reduced visceral fat after 12 weeks of daily oral administration of 1200 mg, and the effect was superior to placebo with no significant adverse events.

Fibroblast growth factor receptor 4 (FGFR4) is involved in the regulation of fat storage, energy expenditure and overall body weight. It is highly expressed in the liver and other peripheral tissues. The antisense oligonucleotide IONIS-FGFR4Rx lowers the production of FGFR4 [92]. It significantly suppressed liver FGFR4 expression in obese mice after 10 weeks which

was accompanied by a 20% placebo-subtracted weight loss in addition to improved glucose metabolism and lipid levels. The drug distributes to peripheral tissues but poorly enters heart muscle and brain, hopefully avoiding cardiac and CNS side effects. The phase 2 trial for IONIS-FGFR4Rx has been initiated along with the development of four other antisense drugs.

AMP-activated protein kinase (AMPK) is activated by cellular stress such as exercise, when the depletion of cellular ATP levels leads to a concomitant rise in AMP. AMPK activation in liver, muscle and fat tissues increases oxidation of glucose, fatty acids and triglycerides and decreases their storage by switching to catalytic processes [93]. Activation of AMPK by pharmacological means is, therefore, a potential way of reversing the metabolic abnormalities of obesity. The plant-derived AMPK activator resveratrol mimic the beneficial metabolic effects of energy restriction. The AMPK activator MB-11055 is currently in phase 2 trial for obesity.

A long-acting β_2 -adrenergic receptor agonist, salmeterol xinafoate, is administered via series of multiple subcutaneous injections where it reduces the fat by increased lipolysis. In 54 healthy adults after 4 weeks, it reduced abdominal circumference and skin-fold thickness. It is currently in phase 2 trial in 160 obese subjects.

Microsomal triglyceride transfer protein (MTP) is an essential chaperone highly expressed in the intestine and liver that transfers several lipids including triacylglycerols and phospholipids. The triacylglycerol transfer activity of MTP was used to identify several potent antagonists as a potential therapeutic approach for obesity and hyperlipidaemia. However, MTP inhibition results in triacylglycerol build-up and gastrointestinal disturbances, but more important leads to steatosis and increases levels of transaminases [94]. SLx-4090 is a compound currently in phase 2 clinical trial. In 24 patients with dyslipidaemia, this drug demonstrated reductions in postprandial triacylglycerols and clinically significant weight reduction with no effect on liver. Another selective intestinal MTP inhibitor, JNJ-16269110, showed dose-related weight loss reduction after 12 weeks in 321 nondiabetic obese subjects.

Sirtuins (silent information regulator 1 proteins, SIRT1) participate in critical pathways such as lipid metabolism and insulin secretion. SIRT1 suppresses the expression of the nuclear receptor peroxisome proliferator-activated receptor- γ (PPAR γ) that is responsible for fat storage. Resveratrol is an allosteric activator of SIRT1 that increases the mitochondrial activity in brown adipose tissue and skeletal muscle. Besides resveratrol, several other sirtuin activators are in early clinical development. Phase 2 study for SRT2104 gave optimistic data on the metabolic profile of elderly patients [95].

4.3. Agents acting through central and peripheral mechanisms

Since the approval of liraglutide, several long-acting GLP-1 receptor analogues are being evaluated with the goal to provide a more stable blood profile of the peptide and avoid the peak levels more likely to cause side effects, in particular, nausea [96]. Langelanotide is intended for weekly/monthly subcutaneous administration, which achieved placebo-subtracted body weight loss of 7.3 kg in a phase 2 trial in 297 obese patients. Semaglutide has completed a phase 3 trial in 632 obese patients with type 2 diabetes, where a daily intake of 40 mg semaglutide for 26 weeks resulted in improved glycaemic control and placebo-subtracted bodyweight reductions of 5.7 kg.

Glucagon increases resting energy expenditure by inducing thermogenesis [97]. This effect in combination with GLP-1-mediated reduced appetite has demonstrated interesting results in preclinical trials. The natural gut hormone oxyntomodulin (OXM) acts at both the GLP-1 and glucagon receptor. The crucial step in developing OXM analogues is to identify the ratio of GLP-1/glucagon receptor co-agonism with maximal weight loss and without impairing glucose tolerance. Six agonist agents are in phase 1 trial, and at least one agent is in phase 2 for diabetes and obesity.

Although leptin replacement successfully treats obesity caused by congenital leptin deficiency, results are still disappointing in obese patients because of leptin resistance in obesity. Some molecules may re-establish responsiveness to leptin. Different combinations of leptin analogues and other weight loss agents are now in preclinical development [98].

5. Challenges in obesity drug development

Due to the unmet need for the right medication, growing of the antiobesity market created the clinical development of several promising weight loss therapies. When choosing a weight loss drugs, several things, such as safety, cost and effectiveness, must be equally considered. Several challenges need to be overcome to achieve substantial weight loss. Improved medications are needed to help reduce costs of obesity to society. These new pharmacological agents will either replace the existing ones or be used in combination with them. The advantage of combinational therapy is the need for lower doses of the individual drug, which lowers the rate of side effects and suppression of counter-regulation and increases the weight loss effect. Like any other chronic condition, polytherapeutic and long-term treatment strategies are required to achieve the sustained efficacy of weight loss therapies, although the probability of maintaining weight loss is low with an available drugs [77].

A significant challenge for the development of new antiobesity drugs is the different clinical efficacy criteria by FDA and EMA. For the FDA, the placebo-subtracted weight loss should be 5%, or that amount of weight loss from the baseline of body weight must be achieved in 35% of patients. Both those differences between drug and placebo effects must be statistically significant. EMA puts the accent on weight loss from baseline as more clinically relevant than the weight loss compared to placebo, so the primary criterion is 10% weight reduction regarding to baseline. Another factor that discourages pharmaceutical industry in obesity research is the reluctance of medical insurers to pay for antiobesity drugs, which directly affect the sales of new drugs. An additional reason for drug sales to be disappointing is the expectations of prescribers and patients [99]. Current antiobesity drugs can produce modest weight loss of 2–9 kg, which is not satisfactory to the majority of obese patients [100]. They end up discontinuing medication to avoid the cost and side effects connected to the potential lifelong treatment. The major problem with stopping the medication is weight loss maintenance because patients can weight more than before the intervention [101]. From this fact, it is evident that weight reduction of 5–10% will not be enough for many patients and physicians—any new entrant to the obesity market will be considered clinically effective only if the reduction in body weight will be 10–15%.

Now that most drugs target endogenous pathways regulating energy homeostasis, the off-target effects are hard to avoid. The withdrawal of several previously marketed antiobesity medications encouraged the regulatory authorities to require pre-specified safety data for all antiobesity drugs. Monitoring of adverse cardiovascular and psychiatric effects is of particular importance for drugs modulating the monoamine system. Additionally, some of the peripherally acting drugs work in multiple tissues by poorly understood mechanisms, which can cause the appearance of unexpected adverse effects. Besides acceptable safety profile, an antiobesity drug must meet minimum efficiency criteria to be considered relevant. Those criteria apply not only to weight loss but also to beneficial effects on the comorbidities such as diabetes type 2, cardiovascular disease and NASH [102]. In the end, treatment must be effective in the long term to sustain weight loss, which can be limited by receptor desensitisation and tachyphylaxis [103]. The progression of active weight loss to its maintenance will cause retention of patients to antiobesity drug especially when the patient has to contribute to the cost of the drug. All these unmet needs will have to be covered by any new medication entering the market to have a chance of being the commercial success.

6. Conclusion

Given the increasing prevalence of obesity worldwide, researchers are putting a lot of effort to find new therapeutical strategies for treatment of the disease and its prevention. Rising understanding of the body weight control and the molecules participating in this process lead to the breakthrough of potential novel therapeutic opportunities. Also, genome-wide studies are developing to give insights and answer to a lot of remaining questions. Furthermore, recently established clinical guidelines and increased number of monotherapy and combination medications enabled an individual approach to different types of obesity. However, there is still a long way to win a battle against obesity by pharmaceutical means. As for any other chronic condition, there is a small chance that super drug will ever appear to resolve obesity. Thus, we must focus on individually tailored approach with the accent on the metabolic, genetic and molecular background.

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