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COMPREHENSIVE REVIEW

Anti-obesity peptides from food: Production, evaluation, sources, and commercialization

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Abstract

The global obesity epidemic has heightened interest in natural solutions, with anti-obesity peptides emerging as promising candidates. Derived from food sources such as plants, algae, marine organisms, and products like milk and eggs, these peptides combat obesity through various mechanisms but face challenges in production and scalability. The aim of this review is to explore their sources, mechanisms, measurement, and synthesis methods, including innovative approaches such as de novo synthesis, proteomics, and bioinformatics. Its unique contribution lies in critically analyzing the current state of research while highlighting novel synthesis techniques and their practical relevance in addressing commercialization challenges, offering valuable insights for advancing anti-obesity peptide development. Diverse methods for assessing the anti-obesity properties of these peptides are discussed, encompassing both in vitro and in vivo experimental approaches, as well as emerging alternatives. The review also explores the integration of cutting-edge technologies in peptide synthesis with the potential to revolutionize scalability and cost-effectiveness. Key findings assert that despite the great potential of peptides from various food sources to fight against obesity and advances in their identification and analysis, challenges like scalability, regulatory hurdles, bioavailability issues, high production costs, and consumer appeal persist. Future research should explore the use of bioinformatics tools and advanced peptide screening technologies to identify and design peptides with enhanced efficacy and bioavailability, efficient and cost-effective extraction and purification methods, sustainable practices such as utilizing byproducts from the food industry, and the efficacy of products containing isolated anti-obesity peptides versus whole materials in clinical settings.

KEYWORDS
anti-obesity assays, de novo methods, enzymatic hydrolysis, obesity, peptides

1 | INTRODUCTION

Obesity has emerged as one of the most critical public health challenges globally, marked by the excessive accumulation of body fat that significantly elevates health risks. The global prevalence of obesity has nearly tripled since 1975, with over 650 million adults classified as obese by 2016 (Suryaningtyas & Je, 2023). This alarming trend imposes a substantial burden on healthcare systems worldwide, as obesity is closely associated with numerous comorbidities, including diabetes and cardiovascular diseases (CVDs) (Wang, Xiang, et al., 2022). Obesity arises from multifactorial causes, involving intricate interactions among genetic, hormonal, and environmental factors. Although genetic predisposition contributes to individual susceptibility, environmental factors like lifestyle, dietary habits, and other external influences play a more substantial role in its development (Chen, Wang, et al., 2024). The complexity of obesity, driven by genetic, environmental, and behavioral factors, makes its management particularly challenging (Xu et al., 2023).

Obesity treatment typically involves lifestyle modifications, pharmacotherapy, or surgical interventions. However, sustaining weight loss remains a significant challenge due to central dysregulation of appetite and metabolic counter-regulation (Aguiar et al., 2024). Given the limitations and side effects of current treatments, there is an increasing focus on natural bioactive compounds as safer and more effective alternatives with promising anti-obesity properties (Singh et al., 2022). In recent years, bioactive peptides derived from natural sources have garnered significant attention as potential anti-obesity agents. These peptides, composed of short chains of amino acids, are naturally present in various foods, including milk, eggs, fish, and plants (Chelliah et al., 2021). Their mechanisms include suppressing appetite, regulating fat absorption, modulating metabolic pathways to improve energy balance, and promoting satiety, all of which contribute to effective weight management (Suryaningtyas et al., 2025). Anti-obesity peptides can be obtained through conventional methods, such as fermentation, chemical and enzymatic hydrolysis, or green technologies like subcritical water extraction. Advanced techniques, including de novo synthesis, proteomic analysis, and bioinformatics, further aid in their design, optimization, and functional characterization. These approaches underscore the practical potential of food-derived peptides as natural solutions for managing obesity, with applications in functional foods and dietary supplements. They are released during digestion or through enzymatic hydrolysis and have been shown to exhibit a wide range of biological activities, such as anti-obesity, antioxidant, antimicrobial, antihypertensive, and anti-inflammatory effects (Wang, Yang, et al., 2022).

This review offers a thorough examination of anti-obesity peptides acquired from a diverse array of food sources, including plants, algae, marine organisms, terrestrial species, and products like milk and eggs. The main objective is to analyze the potential of food-derived peptides for obesity management by investigating their synthesis, evaluation methods, sources, and commercialization. It explores the synthesis of these peptides, emphasizing innovative methods that utilize proteomics and bioinformatics, alongside traditional techniques such as enzymatic hydrolysis and de novo synthesis. The review assesses various strategies for evaluating the anti-obesity effects of these peptides, encompassing in vitro and in vivo techniques, as well as emerging methodologies. Additionally, it investigates the commercialization potential of these peptides, considering factors like scalability, regulatory challenges, and market viability. The article concludes with a critical summary of current research advancements and outlines future directions for developing food-derived peptides as sustainable and natural solutions for obesity management.

In preparing this review, a systematic search of the literature was conducted using different online databases such as Google Scholar and Science Direct, focusing on studies published particularly in the last decade. Keywords such as "anti-obesity peptides," "bioactive compounds from food," and "peptide synthesis" were used to identify relevant articles. Each study was meticulously evaluated for its findings, with priority given to those providing experimental insights into peptide synthesis, bioactivity evaluation, and commercialization feasibility. Studies lacking sufficient experimental detail or relevance to food-derived peptides were excluded.

2 | MECHANISMS OF OBESITY

This section provides an overview of key mechanisms of obesity, including energy homeostasis, metabolic regulation, adipogenesis, lipid metabolism, inflammation, genetic and epigenetic influences, and the gut–brain axis. While the primary focus here is on food and nutrition, a concise explanation of these mechanisms is provided to contextualize the underlying factors contributing to obesity. An overview of obesity mechanisms in the human body is illustrated in Figure 1.

2.1 | Energy homeostasis and metabolic regulation

Energy balance represents the equilibrium between the energy acquired (through food and drink) and the energy

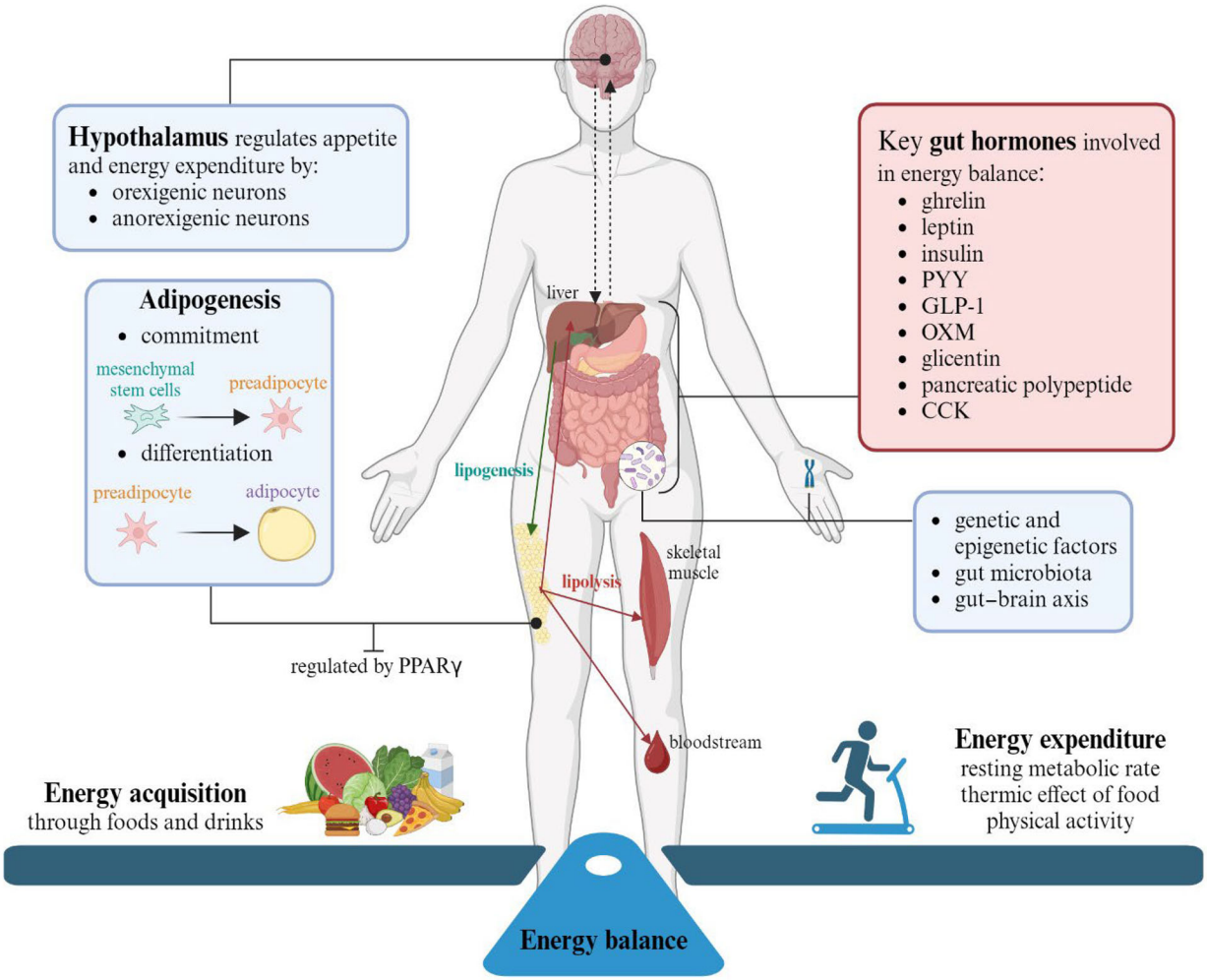

FIGURE 1 An overview of mechanisms of obesity in the human body.

expended (through metabolic processes and physical activity). Achieving stable body weight hinges on maintaining this delicate balance over time. The type and quantity of food consumed directly impact energy intake. On the other hand, energy expenditure includes resting metabolic rate (RMR) (the energy needed for basic bodily functions at rest), the thermic effect of food (the energy cost of digesting, absorbing, and metabolizing food), and physical activity (Wali et al., 2025). This balance is regulated by intricate mechanisms involving the central nervous system, peripheral tissues, and endocrine signals (Hall et al., 2022).

The hypothalamus, particularly the arcuate nucleus in the mediobasal hypothalamus, plays a central role in regulating appetite and energy expenditure by modulating appetite, fat storage, and energy consumption based on peripheral signals. Two distinct neuron populations in the arcuate nucleus, namely orexigenic (appetite promoters) and anorexigenic (appetite suppressers), control food intake by responding to metabolic hormones (e.g., leptin, insulin, and ghrelin) and nutrients (Dimitri, 2022). Key gut hormones involved in energy balance include ghrelin, peptide YY, glucagon-like peptide 1 (GLP-1), oxyntomodulin, glicentin, pancreatic polypeptide, amylin, and cholecystokinin (CCK). Ghrelin is the only one that is orexigenic, whereas the others possess anorexigenic characteristics (Koliaki et al., 2020). Circulating metabolic molecules are either short-acting (e.g., amino acids, fatty acids, gastrointestinal peptide hormones) or long-acting (e.g., leptin, insulin). The former regulate food intake in the short term, while the latter reflect fat storage and glucose levels, sending peripheral energy signals to the central nervous system to control energy homeostasis. Leptin, secreted by adipocytes in proportion to fat mass, and insulin, secreted by pancreatic beta cells in response to glucose levels, activate specific neurons to regulate calorie intake and energy consumption (Chen, Xiao, et al., 2022). The network formed by central neurons and peripheral metabolic signals is essential for regulating energy homeostasis. In obesity, central neurons often become resistant to these signals, leading to insulin and leptin resistance.

Insulin, released by pancreatic β cells, is essential for glucose and lipid homeostasis (Li, Chi, et al., 2022). Insulin resistance, a reduced responsiveness of cells to insulin, can lead to elevated blood sugar levels and is influenced by hormonal imbalances, inflammation, and cellular dysfunction. It arises from a combination of endocrine, inflammatory, neural, and intracellular mechanisms (Tong et al., 2022) and is linked to lifestyle factors, including diet (Bruckner et al., 2024). Leptin, produced by adipose tissue, regulates appetite, metabolism, and sexual maturation. Obese individuals often exhibit high leptin levels, suggesting resistance to its appetite-suppressing and metabolic effects. There are positive correlations between plasma leptin levels and body fat percentage (Obradovic et al., 2021). Leptin resistance, characterized by the hormone's inability to inhibit energy intake and increase energy expenditure, contributes to overeating and reduced satiety, leading to increased total body mass. Despite this, activating the leptin receptor and its signaling pathway in the brain might still make leptin a viable option for weight loss (Izquierdo et al., 2019). Other hormones, such as ghrelin (Marzullo et al., 2004), peptide YY (Tan et al., 2021), CCK (Miller et al., 2021), adiponectin (Achari & Jain, 2017), and GLP-1 (Temporelli, 2024), also regulate energy balance and body weight. Glucagon-like peptide-1 receptor (GLP-1R) agonists, used in peptide-based drugs, help suppress appetite, delay gastric emptying, and boost insulin secretion. However, in obese individuals, both fasting GLP-1 levels and nutrient-stimulated secretion are lower compared to lean individuals (Koliaki et al., 2020).

2.2 | Adipogenesis and lipid metabolism

Obesity is influenced by adipogenesis and lipid metabolism, which control fat cell creation and lipid storage in the body. Understanding these processes is essential for developing effective approaches to combat obesity and its associated health issues. Adipogenesis involves the differentiation of preadipocytes into mature adipocytes, responsible for storing fat. This process includes commitment, where mesenchymal stem cells become preadipocytes, and differentiation, where preadipocytes become mature adipocytes. Transcription factors involved in this process such as peroxisome proliferator-activated receptor gamma (PPARγ) orchestrate the expression of genes necessary for lipid uptake, storage, and insulin sensitivity (Jakab et al., 2021).

Lipid metabolism includes lipogenesis (synthesis of fatty acids and their storage as triglycerides) and lipolysis (breakdown of triglycerides into free fatty acids (FFAs) and glycerol for energy). Insulin regulates lipogenesis by enhancing glucose uptake and providing substrates for fatty acid synthesis, involving enzymes like acetyl-CoA carboxylase (ACC) and fatty acid synthase (FAS) (Saponaro et al., 2015). Lipolysis, stimulated by catecholamines (Takeuchi et al., 2021) and inhibited by insulin (Sancar et al., 2022), involves enzymes like hormone-sensitive lipase and adipose triglyceride lipase (Trites & Clugston, 2019).

In obesity, the balance between adipogenesis and lipid metabolism is disrupted, leading to excessive fat accumulation and adipocyte dysfunction. Increased adipogenesis results in more and larger adipocytes, which are less responsive to insulin and more prone to inflammation, contributing to metabolic dysregulation (Ghaben & Scherer, 2019). Obesity disrupts lipid metabolism by increasing lipogenesis, the process of fat synthesis and storage, while simultaneously decreasing lipolysis, the breakdown of stored fat (Sekar & Thirumurugan, 2022), which brings about the accumulation of excess fat in adipocytes. Hyperinsulinemia exacerbates this condition by stimulating lipogenesis and suppressing lipolysis, leading to increased fat storage and reduced fat utilization (Bu et al., 2018). Additionally, dysfunctional adipocytes release pro-inflammatory cytokines and FFAs, which impair insulin signaling and contribute to chronic inflammation and metabolic disturbances (Kim et al., 2017). This further deteriorates the overall metabolic profile in obese individuals.

Dysregulation of cholesterol metabolism, crucial for cellular function and overall metabolic health, contributes to obesity-related complications like CVD (Duan et al., 2022). Cholesterol metabolism involves synthesis, absorption, transport, and excretion of cholesterol (Röhrl & Stangl, 2018). Obesity leads to dyslipidemia, characterized by abnormal lipid levels in the blood (Klop et al., 2013), increased hepatic cholesterol synthesis (Röhrl & Stangl, 2018), elevated levels of low-density lipoprotein cholesterol (LDL-C) and triglycerides, and reduced levels of high-density lipoprotein cholesterol (HDL-C), which contribute to atherosclerosis and CVD (Klop et al., 2013). In obesity, HDL functionality is often compromised, impairing reverse cholesterol transport and exacerbating lipid accumulation and atherosclerotic risk (Lecerf & De Lorgeril, 2011). Therefore, understanding adipogenesis, lipid metabolism, and their interactions is crucial for addressing obesity-related health issues.

2.3 | Other factors

Genetic factors can predispose individuals to obesity through variants in genes related to appetite regulation, energy expenditure, adipogenesis, and lipid metabolism. For example, the fat mass and obesity-associated

protein (*FTO*) gene is strongly linked to energy and lipid metabolism through its regulatory effects on genes such as *IRX3* and *IRX5*. Variants in the *MC4R* gene influence appetite and satiety, contributing to a heightened risk of weight gain. Together, these genes, along with others involved in adipogenesis, lipid metabolism, and energy regulation, impact a range of physiological pathways that influence fat cell development, energy storage, and metabolic processes related to obesity (Keller et al., 2023). However, environmental factors, such as lifestyle choices and eating habits, play a more significant role. Epigenetic modifications, including deoxyribonucleic acid (DNA) methylation, histone modification, and noncoding ribonucleic acid (RNA), also regulate genes involved in metabolism and adipogenesis (Chen, Wang, et al., 2024). In addition, gut microbiota impacts energy balance and metabolism. It helps extract calories by breaking down complex carbohydrates and dietary fibers, releasing short-chain fatty acids as an energy source. Obesogenic bacteria, including *Ruminococcus*, *Clostridium*, and *Lactococcus*, contribute to obesity by producing these fatty acids and causing low-grade inflammation, which increases fat storage and insulin resistance. Conversely, beneficial bacteria like *Bifidobacterium* and *Akkermansia muciniphila* support weight loss and metabolic health by enhancing gut barrier function, reducing inflammation, and improving insulin sensitivity (Islam et al., 2023). The gut–brain axis, involving neural, hormonal, and immune pathways, also regulates appetite and energy balance (Jia et al., 2023).

3 | PRODUCTION OF ANTI-OBESITY PEPTIDES

Peptides with bioactive properties, such as anti-obesity effects, can be produced using conventional or de novo methods. Conventional methods involve breaking down protein chains through fermentation, chemical hydrolysis, enzymatic hydrolysis, and subcritical water hydrolysis, sometimes enhanced with treatments like ultrasound or microwave. These processes yield hydrolysates containing whole proteins, peptides of varying sizes, and free amino acids. Further purification using membrane filtration and/or chromatographic methods yields more homogeneous peptide fractions. De novo methods include design and analysis (computational and experimental approaches to create and optimize peptide sequences) and synthesis (chemical and biological methods to construct these peptides) techniques. This section provides an overview of both conventional and de novo peptide generation methods (Figure 2). These methods are crucial for developing innovative strategies to harness food-derived bioactive peptides to combat obesity. This aligns with the review's objective to explore advancements in peptide production and their potential applications in functional foods and dietary interventions.

3.1 | Conventional methods

3.1.1 | Fermentation

Microbial production through the fermentation of proteins represents a promising approach to producing bioactive peptides with diverse biological activities and applications in the food and nutraceutical industries (Arteaga et al., 2022). Microorganisms cultured on a medium containing protein substrate produce proteolytic enzymes that break down peptide chains. The extent of hydrolysis depends on factors such as fermentation time, microbial species, protein concentration, and peptide yield. While *Lactobacillus* species are primarily used for peptide generation through fermentation, other species may also be cultured alongside to enhance hydrolysis efficiency (Khakhariya et al., 2023). Bioactive peptides from fermented protein substrates offer various health benefits (Nasri et al., 2022). Manzanarez-Quin et al. (2023) found that peptides in milk fermented with *Limosilactobacillus fermentum* have anti-obesity properties by inhibiting pancreatic lipase activity and lipid accumulation in a 3T3-L1 cell line. Furthermore, Tiss et al. (2020) reported that soy milk fermented with kefir grains inhibits pancreatic lipase and α-amylase activities, leading to lower LDL-C and total cholesterol (TC) levels, higher HDL-C levels, and decreased body weight in rats. Nevertheless, the scalability of fermentation for industrial applications remains a challenge, as does consumer acceptance and bioavailability of the peptides in final products. Therefore, while promising, the application of fermentation for producing bioactive peptides needs further optimization and validation. Additionally, the use of genetically modified microorganisms offers promising potential for enhancing peptide yields and tailoring anti-obesity properties, paving the way for scalable production.

3.1.2 | Chemical hydrolysis

Chemical hydrolysis breaks down proteins using acids like hydrochloric acid (Ashaolu, 2020) or bases such as calcium, sodium, or potassium hydroxide (Álvarez-Viñas et al., 2020). Despite being cost-effective and rapid, chemical hydrolysis lacks precise control over hydrolysate consistency and leads to variations in amino acid profiles due to nonspecific hydrolysis (Siddik et al., 2021). No

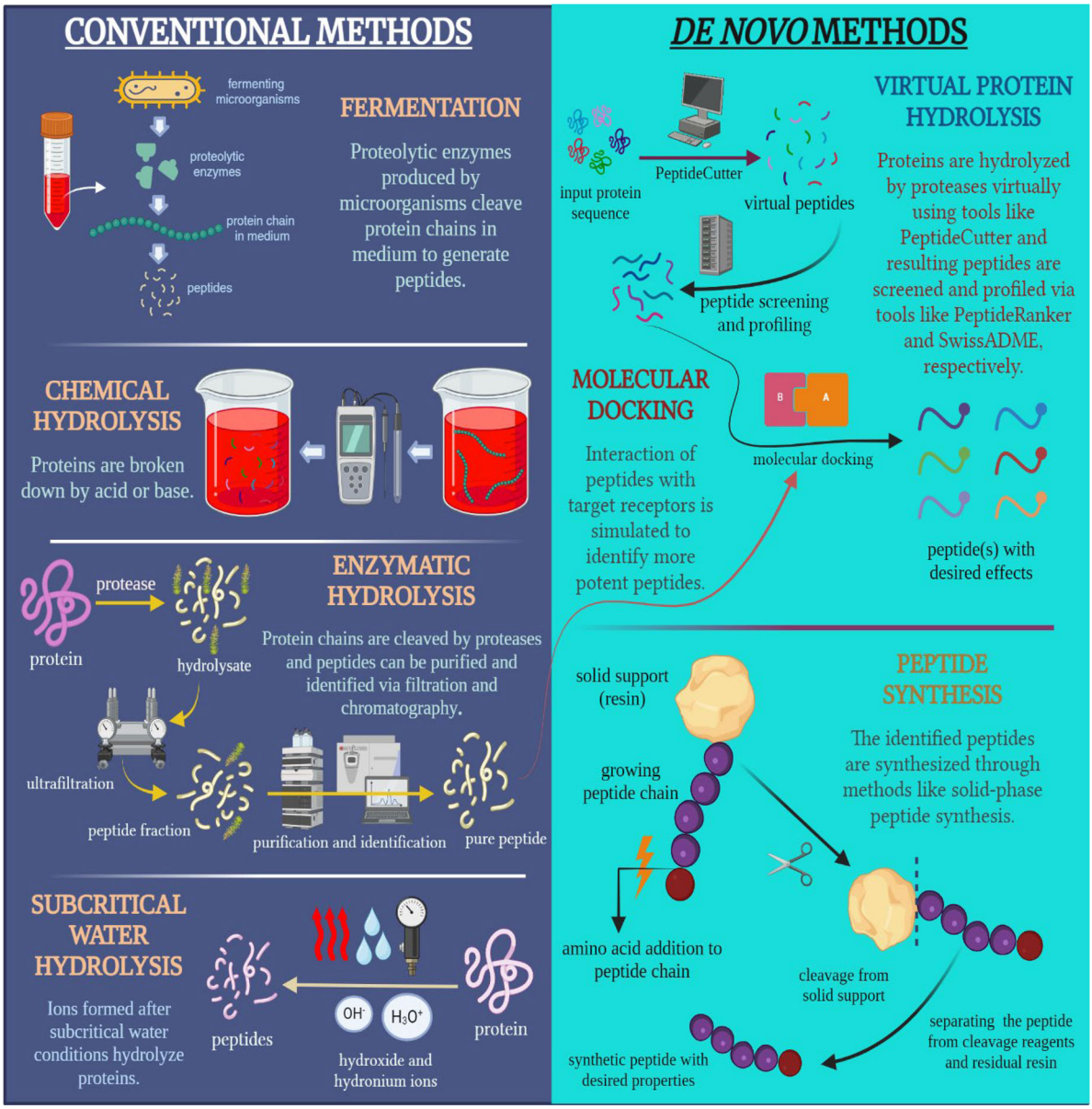

FIGURE 2 Conventional and de novo methods for production of anti-obesity peptides.

published articles were found on the anti-obesity effects of peptides from chemical hydrolysis, presenting an area for future research. For example, this method can be applied to obtain anti-obesity peptides from algae by breaking the rigid cell walls. However, the unfavorable pH levels and potential destruction of amino acids during hydrolysis must be considered.

3.1.3 | Enzymatic hydrolysis

Enzymatic hydrolysis is widely used in food and pharmaceutical industries to create protein hydrolysates with specific characteristics. Proteases break peptide bonds, and enzyme choice and conditions (temperature, pH, time) affect the process. This method can customize protein hydrolysates by adjusting reaction parameters, hydrolysis degree, peptide size, and bioactive content (Hajfathalian et al., 2018). The resulting peptides can be further purified using techniques such as ultrafiltration and fast protein liquid chromatography and identified using liquid chromatography–mass spectrometry (LC–MS). For example, Chaipoot et al. (2023) produced protein hydrolysate from black sesame cake using a commercial protease and obtained three fractions (<3, 3–10, and >10 kDa), where the fraction with the smallest peptides showed the highest inhibitory activity against α-amylase and α-glucosidase. Fan et al. (2018) enzymatically hydrolyzed *Spirulina platensis* and purified the resulting peptides and found that fraction containing 3–5 kDa peptides obtained with pepsin efficiently inhibited lipase and 3T3-L1 preadipocytes. Overall, enzymatic hydrolysis is

favored for bioactive peptide production due to its high specificity, mild processing conditions, and absence of residual organic solvents and toxic chemicals. However, its applicability is limited by the high cost of enzymes (Ulug et al., 2021). For example, the studies on black sesame cake and algae used relatively high enzyme concentrations (1%–6% enzyme-to-substrate ratio), which may not be feasible for industrial applications. Future research should explore using smaller enzyme concentrations and developing cost-effective peptide purification procedures. Readers are referred to Section 5 for more studies on anti-obesity peptides and hydrolysates acquired after enzymatic hydrolysis.

3.1.4 | Subcritical water hydrolysis

This technique uses water heated above its boiling point at 1 atm but below its critical point, with enough pressure to keep it liquid. The increased presence of hydroxide and hydronium ions boosts the water's reactivity, enabling it to function as an acid, base, or double catalyst in hydrolysis reactions. Subcritical water hydrolysis is chosen for its unique capability to enable rapid and selective hydrolysis without requiring additional chemical catalysts, making it environmentally friendly. Compared to traditional enzymatic or chemical hydrolysis methods, subcritical water hydrolysis significantly reduces extraction times, eliminates harmful effluent production, and offers improved scalability and process control (Rivas-Vela et al., 2021). Lee et al. (2017) reported that fish collagen peptides obtained with this method inhibited lipid accumulation in 3T3-L1 preadipocytes and reduced adipocyte sizes in mice fed a high-fat diet (HFD). However, the method's industrial applicability is uncertain due to high operational costs and the need for specialized equipment. The consistency and yield of bioactive peptides also require further validation. Future research should optimize process parameters to enhance efficiency and cost-effectiveness.

3.2 | De novo methods

De novo peptide production involves designing and synthesizing new peptide sequences using advanced computational techniques like deep learning and in silico analyses, complemented by experimental approaches such as molecular docking and phage display. Optimal sequences are synthesized using traditional chemical techniques, like solid-phase and liquid-phase peptide synthesis, or innovative strategies like gene recombination. This integrated workflow facilitates the production of peptides with tailored characteristics for various applications. While many de novo methods focus on therapeutic peptides, this review emphasizes designing, analyzing, and producing anti-obesity peptides for food or dietary supplements.

3.2.1 | Design and analysis

De novo methods for designing and analyzing anti-obesity peptides involve in silico procedures to select appropriate enzymes, mimic gastrointestinal digestion, and predict bioactive and even undesirable (e.g., allergic and toxic) properties of peptides (Peredo-Lovillo et al., 2022). These methods include deep learning, parametric generation, and computational modeling and simulation, but most of them are primarily used for therapeutic peptides. For instance, deep learning algorithms can design peptide sequences with specific characteristics, as seen in the creation of dual agonists of the human glucagon receptor (GCGR) and GLP-1R to combat obesity (Puszkarska et al., 2024). Nevertheless, since the focus of the present review is on the bioactive peptides from different sources that can be administered as food and dietary supplements, the focus here is on the in silico methods to design and analyze these peptides.

In silico methods predict peptide release from protein sources and use molecular docking to screen active peptides and predict their interactions. This enhances the discovery of bioactive peptides and provides insights into their mechanisms. For instance, sesame proteins were subjected to virtual proteolysis with three proteases (pepsin, trypsin, and chymotrypsin) using PeptideCutter; then, peptide screening by PeptideRanker and ADME (absorption, distribution, metabolism, and excretion) profiling by SwissADME yielded six peptides. Afterward, the potential interactions of these peptides with key sites of pancreatic lipase (leading to the inhibition of the enzyme) were predicted via molecular docking (Wang, Ai, et al., 2022). Anti-obesity peptides from canary seed were purified after enzymatic hydrolysis and simulated gastrointestinal digestion. Four peptides (VPPR, LADR, LSPR, and TVGPR) showed strong inhibitory activity against pancreatic lipase, especially through their content of arginine, glycine, and hydrophobic amino acids (Urbizo-Reyes et al., 2022). Anti-obesity peptides from lupin protein were analyzed in silico using PeptideRanker and molecular docking. The peptides showed higher inhibitory activity against α-glucosidase than α-amylase, with strong binding to the enzymes' catalytic sites (Fadimu et al., 2022). This finding aligns with structural–activity relationship analysis of pinto bean peptides, which also showed strong binding to lipase catalytic residues (Ngoh et al., 2017).

Phage display is another technique for identifying anti-obesity peptides where peptides or proteins are displayed on the surface of bacteriophages, linking the displayed

peptides to their genetic information. This method is effective for identifying peptides that selectively bind to targets like enzymes and cell-surface receptors (Song et al., 2024). It was used to identify peptide inhibitors for α-amylase from pinto bean (Ngoh et al., 2016) and cumin seed (Siow et al., 2017). However, computational models rely on data quality and algorithms, often missing biological complexities like digestion and bioavailability. Peptides effective in simulations may fail in real-world conditions, needing extensive validation for safety and efficacy. Reduced regulatory scrutiny for food and supplements risks overreliance on predictions without enough empirical data, leading to misleading health claims. Consumer skepticism toward synthetic origins and potential bioavailability issues could compromise product credibility. Additionally, unpredictable interactions with other dietary components and side effects may not be fully captured by the in silico models.

3.2.2 | Synthesis

After designing anti-obesity peptides, the next step is to synthesize them and validate their effectiveness through in vitro or in vivo analyses. Integrating in silico and in vitro (and/or in vivo) techniques enhances our comprehension of alternative methods for obesity research (Pamplona et al., 2023). Common peptide synthesis methods for food include liquid (or soluble)- and solid-phase peptide synthesis. Liquid-phase synthesis is economical and allows for purification at each step but generates unwanted intermediates, making the process lengthy and challenging. Despite issues like insolubility in organic solvents, extended synthesis times, and chemical waste, it has successfully produced therapeutic peptides. Solid-phase peptide synthesis simplifies production by attaching amino acids to a resin, facilitating mass production. However, it requires significant starting materials and uses environmentally harmful chemicals. This method was applied to synthesize anti-obesity peptides identified in insect larvae protein hydrolysate (Zhang et al., 2024) and hazelnut protein hydrolysate (Wang et al., 2020).

Innovations like Fmoc (fluorenylmethyloxycarbonyl) protection have enhanced solid-phase peptide synthesis, making it the standard for producing bioactive peptides, although environmental concerns persist (Akbarian et al., 2022). This technique employs the Fmoc group to temporarily shield the amine group of amino acids during the sequential assembly of peptides on a solid resin. The Fmoc group can be selectively removed with a mild base like piperidine, enabling the addition of subsequent amino acids without disturbing the existing peptide chain or other protective groups. Fmoc solid-phase peptide synthesis is highly regarded for its efficiency and adaptability, making it easier to synthesize complex peptides with high purity (Behrendt et al., 2016). This technique was used to generate anti-obesity peptides obtained after in silico gastrointestinal hydrolysis of sesame protein (Wang, Ai, et al., 2022) and anti-obesity peptides identified in ark shell protein hydrolysate (Ahn & Je, 2021). In addition to the chemical methods, the gene recombination approach may potentially be applied to produce anti-obesity peptides. This technique involves integrating a specific gene that encodes the desired bioactive polypeptide into the DNA of a host organism, such as bacteria, yeast, or plants. Once the recombinant DNA is introduced, the host expresses the target peptide, which is then harvested. This approach enables the efficient production of peptides in large quantities with high yield and purity. Compared to chemical synthesis, gene recombination can produce more complex peptides and proteins that may be difficult or expensive to synthesize chemically. This method offers a cost-effective and scalable solution for large-scale peptide production, making it a promising approach for developing food-derived anti-obesity peptides (Gao et al., 2021). It remains to be seen if these approaches can be used to synthesize anti-obesity peptides for food applications, as each has limitations. Solid-phase peptide synthesis may be inefficient for large-scale production due to high reagent costs, peptide aggregation, and expensive purification. Also, the Fmoc-based method may be considered resource-intensive and environmentally harmful. On the other hand, liquid-phase peptide synthesis is labor-intensive and inefficient for long peptides. Also, gene recombination may face purity issues, consumer acceptance challenges, and strict regulations related to genetically modified organisms. Therefore, the application of these methods for synthesizing food-grade anti-obesity peptides needs a balance of cost, scalability, purity, and regulatory compliance.

4 | EVALUATION OF ANTI-OBESITY PEPTIDES

Precise methods for measuring the anti-obesity effects of food-derived peptides are essential in combating obesity. This section reviews anti-obesity evaluation techniques, categorized into in vitro assays (including enzymatic inhibition and cell line studies), in vivo assays (covering metabolic and physiological analyses), and emerging alternative approaches (such as in silico methods and advanced technologies) (Figure 3). It also briefly outlines the hierarchy of methods used to approve substances as anti-obesity agents.

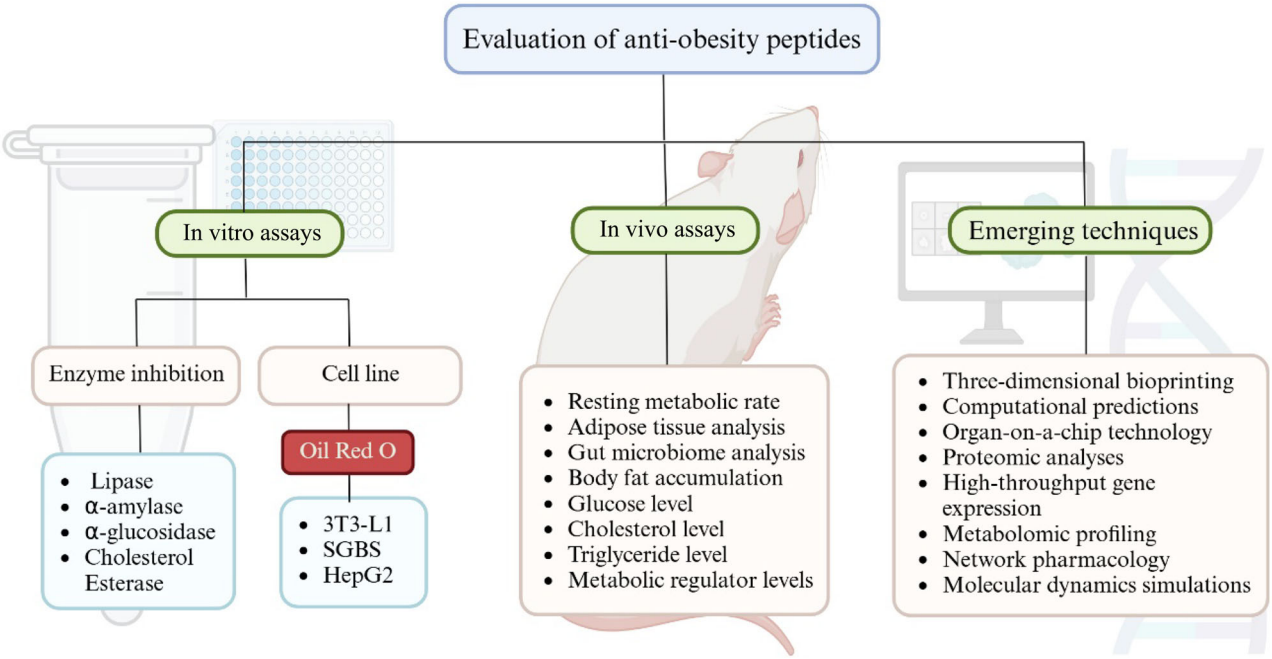

FIGURE 3 Methods for evaluation of anti-obesity peptides from food.

4.1 | In vitro assays

Several techniques have been devised to evaluate anti-obesity effects in vitro. When investigating the anti-obesity attributes of peptides, researchers frequently utilize in vitro assays to examine a range of cellular mechanisms associated with metabolism and fat regulation. What follows is a concise introduction to some commonly used in vitro assays, categorized into enzymatic and cell-based assays, for evaluating the anti-obesity properties of different compounds such as bioactive peptides.

4.1.1 | Enzyme inhibition assays

Enzyme inhibition assays play a crucial role in in vitro anti-obesity research by facilitating the screening of potential inhibitors targeting key enzymes involved in lipid digestion and metabolism (Bandara et al., 2023; Híreš et al., 2018; Singh et al., 2021). Researchers focus on halting the activity of fat-metabolizing enzymes like pancreatic lipase and cholesterol esterase, as well as carbohydrate-metabolizing enzymes such as α-amylase and α-glucosidase (Spínola & Castilho, 2021; Zhang et al., 2020). These enzymes are essential for fat digestion and absorption from the diet (Bandara et al., 2023). Given that lipids constitute a significant portion of dietary calories, targeting these enzymes with bioactive peptides emerges as an effective approach to managing obesity.

Pancreatic lipase inhibition

Lipid metabolism is crucial for maintaining energy balance, with dysregulation leading to obesity and hyperlipidemia, associated with conditions like atherosclerosis, hypertension, and diabetes (Liu et al., 2020). Reducing lipid absorption in the gastrointestinal tract is a potent strategy against obesity (Nayebhashemi et al., 2023). The pancreatic lipase inhibitory assay assesses how compounds, including bioactive peptides, affect pancreatic lipase activity. During the assay, pancreatic lipase is incubated with a test compound and a substrate mimicking dietary fats. The enzyme's activity is measured by quantifying hydrolysis products like fatty acids. A higher inhibition percentage indicates effective lipase activity reduction, suggesting anti-obesity potential (Vo et al., 2022). Pancreatic lipase breaks down ester bonds in triacylglycerols (TGs) to produce FFAs, diacylglycerols, monoacylglycerols, and glycerol. Inhibiting lipase can help manage caloric intake by regulating lipid digestion and absorption, making it a promising approach for preventing obesity and reducing hyperlipidemia-related chronic diseases (Blasi et al., 2023). Orlistat is used as a positive control in this assay. A new method using (normal-phase) high-performance liquid chromatography (HPLC)–evaporative light scattering detector was validated to measure products from in vitro pancreatic lipase digestion as an index of anti-obesity effect. The TG fraction from extra-virgin olive oil was used as a natural substrate to measure pancreatic lipase activity, with Orlistat as the reference inhibitor.

The method's validation by monitoring the remaining TG levels and the formation of FFAs showed high recovery rates (99%–103%) and low relative standard deviation values (2%–7%) for both triolein and oleic acid standards. This method evaluated the inhibitory effect of a polyphenolic extract from apple pomace, showing inhibition comparable to Orlistat (Blasi et al., 2023).

α-Amylase and α-glucosidase inhibition

The α-amylase breaks down glycosidic linkages in carbohydrates, forming oligosaccharides, which are further broken down into glucose by α-glucosidase. Inhibitors of these enzymes can help regulate blood glucose levels in individuals consuming carbohydrate-rich foods (Poovitha & Parani, 2016). The α-amylase inhibitory assay measures a substance's ability to inhibit α-amylase, which breaks down starch into maltose, maltotriose, and limit dextrins. In this assay, α-amylase is incubated with a starch substrate and the test compound, and the enzyme's activity is measured by quantifying the glucose produced. A decrease in glucose production indicates effective α-amylase inhibition, which can slow carbohydrate digestion and reduce blood sugar levels. Similarly, the α-glucosidase inhibitory assay measures a substance's ability to inhibit α-glucosidase, which converts terminal, nonreducing α-D-1,4 linkages into glucose in the small intestine. This assay involves incubating α-glucosidase with a disaccharide substrate and the inhibitor, followed by measuring the glucose released. Lower glucose levels in the presence of the inhibitor suggest that the substance can reduce glucose absorption, aiding in the management of postprandial blood sugar levels (Wang, Zhao, et al., 2022). Inhibiting α-amylase and α-glucosidase can be beneficial in anti-obesity research as it reduces the digestion and absorption of dietary starch, leading to lower postprandial glucose levels. This helps manage blood sugar spikes and may contribute to weight loss (Haguet et al., 2023). Acarbose acts as a reference standard in both α-amylase and α-glucosidase inhibitory assays (Lam et al., 2024).

Cholesterol esterase inhibition

Cholesterol esterase stands as a significant enzyme in industries, facilitating the breakdown of cholesterol ester into cholesterol and fatty acid (Vaquero et al., 2016; Yoshida et al., 2019). Its application as a diagnostic reagent for measuring cholesterol levels in human blood serum underscores its importance in health care (Vaquero et al., 2016). Crucial in lipid digestion and absorption pathways, cholesterol esterase has garnered attention for its role in metabolic processes (Sivashanmugam et al., 2013). Studies have highlighted the cholesterol esterase-inhibitory properties of natural compounds, suggesting their potential as agents in combating obesity (Walters & Conway, 2001). Regarding the contribution of inhibiting this enzyme to reduce obesity, cholesterol esterase is predominantly found in the small intestine. Its main function is to hydrolyze cholesteryl esters, resulting in the production of cholesterol and FFAs. Subsequently, the unesterified cholesterol and FFAs are readily absorbed into the bloodstream by the intestine. Consequently, inhibiting cholesterol esterase activity is essential for restricting dietary cholesterol absorption and preventing obesity development (Zhao et al., 2024). In this assay, cholesterol esterase is incubated with a cholesterol ester substrate (such as cholesterol oleate) and the test compound. The enzyme's activity is measured by determining the amount of free cholesterol produced after the hydrolysis reaction. The degree of inhibition is assessed by comparing the amount of free cholesterol generated in the presence of the inhibitor to a control without the inhibitor. Effective inhibitors will show reduced levels of free cholesterol, indicating their potential to influence cholesterol metabolism (Kim, 2010). As in the pancreatic lipase inhibitory assay, Orlistat can serve as a positive control in the cholesterol esterase inhibitory assay.

4.1.2 | Cell line assays

Cell line assays serve as valuable tools for investigating potential anti-obesity interventions. These assays utilize cultured cells, representing adipocytes, hepatocytes, and other relevant cell types, to explore lipid metabolism, adipogenesis, and molecular pathways. By assessing lipid accumulation and the effects of compounds, researchers gain insights into novel therapeutic targets. These cell-based models contribute significantly to our understanding of obesity-related mechanisms and guide the development of effective strategies (Ruiz-Ojeda et al., 2016). Cell-based assays are commonly employed to screen compound libraries, assessing whether the test molecules induce changes in cell proliferation or demonstrate direct cytotoxic effects that may result in cell demise (Riss et al., 2013). Research has shown that cell viability assays are vital for evaluating the anti-obesity attributes of peptides (Halim, 2020; Herling, 2014; Kumar, 2019; Posimo et al., 2014; Riss et al., 2013). For instance, various in vitro models, such as the mouse 3T3-L1 cell line and the human Simpson–Golabi–Behmel syndrome (SGBS) cell line, are commonly utilized to study the molecular mechanisms of adipogenesis (Li, Jin, et al., 2023). What follows is a brief introduction to three cell line assays for measuring anti-obesity properties of compounds, along with a concise overview of a staining method used in in vitro cell line assays.

3T3-L1

The 3T3-L1 cell line, introduced in the mid-1970s, has significantly advanced our understanding of adipose biology and obesity-related processes (Kuri-Harcuch et al., 2019). As a mouse pre-adipocyte model, 3T3-L1 cells are crucial for studying adipogenesis (Ahmed & Kim, 2019). Key proteins and transcription factors like PPARγ and CCAAT/enhancer-binding protein alpha (C/EBPα) play roles in this process, while adenosine monophosphate-activated protein kinase (AMPK) inhibits differentiation. Inhibition of these cell lines by certain peptides can demonstrate their anti-obesity effects by reducing lipid accumulation and downregulating adipogenesis transcription factors (Hirose et al., 2024). These cells are cultured in Dulbecco's Modified Eagle Medium supplemented with fetal calf serum and antibiotics, maintained at 37°C with 5% CO_2, and regularly passaged to prevent over-confluence (Zhao et al., 2019). For differentiation, 3T3-L1 cells are treated with specific cocktails containing fetal bovine serum, 3-isobutyl-1-methylxanthine, dexamethasone, and insulin, which leads to the formation of mature adipocytes, allowing the study of lipid accumulation and adipocyte-specific gene expression (Zebisch et al., 2012).

SGBS

The SGBS cell line, derived from the subcutaneous adipose tissue of an infant suspected of having SGBS, serves as a valuable tool in anti-obesity research. Universally acknowledged as a paradigmatic in vitro model of human white pre-adipocytes (Tews et al., 2022; Yeo et al., 2017), SGBS cells reliably replicate clinical manifestations associated with the syndrome. These encompass a spectrum of congenital abnormalities, prenatal and postnatal overgrowth, distinctive craniofacial features, macrocephaly, and organomegaly. The syndrome's etiology is ascribed to mutations in the glypican 3 gene, situated on the X chromosome (Xq26), and manifests with substantial clinical heterogeneity (Tenorio et al., 2014). SGBS assessment encompasses diverse methodologies, including genetic screening to identify gene mutations (Rifai et al., 2023), phenotypic scrutiny for signs of overgrowth and dysmorphic traits (Ali et al., 2023), and employing high-throughput RNA sequencing to delineate transcriptomic alterations during adipogenic differentiation (Peng et al., 2023). Moreover, prenatal diagnosis in SGBS entails discerning ultrasound markers such as a thick nuchal fold, hepatomegaly, and fetal anomalies (Argubi et al., 2022). Delving deeper, investigating the effects of natural compounds on SGBS cells offers insights into their modulation of lipid droplets, mitochondrial dynamics, and the expression of genes pertinent to browning adipocytes (Colitti et al., 2022), which provide clearer insights into anti-obesity properties of different compounds.

HepG2

Accumulation of fat in HepG2 cells can be used for studying mechanisms related to obesity (Huggett et al., 2019; Singh et al., 2021; Zhang et al., 2010). Natural extracts and compounds have shown promise in mitigating fat accumulation in HepG2 cells, suggesting potential therapeutic benefits in addressing obesity (Cohen et al., 2021). Therefore, utilizing HepG2 cells in fat accumulation assays can yield valuable insights into obesity mechanisms and potential therapeutic interventions. Notably, HepG2 is a human liver cancer cell line widely used in research to study metabolic processes, particularly the antioxidant and cytoprotective effects of peptides. Peptides can reduce reactive oxygen species (ROS) and enhance superoxide dismutase activity in HepG2 cells (Wang et al., 2025). In obesity-related research, HepG2 cells are valuable for studying the effects of peptides on cholesterol homeostasis due to their potential hypocholesterolemic activity, which is relevant to obesity and associated metabolic disorders (Dwivedi et al., 2024).

Oil Red O

The Oil Red O technique is commonly utilized in in vitro cell line assays to visualize and quantify lipid droplets (Sabiorodriguez & Sabiorodriguez, 2023). This staining method stands as a cornerstone for detecting lipid content in cells or tissues. This entails the preparation of an Oil Red O solution through diverse methodologies to ensure effective staining (Ahn et al., 2022). The staining protocol typically involves treating the sample with Oil Red O, followed by the measurement of the stained lipids. For instance, in a study focusing on *Acremonium chrysogenum*, lipids were stained using a modified Oil Red O method, with staining intensity serving as a quantitative indicator of lipid levels (Du et al., 2023). The staining intensity or lipid content can be quantitatively evaluated by analyzing the color intensity of the stained lipids, with methods such as RGB (Red, Green, and Blue) values or correlation values offering avenues for further analysis (Shin et al., 2011).

In this review, various in vitro anti-obesity assays have been explored as exemplars of evaluation methods. While acknowledging the breadth of available assays, the focus of this paper necessitates selective coverage. On the other hand, the categorization of in vitro anti-obesity assays in terms of enzymatic and cell line assays might not encompass all available in vitro assays. For instance, the cholesterol micellar solubility assay, which was employed to evaluate the hypolipidemic activity of peptides (Yang et al., 2021), does not neatly fit into either of our existing categorizations. Thus, the assays discussed serve as representative examples, albeit not exhaustive, of in vitro approaches to assess anti-obesity properties. These assays provide insights into mechanisms like fat digestion

inhibition but often oversimplify obesity's complex nature. In vitro conditions rarely mimic the in vivo environment, where peptides must endure digestion and absorption and reach the target enzyme in their active form. Other assays, assessing peptide stability and metabolic effects, offer complementary insights but also struggle to replicate human digestion. Despite their utility in high-throughput screening, these assays may not translate well to in vivo efficacy, and therefore, combining them with animal models or human clinical trials is essential to thoroughly assess the anti-obesity potential of food-derived peptides.

4.2 | In vivo assays

In vivo assays are crucial for evaluating anti-obesity interventions, providing insights into the physiological effects of compounds in living organisms. These methods assess metabolic function, adipose tissue dynamics, glucose homeostasis, and lipid metabolism. Animal models, like mice and rats, are essential for studying obesity and testing treatments, though they have limitations due to metabolic differences and ethical concerns (Lakshmanan et al., 2022). Human clinical trials are vital for translating animal research into real-world applications, with randomized trials offering the most reliable data on treatment efficacy and safety (Duffuler et al., 2022). This section explores various in vivo assays used in anti-obesity research, highlighting their importance in understanding obesity.

4.2.1 | Resting metabolic rate

RMR refers to the energy expenditure required by an organism at rest to maintain basic physiological functions. In the context of obesity research, RMR plays a pivotal role in understanding energy balance and potential interventions. Research has shown that individual differences in energy expenditure, influenced by RMR and physical activity, closely correlate with dietary intake (Johnston et al., 2007). Thus, RMR measurements in mice serve as a valuable tool for assessing the impact of anti-obesity interventions and understanding the complex interplay between metabolism and weight regulation. However, this method requires specialized equipment and may not fully capture overall metabolic activity, necessitating careful interpretation of results (Jafrin & Lakshmanan, 2022).

4.2.2 | Adipose tissue analysis

Adipose tissue analysis entails evaluating the size, distribution, and gene expression within adipose tissue, providing crucial insights into fat accumulation and responses to various treatments. Although this method enhances comprehension of adipose tissue dynamics, its application is constrained by invasive procedures and is primarily confined to animal studies (Pamplona et al., 2023). Techniques utilized for adipose tissue analysis include histological staining (Tordjman, 2013), gene expression analysis methods (Hua et al., 2023), and imaging techniques such as magnetic resonance imaging (Huber et al., 2020).

4.2.3 | Gut microbiome analysis

Gut microbiome analysis reveals distinct microbial signatures related to obesity. Researchers analyze metagenomic data from obese and nonobese individuals, identifying specific microbial species and functional differences. Understanding these signatures informs microbiome-based diagnostics and interventions. In recent years, there has been a surge in interest surrounding microbiome–metabolome investigations of the human gut. This heightened attention is primarily attributed to the mounting evidence uncovering the complex interactions among gut microbes, metabolites, and host well-being (Muller et al., 2021; Park et al., 2021). In vivo anti-obesity assays incorporating gut microbiome analysis have demonstrated promising outcomes in combating obesity. Research has identified specific microbial species associated with metabolically healthy obesity in both human subjects and murine models, underscoring the potential significance of gut microbiota in obesity pathogenesis (Chen, Tang, et al., 2022; Oh et al., 2022). Furthermore, therapeutic manipulation of gut microbiota has been explored as a strategy for preventing and treating obesity-related metabolic disorders. Interventions such as dietary modifications, probiotic treatments, fecal microbiota transplantation, and bariatric surgery have exhibited metabolic improvements in obese populations (Carrera-Quintanar et al., 2018; Santos-Paulo et al., 2022). The utilization of next-generation probiotics and fecal microbiota transplantation presents a novel approach to target obesity by leveraging the gut microbiota, thereby offering promising avenues for anti-obesity interventions (Chang et al., 2019).

4.2.4 | Body fat accumulation

Body fat analysis is integral to anti-obesity strategies, with in vivo methods playing a pivotal role in this endeavor. A variety of techniques, including bioelectrical impedance analysis, quantitative magnetic resonance imaging, dual-energy X-ray absorptiometry, computed tomography (CT), and anthropometry, are employed to precisely assess fat

percentage (Kuk & Ross, 2007; Lemos & Gallagher, 2017). These methodologies enable the evaluation of fat, fat-free mass, bone mineral content, total body water, and distinct fat depots, providing insights into metabolic functions and facilitating the monitoring of health changes. In this regard, the assessment of total body fat percentage as well as android and gynoid fat percentages by using existing CT data was recently proposed through quantitative computed tomography (Mai et al., 2024).

Abdominal fat accumulation is a key factor in obesity, resulting from an imbalance between energy intake and expenditure. In vivo anti-obesity assays measure parameters like weight gain, fat deposition, lipid levels, gene expression, and inflammatory markers. Several compounds have demonstrated efficacy in reducing abdominal fat in animal models (Chen et al., 2019; He et al., 2016; Li, Zhang, et al., 2020; Tamura et al., 2017). For instance, osmotin, a plant-derived protein, was found to suppress abdominal fat accumulation by regulating energy metabolism in mice fed an HFD (Jo et al., 2019). Imaging modalities also play a pivotal role in quantitatively evaluating abdominal fat levels, which are strongly associated with metabolic syndrome and its associated health risks (De Melo et al., 2009).

Lots of investigations have delved into the mechanisms underlying lipid accumulation using various models. Lipomas, characterized by heightened adipocyte proliferation and increased lipid accumulation, serve as a valuable model for understanding altered lipolysis mechanisms associated with obesity (Le Duc et al., 2021; Vekic et al., 2023). Moreover, *Caenorhabditis elegans* has emerged as a model organism for studying the inhibition of lipid accumulation by plant extracts, demonstrating its utility in anti-obesity screening assays (Sulistiyani et al., 2017). Additionally, in vivo studies employing radiolabeled lipids have been conducted to monitor the distribution of dietary lipids in tissues, aiding in the identification of dysregulated pathways contributing to obesity-related lipid accumulation (Aurrekoetxea et al., 2023). These diverse approaches collectively illuminate lipid accumulation processes and provide crucial insights for the development of effective anti-obesity interventions.

4.2.5 | Glucose level

Blood glucose levels are critical indicators for assessing anti-obesity strategies. Researchers monitor these levels to evaluate interventions and their impact on metabolic health. Elevated blood glucose poses a significant challenge in obesity. Scientific investigations focus on in vivo glucose monitoring for individuals with carbohydrate metabolism disorders. Excessive glucose consumption exacerbates obesity and type-2 diabetes, affecting intestinal enterocytes and influencing gene expression patterns (Boztepe & Gulec, 2018). Continuous blood glucose monitoring methods offer real-time surveillance of obesity (Camastra et al., 2017), helping identify novel therapeutic targets and develop strategies to mitigate hyperglycemia's effects on metabolic health. Glucose tolerance test (GTT) is used clinically to diagnose impaired glucose tolerance and assess carbohydrate metabolism and insulin secretion. It involves oral administration of glucose, followed by monitoring plasma glucose and insulin levels over time. GTT provides insights into glucose processing and can evaluate the effects of compounds on glucose regulation and insulin sensitivity (Jafrin & Lakshmanan, 2022). However, it requires fasting and may not fully capture long-term effects (American Diabetes Association, 2018).

4.2.6 | Cholesterol level

In vivo studies have demonstrated a direct relationship between cholesterol levels and obesity in mice (Duong et al., 2018). This relationship can be leveraged to assess the anti-obesity effects of natural compounds in animal models, such as rats fed a high-cholesterol diet (Lee et al., 2012). Notably, cholesterol levels in the serum lipid profile serve as an indication of the anti-obesity properties of these natural compounds (Faris Abdulghani et al., 2024).

TC serves as a pivotal metric within anti-obesity assessments (Evans & Colls, 2009). It is integral to various cellular functions and, in addition to obesity, contributes to the pathogenesis of both CVD and cancer (Kuzu et al., 2016; Lin et al., 2018). Understanding TC levels is crucial in evaluating the effectiveness of anti-obesity peptides, as they can directly impact TC levels, leading to decreased obesity and subsequent reduction in cardiovascular risk. Moreover, elevated levels of LDL-C have been linked to obesity (Fan et al., 2019). For a while now, HDL-C has been hailed as "good cholesterol," with recognized benefits for overall health (Jomard & Osto, 2020; Khan et al., 2018; Von Eckardstein & Widmann, 2014). Obesity impacts not only the plasma concentration of HDL-C but also its functionality (Wang & Peng, 2011). Therefore, bioactive peptides that enhance HDL levels can contribute to improving lipid metabolism and reducing the risk of obesity-related complications.

Moreover, assessing very low-density lipoprotein cholesterol (VLDL-C) kinetics is also a common approach in in vivo measurements related to anti-obesity strategies. Recent research has demonstrated that interventions such as dietary control can influence VLDL-C turnover rates, with standardized macronutrient intake showing promise in improving study outcomes (Johansen et al., 2017).

Additionally, in cases of obesity, VLDL receptor deficiency can mitigate cardiac lipotoxicity by decreasing lipid deposition and enhancing insulin sensitivity (Fungwe et al., 2019). Recognizing the interplay between HDL catabolism, VLDL kinetics, liver fat, and visceral adiposity is crucial, particularly in the context of abdominal obesity (Vergès et al., 2014). Therefore, analyzing in vivo cholesterol levels in terms of TC, LDL-C, HDL-C, and VLDL-C can be considered to study the anti-obesity properties of natural compounds.

4.2.7 | Triglyceride level

Elevated triglyceride levels are associated with obesity and metabolic disorders, making their measurement crucial for evaluating the efficacy of anti-obesity interventions. Researchers analyze blood samples from animal models (Jeong et al., 2023; Park et al., 2024) or human subjects (Kim et al., 2024; Wilson et al., 2021) to track changes in triglyceride levels in response to various treatments. This provides insights into lipid metabolism and overall metabolic health. These assays not only allow for the direct evaluation of the effectiveness of anti-obesity therapies but also aid in the identification of potential therapeutic targets and biomarkers. Through in vivo triglyceride assays, researchers gain a deeper understanding of the complex mechanisms underlying obesity and can develop more targeted and personalized approaches to its management. It is noteworthy that triglyceride evaluation can also be applied in in vitro anti-obesity studies by measuring cellular triglyceride, for example, through special quantification kits (Kim et al., 2016).

4.2.8 | Metabolic regulator levels

In obesity research, various metabolic regulators play crucial roles in shaping metabolic processes and adipose tissue dynamics (Jones et al., 2008; Nakayama et al., 2020). In vivo evaluations of these metabolic regulators would involve (i) animal models fed an HFD to induce obesity in order to assess the effects of activating or inhibiting these regulators on adipose tissue development, insulin sensitivity, and overall metabolic health; (ii) administering their agonists or antagonists to animals to measure changes in body weight, fat distribution, glucose metabolism, and other relevant parameters; and (iii) analyzing gene expression patterns in adipose tissue, liver, and other relevant organs to identify their target genes in the context of obesity.

For example, PPARs play a crucial role in lipid metabolism and have been studied extensively in the context of obesity and related diseases. PPARs are nuclear receptors that regulate gene expression in response to fatty acids and other lipid molecules. They are therapeutic targets for various lipid metabolic disorders, including obesity, atherosclerosis, diabetes, hyperlipidemia, and nonalcoholic fatty liver disease (Xu et al., 2018). PPARs are involved in adipogenesis and lipid storage in adipocytes, and their activation can lead to improved insulin sensitivity and even induce browning of white fat (Ma et al., 2018). There are three main subtypes of PPARs: PPARα, PPARβ/δ, and PPARγ. PPARα is associated with fatty acid catabolism and triglyceride metabolism (Moller & Berger, 2003), and PPARγ is highly expressed in adipocytes and is a master regulator of adipogenesis, lipid storage, and insulin sensitivity. Full agonists of PPARγ, such as thiazolidinediones, improve insulin sensitivity but may also lead to weight gain and visceral obesity (Ma et al., 2018). Overall, PPARs play a critical role in obesity and related metabolic disorders. Their intricate interactions with other receptors provide potential avenues for therapeutic interventions.

Another example of metabolic regulators studied in vivo in the context of obesity is nuclear factor erythroid 2-related factor 2 (Nrf2). Nrf2 is a transcription factor that plays a critical role in cellular defense against oxidative stress (Li, Eguchi, et al., 2020). In obesity, excessive fat accumulation leads to oxidative stress due to increased production of ROS in adipose tissue (Xia et al., 2022). Nrf2 activation helps counteract oxidative damage by upregulating antioxidant enzymes and detoxification pathways (Bayliak & Abrat, 2020). The activation of Nrf2 using pharmacological or natural products is a promising therapeutic approach for obesity (Gutiérrez-Cuevas et al., 2022) and therefore can be considered for in vivo anti-obesity experiments.

In addition to PPARs and Nrf2, other metabolic regulators have also been highlighted for their role in obesity research, including leptin receptor whose activation could be a therapeutic approach for obesity, affecting metabolic pathways related to food intake, lipid metabolism, and energy balance; protein tyrosine phosphatase 1B, which negatively impacts the leptin signaling pathway when activated, and its inhibition can be considered in obesity research due to its role in reducing sensitivity to both leptin and insulin; *FTO*, an RNA demethylase found in various tissues and organs, which can be a potential anti-obesity target; lingual cluster of differentiation 36, a transmembrane protein with high affinity for long-chain fatty acids, which is investigated as an anti-obesity target in pharmacological studies; ACC, whose inhibition is regarded as a promising therapeutic strategy for managing metabolic disorders; and type 1 cannabinoid receptor whose antagonists are investigated as a potential therapy for obesity by reducing appetite and body weight. For detailed

explanations regarding the analysis of these metabolic regulators in the context of obesity research, readers are referred to de Medeiros et al. (2024).

4.3 | Emerging alternative techniques

Obesity research methodologies continue to evolve, making it challenging to cover all existing and emerging approaches comprehensively within a single review article. For instance, while two-dimensional in vitro models have long been used, recent focus has shifted to three-dimensional in vitro models for studying compounds targeting adipose metabolic activity (Hamel et al., 2024). Three-dimensional bioprinting technology, which constructs tissues using bioink (cells embedded in a hydrogel), offers innovative ways to create three-dimensional structures (Pamplona et al., 2023). Additionally, researchers are increasingly integrating in silico methods with in vivo and in vitro analyses. Researchers are increasingly integrating in silico methods with in vivo and in vitro analyses, offering a cost-effective means of screening and predicting peptide functionalities (de Medeiros et al., 2024). This process typically involves the following steps: (i) retrieving target structures from the RCSB Protein Data Bank (PDB); (ii) generating peptide structures using tools like UniProt, PeptideCutter, Marvin Sketch, ChemBio3D, and Hyperchem; (iii) docking peptides with receptors using software such as GOLD, AutoDock, HADDOCK, and MOE; and (iv) evaluating interactions with visualization programs like LigPlot and Studio Discovery. Theoretical models are validated using tools like MolProbity and refined through bond and angle analyses. Validated structures can be added to databases like BIOPEP, which contains bioactive peptide sequences (Aguiar et al., 2024). Furthermore, micro-physiological systems, also known as organ-on-a-chip technology, have transformed preclinical research. These miniaturized cell culture platforms use living cells in controlled microenvironments, closely mimicking tissue and organ functions, and provide more physiologically relevant, predictive, and ethical methods for drug discovery and development (Pamplona et al., 2023).

Moreover, de novo techniques in anti-obesity research encompass innovative methodologies specifically tailored to elucidate the complex mechanisms underlying obesity development and progression, as well as to identify novel therapeutic targets and interventions. These approaches leverage a diverse array of techniques, including advanced proteomic analyses (Masood et al., 2018), high-throughput gene expression studies (Ke et al., 2021), comprehensive metabolomic profiling (Telle-Hansen et al., 2020), and network pharmacology combined with molecular docking and dynamics simulations (Liu et al., 2024). By harnessing these cutting-edge tools, researchers aim to unravel the intricate interplay of genetic, environmental, nutritional, and behavioral factors contributing to obesity pathogenesis. Overall, the integration of de novo approaches holds promise for revolutionizing our understanding of obesity and catalyzing the development of more effective preventive and therapeutic interventions to combat this global health epidemic.

4.4 | Hierarchy, limitations, and human relevance of measurement methods

When evaluating the effectiveness of anti-obesity peptides, it is essential to understand the hierarchy of research methods used to establish their biological effects (Figure 4). This hierarchy begins with in vitro studies, where the peptide's mechanisms are first explored in a controlled environment, such as test tubes. These studies allow researchers to investigate the fundamental interactions and biochemical pathways influenced by the peptide, providing initial insights into its potential efficacy. In vitro studies are useful for the preliminary evaluation of bioactive peptides, as they provide controlled settings to examine their mechanisms of action. Nonetheless, they do not replicate the physiological complexity of living organisms, including digestive processes and systemic interactions, which limits their direct relevance to human scenarios. Next, cell models are employed to provide a more complex and physiologically relevant context. These models mimic aspects of human physiology, enabling researchers to observe how peptides interact with specific cell types and tissues. This step is crucial for understanding the peptide's cellular mechanisms and potential therapeutic targets. Following cell models, animal studies are conducted to offer preclinical evidence of the peptide's effects in a living organism. These studies are essential for assessing the peptide's systemic impact, including its pharmacokinetics (how the body absorbs, distributes, metabolizes, and excretes the peptide) and pharmacodynamics (the biological effects and mechanisms of action). Animal studies also help identify potential side effects and safety concerns, providing a comprehensive understanding of the peptide's efficacy and safety profile. Although cell models enable targeted investigation of lipid metabolism, peptide absorption, and signaling pathways, they lack the systemic complexity of living organisms, including organ interactions and metabolic pathways, and do not replicate in vivo digestive processes. In vivo animal models address these gaps by providing insights into peptide absorption, metabolism, and systemic effects within a living organism. However, physiological, genetic, and metabolic differences between animals and humans limit the direct

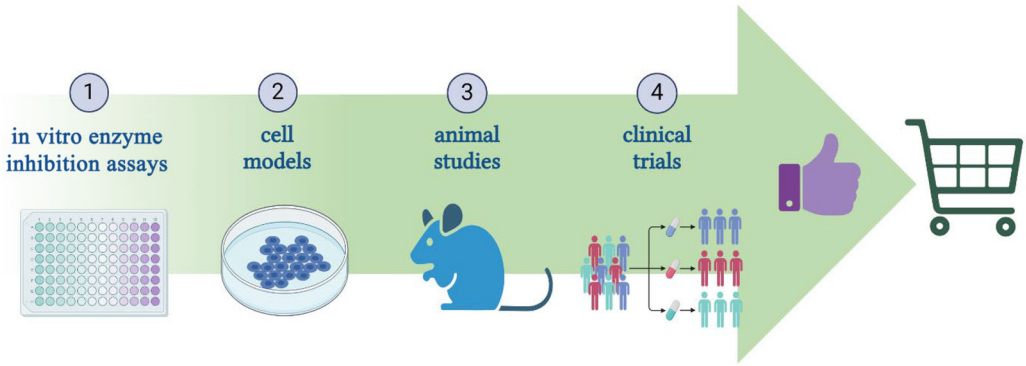

FIGURE 4 Hierarchy of methods for validating anti-obesity peptides.

applicability of these findings to clinical settings, and ethical concerns pose additional challenges. Finally, clinical studies in humans represent the highest level of evidence. These studies are necessary for regulatory approval of peptides as anti-obesity agents. Clinical trials are conducted in multiple phases to evaluate efficacy, safety, and potential side effects in the target population. These studies are critical for confirming the peptide's effectiveness in real-world settings and ensuring it meets the stringent safety and efficacy standards required for approval by regulatory agencies. However, human trials are often limited by small sample sizes, genetic variability within populations, and high costs. By progressing through this hierarchy of research methods, robust evidence can be systematically gathered to support the development and approval of anti-obesity peptides, ensuring they are both effective and safe for human use.

5 | SOURCES OF ANTI-OBESITY PEPTIDES

In the past few years, many bioactive peptides have been found to occur naturally or be produced from proteins in various foods, including milk, eggs, soybeans, fish, marine products, and meat (Baek et al., 2023; Chelliah et al., 2021; Suryaningtyas & Je, 2023). Peptides showcase promising attributes in regulating adipogenesis, lipid metabolism, gut microbiota, and appetite control, making them potential contenders in the battle against obesity. These peptides offer a natural and secure avenue for obesity management, with the possibility of developing functional foods or nutraceuticals. In this section, studies focusing on obtaining anti-obesity hydrolysates and peptides from various types of resources are reviewed (Table 1). While literature supports the anti-obesity effects of dietary peptides, their effectiveness can vary across populations and dietary contexts. Factors such as age, gender, metabolic health, and diet composition influence peptides' bioavailability, metabolism, and efficacy in promoting weight loss or reducing obesity-related risks. A detailed exploration of the molecular and physiological mechanisms underlying these peptides' anti-obesity activity is essential. Although many studies highlight their role in regulating appetite hormones, enhancing fat metabolism, or modulating gut microbiota, the specific molecular pathways involved remain complex and require further investigation. For instance, some peptides may act on specific receptors or signaling pathways, such as activating brown adipose tissue or inhibiting adipogenesis, providing insights into more targeted interventions. With the vast array of research on anti-obesity peptides, it is unrealistic to assume that this section could encompass every study. Nevertheless, an effort is made to offer a review of selected studies from each category to present a comprehensive perspective on the derivation of anti-obesity peptides from diverse resources.

5.1 | Plant and algae

5.1.1 | Seeds

Seeds serve as a rich source of peptides with anti-obesity effects. Peptides extracted from black sesame cake have shown promise in inhibiting metabolic enzymes such as α-amylase, α-glucosidase, and pancreatic lipase (Chaipoot et al., 2023). The study compared peptide fractions with a commercial anti-obesity agent, acarbose, revealing significant inhibitory activity, particularly in the 3-kDa fraction. Notably, the 3-kDa fraction displayed the highest inhibition (31.08%) of α-amylase activity, while peptide samples exhibited moderate activity against α-glucosidase compared to acarbose (29.45%–35.78% vs. 78.23%). Pancreatic lipase inhibitory activity increased with higher peptide molecular weights, with the >10-kDa fraction demonstrating substantially higher inhibition (57.25%) compared to other fractions (Chaipoot et al., 2023). Furthermore, the

TABLE 1 Peptides and hydrolysates with anti-obesity properties derived from various sources.

Sources		Peptide or hydrolysate specifications	Production methods	Measurement methods	Mechanisms of action	References
Seeds	Sunflower seed	Peptide sizes of <1, 1–3, 3–5 and >5 kDa	Enzymatic hydrolysis: flavorzyme and alcalase	In vitro: anticholesterolemic activity and enzymatic assays	Inhibition of cholesterol micellar solubility and α-amylase and α-glucosidase activity	del Carmen Hernández-Barillas et al., 2025
	Black sesame cake	Peptide sizes of <3, 3–10, and >10 kDa	Enzymatic hydrolysis: flavorzyme	In vitro: enzymatic assays	Inhibition of α-amylase, α-glucosidase, and pancreatic lipase	Chaipoot et al., 2023
	Chia seed and flaxseed	Chia seed and flaxseed protein hydrolysates	Enzymatic hydrolysis: bromelain, alcalase, and papain	In vitro: enzymatic assays	Inhibition of α-glucosidase, pancreatic lipase, and cholesteryl esterase	Mudgil et al., 2023
	Chia seed	Pure peptide 1:NSPGPHDVALDQ, Pure peptide 2:RMVLPEYELLYE, digested total protein (DTP), albumin, and glutelin	Enzymatic hydrolysis: pepsin and pancreatin	In vitro: adipogenesis assays	Decrease in PPAR expression, LPL, FAS, SREBP1, lipase activity, TG, secretion of nitric oxide, PGE2, and TNF	Grancieri et al., 2021
	Quinoa	FGVSEDIAEKLQAKQDERGNIVh, AEGGLTEVWDTQDQQF, YIEQGNGISGLMIPG, AVVKQAGEEGFEW, HGSDGNVF	Enzymatic hydrolysis: pepsin, papain, and pancreatin	In vitro: adipogenesis assays	Downregulation of PPARγ, C/EBPα, aP2, and LPL expression levels	Shi, Hao, et al., 2019
Cereals	Rice bran	VYTPG (memolin)	Enzymatic hydrolysis: pepsin and pancreatin	In vivo: animal study	Improvement of glucose tolerance	Shobako et al., 2023
	Rice	IVPQH, PIVF, IIQGR, QPY, QSPVF	Silico hydrolysis: pepsin, trypsin, and chymotrypsin	In vitro: adipogenesis assay	Downregulation of PPARγ.	Ruiz-López et al., 2023
	Corn	Corn gluten hydrolysate	Enzymatic hydrolysis: alcalase and protamex	In vitro: adipogenesis assays In vivo: animal study	Inhibition of preadipocyte differentiation and reduction in TG, TC, LDL, expression of transcription factors, C/EBPα, C/EBPβ, and PPARγ	Zhang et al., 2022

(Continues)

TABLE 1 (Continued)

Sources		Peptide or hydrolysate specifications	Production methods	Measurement methods	Mechanisms of action	References
		LVHLL, LVHL, LLPPY	Enzymatic hydrolysis: protamex	In vitro: adipogenesis assays In vivo: animal study	Reduction in TG, TC, LDL-C, insulin resistance, and liver lipid accumulation in the liver cell and liver tissue, increase in HDL, and reduction in the expression of PPAR-α and sirtuin 1	Wei et al., 2022
Legumes	Lupin	Lupin protein hydrolysate	Enzymatic hydrolysis: alcalase	In vivo: animal study	Alleviation of the dyslipidemia and insulin resistance, improvement of healthy gut microbiota, and reduction in dysbiosis	Ponce-España et al., 2024
	Black bean	SGNGGGGGASM, KPGGGSPVA, VELVGPK, KPTTGKGALA	Enzymatic hydrolysis: pepsin/pancreatin or alcalase	In vitro: adipogenesis assays	Inhibitory activity against pancreatic lipase	Moreno Valdespino et al., 2019
		A total of 28 peptides identified in hydrolysates from raw, cooked, or fermented substrates.	Fermentation and enzymatic hydrolysis (pepsin and pancreatin)	In vitro: adipogenesis assays, enzymatic assay	Inhibition of the metabolic enzymes α-amylase and α-glucosidase	Flores-Medellín et al., 2021
	Mung bean	Mung bean protein hydrolysate	Enzymatic hydrolysis: alkaline protease	In vitro: enzymatic assay In vivo: animal study	Decline of liver weight, hyperglycemia, hyperlipidemia, and insulin resistance, increase in the gut microbial diversity, and inhibition of the metabolic enzyme α-glucosidase.	Li, Tian, et al., 2022
	Soybean	ALEPDHRVESEGGL, SLVNNDDRDSYRLQSG-DAL	Enzymatic hydrolysis: pepsin and trypsin	In vitro: cholesterol assay In vivo: animal study	Stimulation of TICE and reduction in cholesterol levels, hyperlipidemia, and hepatic cholesterol synthesis	Lee, Shin, et al., 2022
	Soybean	Soya protein preparation	Simulated in vitro gastrointestinal digestion	In vitro: cell line assay In vivo: animal study	Suppression of adipogenesis in 3T3L-1 cells, increased expression of certain genes in hepatocytes, and upregulation of adiponectin gene expression in visceral fat tissue	Sharma et al., 2024

(Continues)

TABLE 1 (Continued)

Sources		Peptide or hydrolysate specifications	Production methods	Measurement methods	Mechanisms of action	References
	Black bean	Black bean protein hydrolysates with a total of 28 peptides	Fermentation (*Bacillus subtilis*) and enzymatic hydrolysis (pepsin)	In vitro: adipogenesis assay, enzymatic assay	Inhibition of α-amylase and α-glucosidase, decrease in free fatty acids, and inhibition of lipid accumulation in 3T3-L1 cell line	Flores-Medellín et al., 2021
	Chickpea	VFVRN, VVYP, VYP, GLAIQK M_w <1 and <5 kDa	Enzymatic hydrolysis: alkaline protease	In vivo: animal study, cholesterol level	Decrease in body weight, adipose tissue size, TG, TC, LDL-C, and the atherogenic index in serum and liver TC and TG, increase in HDL, downregulation of PPARγ and FAS, and restraining obesity caused by estrogen deficiency	Shi, Hou, et al., 2019
	Lupin	GPETAFLR	Enzymatic hydrolysis: alcalase	In vivo: animal study	Decrease in body and liver weight gain, hepatic inflammation, TG, TC, FAS, blood markers, and leptin resistance	Lemus-Conejo et al., 2020
Nuts	Walnut	Walnut meal protein hydrolysate	Enzymatic hydrolysis: different proteases	In vitro: inhibition of cholesterol micellar solubility, enzymatic assay In vivo: animal study	Inhibition of pancreatic lipase and cholesterol micellar solubility, reduction in TG, TC, LDL, and hepatic steatosis, increase in HDL, normalization of elevated apolipoprotein (Apo)-B, and reduction in Apo-A1.	Yang et al., 2021
	Hazelnut	RLLPH	Enzymatic hydrolysis: alcalase	In vitro: cell line, enzymatic assay, gastrointestinal digestion stability	Inhibition of pancreatic lipase and intracellular lipid accumulation, reduction in TG, TC, and FAS, and downregulation of PPARγ and/or C/EBPα	Wang et al., 2020
Fruit	Sacha inchi	NLYYKVV, WWYVK, WLLMWPYK, EGLLMWPY, FPFFGYVWK	Enzymatic hydrolysis: pepsin, papain, and trypsin	In vitro: enzymatic assay	Inhibition of pancreatic lipase	Wang, Liu, et al., 2023
	Saskatoon berry	Free amino acids	Milling dried fruit to obtain homogeneous powder	In vitro: enzymatic assay	Inhibition of α-amylase, α-glucosidase, and pancreatic lipase	Lachowicz et al., 2019
Aquatic organisms	*Chlorella pyrenoidosa*	LLVVYPWTQR	Enzymatic hydrolysis: pepsin, papain, trypsin, and alcalase	In vitro: enzymatic assay, cell line assay	Inhibition of porcine pancreatic lipase, fatty acid synthesis, and fat accumulation in adipocytes	Zhang et al., 2019

(Continues)

TABLE 1 (Continued)

Sources		Peptide or hydrolysate specifications	Production methods	Measurement methods	Mechanisms of action	References
	Spirulina platensis	NALKCCHSCPA, LNNPSVCDCD-CMMKAAR, NPVWKRK, CANPHELPNK	Enzymatic hydrolysis: trypsin, alcalase, pepsin, papain, and protamex	In vitro: adipogenesis assays, enzymatic assay	Inhibition of preadipocytes proliferation and porcine pancreatic lipase	Fan et al., 2018
	Asian hard clam (*Meretrix lusoria*)	Peptide sizes of ≤1 kDa	Enzymatic hydrolysis: protamex	In vivo: animal study	Reduction in serum total cholesterol, triglycerides, and LDL-C, increased expression of the AMPK protein, and reduced lipogenic gene expression	Chilakala et al., 2024
	Monkfish	Monkfish peptides (<1 kDa)	Enzymatic hydrolysis: neutral protease	In vivo: animal study	Reduction in body wight, liver wight, TG, TC, FAS, and LDL, increase in HDL, and upregulation of AMPK	Ye et al., 2022
	Chum salmon milt	RPR	In silico hydrolysis (pepsin)	In vivo: animal study	Increase in adipocyte PPARy1 mRNA, upregulation of hepatic PPARa mRNA, and decrease in hepatic stearoyl-CoA desaturase 1 mRNA through SREBP1 mRNA and adipocyte FAS mRNA downregulation	Mijiti et al., 2021
	Oyster (*Crassostrea gigas*)	189 heptapeptides, 145 octapeptides, and 100 nonapeptides	In vitro gastrointestinal digestion	In vivo: animal study	Downregulation of Acc, FAS, and SREBP1, improvement of dyslipidemia, inhibition of enzyme activity, and regulation of fatty acid uptake	Chen, Dong, et al., 2024
	Blue mussel	Blue mussel hydrolysate	Enzymatic hydrolysis: pepsin	In vitro: cell line assay	Increase in lipolysis and downregulation of adipogenic transcription factors including PPARγ, C/EBPα, and SREBP1	Oh et al., 2020
Terrestrial animals	Porcine skin	Porcine collagen peptide	Commercially available product	In vitro: cell line assay In vivo: animal study	Downregulation of leptin, C/EBPα, PPARγ, and FAS and decrease in weight gain, TG, TC, and adipocyte size	Lee, Bang, et al., 2022
Milk and egg	Casein and whey	Casein and whey hydrolysates	Simulated in vitro gastrointestinal digestion	In vitro: cell line assay In vivo: animal study	Suppression of adipogenesis and stimulated adipolysis in 3T3L-1 cells and upregulation of adiponectin gene expression in visceral fat tissue	Sharma et al., 2024

(Continues)

TABLE 1 (Continued)

Sources		Peptide or hydrolysate specifications	Production methods	Measurement methods	Mechanisms of action	References
	Whey	Whey protein hydrolysate	Commercially available product	In vivo: animal study	Reduction in TG, TC, and LDL, increase in HDL, decrease in body weight gain, inhibition of lipid accumulation in the liver, downregulation of cholesterol biosynthesis genes, and upregulation of cholesterol uptake and excretion genes in the liver.	Wang, Fu, et al., 2022
	Whey	Hydrolysate obtained by hydrolysis of protein suspensions containing bovine serum albumin and whey protein powder	Enzymatic hydrolysis: pepsin	In vitro: cell line assay	Increase in lipolysis and mitochondrial content and fat oxidation in adipocytes and skeletal myotubes, upregulation of PPARγ and PPARδ, reduction in insulin, ER stress, inflammation, and DG accumulation, and improvement of palmitate-induced insulin resistance	D'Souza et al., 2020
	Nonfat milk	Water-soluble fraction of fermented milk (<3 kDa)	Fermentation: lactic acid bacteria isolated from cheese	In vitro: cell line assay, enzymatic assay	Inhibition of pancreatic lipase and lipid accumulation during adipogenesis	Manzanarez-Quin et al., 2023
	Goat milk	Whey protein hydrolysate	Enzymatic hydrolysis: proteases	In vitro: enzymatic assays	Inhibition of α-amylase and α-glucosidase	Du et al., 2022
	Camel milk	KDLWDDFKGL, MPSKPPLL	Enzymatic hydrolysis: alcalase, bromelain, and papain	In vitro: enzymatic assays	Inhibition of porcine pancreatic lipase and α-amylase	Mudgil et al., 2018
	Camel milk	KFQWGY, SQDWSFY, YWYPPQ, WPMLQPKVM	Enzymatic hydrolysis: alcalase, bromelain, and papain	In vitro: enzymatic assay	Inhibition of cholesterol esterase	Mudgil et al., 2019
	Chicken egg white	WEKAFKDED, QAMPFRVTEQE, ERYPIL, VFKGL	Enzymatic hydrolysis: thermolysin and pepsin	In vitro: cell line assay	Upregulation of PPARγ	Jahandideh et al., 2018

(Continues)

TABLE 1 (Continued)

Sources		Peptide or hydrolysate specifications	Production methods	Measurement methods	Mechanisms of action	References
Synthetic peptides	D3	Nine-amino-acid peptide	Solid-phase synthesis	In vivo: animal study	Suppression of appetite, regulation of gut microbiota, amelioration of insulin, and decrease in leptin resistance	Li, Zhang, et al., 2023
	Hemp seed	YNLPILRF, NQANQLDQF, and YNLPILSF	Solid-phase synthesis	In vitro: enzymatic assays In silico: molecular docking	Inhibition of pancreatic lipase through hydrogen bonds, electrostatic action, and hydrophobic interaction	Zhang et al., 2025
	Baltic herring	PPVEEP, GPAGDPA, (HyP)-HyP-GRPGF, GADPEDVIVS	Synthesized by a company	In vivo: animal study	Improvement of glucose tolerance and insulin tolerance and increase in plasma GLP-1 content	Wang et al., 2024
	Erythrina edulis seed protein	YPSY, AALWE, DGLGYY, SQLPGW TWVV, CCGDYY, YDLHGY, MFTGPY, GSYHDSK	Solid-phase synthesis	In vitro: enzymatic assay	Inhibition of α-amylase, α-glucosidase, and pancreatic lipase	Rodríguez-Arana et al., 2022
	Lupin	GPETAFLR	Solid-phase synthesis	In vitro: lipolysis assays In vivo: animal study	Decrease in body and liver weight gain, hepatic inflammation, TG, TC, FAS, blood markers, and leptin resistance	Lemus-Conejo et al., 2020
	Hazelnut	RLLPH	Solid-phase synthesis	In vitro: adipogenesis assay, enzymatic assay	Inhibition of pancreatic lipase activity, intracellular lipid accumulation, TG, TC, and FAS and downregulation of PPARγ and/or C/EBPα	Wang et al., 2020
	Lemon basil seeds	GRSPDTHSG	Fmoc solid-phase synthesis	In silico: molecular docking In vitro: cell line assay	Disruption of the stability of the lipase–colipase complex and reduction in adipogenesis by downregulating SREBP1c and PPAR-γ in 3T3-L1 adipocytes	Kuptawach et al., 2024
	Sacha inchi	FPFFGYVWK (FK-9), EGLLMWPY (EY-8), KLVFVTS (KS-7), and KDIPWLY (KY-7)	Solid-phase synthesis	In vitro: cell line assay, enzymatic assay	Inhibition of pancreatic lipase activity and fat accumulation and regulation of lipid metabolism	Wang, Liu, et al., 2023
	Chlorella pyrenoidosa	LLVVYPWTQR	Synthesized by a company	In vitro: adipogenesis assays, 3T3L-1 adipocytes	Decrease in accumulation of intracellular triacylglycerol and inhibition of lipid accumulation and fatty acid synthesis	Zhang et al., 2019

(Continues)

TABLE 1 (Continued)

Sources		Peptide or hydrolysate specifications	Production methods	Measurement methods	Mechanisms of action	References
	Spirulina platensis	NALKCCHSCPA, LNNPSVCDCD-CMMKAAR, NPVWKRK, CANPHELPNK	Solid-phase synthesis	In vitro: adipogenesis assay, enzymatic assay	Inhibition of 3T3-L1 preadipocytes proliferation and porcine pancreatic lipase	Fan et al., 2018
	Blue mussel	PIISVYWK and FSVVPSPK	Synthesized by a company	In vitro: cell line assay In vivo: animal study	Downregulation of C/EBPα, SREBP1, and PPAR-γ, activation of AMPK and hormone-sensitive lipase, reduction in adipogenic and lipogenic biomarkers and pro-inflammatory cytokine production, and enhancement of serum cholesterol levels and lipolysis	Suryaningtyas et al., 2025
	Human milk	AVPVQALLLNQ	Synthesized by a company	In vitro: adipogenesis assays In vivo: animal study	Reduction of adipose tissue mass, regulation of PPARγ expression, and improvement of glucose metabolism, MAPK signaling, and metabolic status	Li et al., 2021
	Chicken egg	IRW, IQW	Synthesized by a company	In vivo: animal study	Reduction of TG, TC, LDL, glucose, malonaldehyde, and leptin levels and increase in glucose tolerance and insulin resistance	Liu et al., 2022
	Egg white-derived peptide	QAMPFRVTEQE (Peptide 2)	Synthesized by a company	In vivo: animal study	Improvement of insulin sensitivity and decrease in hepatic steatosis, liver TG, and hepatic cholesterol accumulation	de Campos Zani et al., 2023
	Skipjack tuna dark muscle	AINDPFIDL, FLGM, GLLF, and WGPL	Synthesized by a company	In vitro: enzymatic assays	Inhibition of pancreatic lipase and cholesterol esterase by binding to their active sites	Huang et al., 2024
	White chicken egg	WEKAFKDED, QAMPFRVTEQE, ERYPIL, and VFKGL from ovalbumin	Synthesized by a company	In vitro: adipogenesis assay	Upregulation of PPARγ	Jahandideh et al., 2018
	Rosa roxburghii	LFCMH, RIPAGSP, and YFRPR	Synthesized by a company	In vitro: enzymatic assays In silico: molecular docking	Inhibition of pancreatic lipase and cholesterol esterase by binding to active sites of enzymes	Yin et al., 2024

Abbreviations: AMPK, adenosine monophosphate-activated protein kinase; Apo, apolipoprotein; C/EBP, CCAAT/enhancer-binding protein; DG, diacylglycerol; ER, endoplasmic reticulum; FAS, fatty acid synthase; HDL, high-density lipoprotein; LDL, low-density lipoprotein; LDL-C, low-density lipoprotein cholesterol; LPL, lipoprotein lipase; MAPK, mitogen-activated protein kinase; PGE2, prostaglandin 2; PPAR, peroxisome proliferator-activated receptor; SREBP, sterol regulatory element-binding protein; TC, total cholesterol; TG, triacylglycerol; TICE, trans-intestinal cholesterol excretion; TNF, tumor necrosis factor.

1–3 kDa peptide fraction from sunflower seeds demonstrated enhanced hypocholesterolemic activity, whereas the peptide fractions smaller than 1 kDa were more effective at inhibiting α-amylase (del Carmen Hernández-Barillas et al., 2025). The comparison between chia seeds and flaxseeds in terms of their anti-obesity effects reveals significant differences. Under identical experimental conditions, chia seeds exhibited higher levels of free amino nitrogen and demonstrated superior hydrolysis by alcalase, leading to the production of peptides with more potent inhibition of pancreatic lipase compared to peptides from flaxseeds (Mudgil et al., 2023). The study highlights the superior pancreatic lipase inhibitory potential of enzyme-catalyzed hydrolysates from chia seed and flaxseed proteins compared to unhydrolyzed proteins. Overall, the data suggest that peptides obtained from chia seeds exhibit more potent anti-obesity effects compared to peptides obtained from flaxseeds (Mudgil et al., 2023). Additionally, peptides derived from chia seeds, such as digested total protein, digested albumin, glutelin, and pure peptides (Pep1 and Pep2), efficiently reduced lipid accumulation during adipogenesis development, with digested toral protein showing the highest efficacy in reducing triglyceride, sterol regulatory element-binding protein 1 (SREBP1), and lipoprotein lipase (LPL). Conversely, digested glutelin, digested albumin, and Pep2 were more effective in reducing the activity of lipids, FAS, and PPARγ, respectively (Grancieri et al., 2021). Similarly, the degree of protein hydrolysis by pepsin was notably higher compared to papain and pancreatin in quinoa-derived protein hydrolysates. Specifically, protein hydrolysates prepared using pepsin for 120 min exhibited remarkable solubility and effectively suppressed 3T3-L1 cell differentiation via the PPARγ pathway, indicating their potential as functional foods for managing obesity in the future (Shi, Hao, et al., 2019).

5.1.2 | Cereals

Cereals, as staple components of the global diet, have emerged as a significant source of bioactive peptides with potential anti-obesity properties. For instance, peptides derived from rice bran exhibited high insulin production linked with insulin resistance, impacting fat storage and weight gain, as well as cognitive function. Rice-memolin improves glucose tolerance, potentially enhancing cognitive function through AMPK activation and improved hippocampal function (Shobako et al., 2023). Rice protein hydrolysates were analyzed in silico for antiadipogenic effects, identifying specific amino acid sequences like IVOH, PIVE, and HOGR from rice glutelin, along with two prolamin sequences (QPY and QSPVF). These sequences showed potent downregulation of PPARγ (Ruiz-López et al., 2023). Corn protein treatment suppressed lipid accumulation in 3T3-L1 preadipocytes and effectively controlled fat accumulation, mitigating HFD-induced obesity and resulted in a notable reduction in body weight in mice when combined with exercise, demonstrating superior efficacy compared to sole administration (Zhang et al., 2022). Mice fed with corn peptides alongside an HFD mitigated weight gain and adverse effects on the liver. Ten amino acid sequences were identified in the peptide fraction less than 3 kDa, with the anti-obesity effects of corn peptides notably correlating with elevated levels of branched-chain amino acids (BCAAs). Among these sequences, LVHLL, LVHL, and LLPPY emerged as the most efficacious (Wei et al., 2022).

5.1.3 | Legumes

Peptides derived from various legumes show promising effects on combating obesity-related issues. Black bean peptides, obtained through different enzymatic processes, significantly impacted lipid metabolism, suggesting their potential as functional foods (Moreno Valdespino et al., 2019). Protein hydrolysates produced by solid-state fermentation from raw beans exhibited high efficacy in inhibiting α-amylase. Raw bean protein hydrolysates, at a concentration of 0.1 mg protein/mL, demonstrated notable potential by inhibiting lipid accumulation (27.9%) in the 3T3-L1 cell line (Flores-Medellín et al., 2021). Additionally, mung bean peptides have demonstrated anti-obesity effects by mitigating weight gain, elevated blood sugar levels, insulin resistance, and other related conditions in diabetic mice. These effects are partly attributed to their impact on intestinal flora composition, as indicated by the reversal of changes in the gut microbiota (Li, Tian, et al., 2022). Furthermore, soybean hydrolysates containing specific bioactive peptides promoted cholesterol efflux and regulated systemic lipid metabolism (Lee, Shin, et al., 2022). Additionally, peptides derived from chickpeas demonstrated significant potential in addressing metabolic disorders associated with lipid metabolism and adipose tissue dimensions in ovariectomized rat models. The hypolipidemic impact of these chickpea peptides is attributed to a noteworthy decline in the activities of FAS and HMG-CoA reductase, as well as the modulation of protein expressions (Shi, Hou, et al., 2019). Similarly, GPETAFLR from lupin showed promise in mitigating nonalcoholic fatty liver disease by modulating hepatic enzyme profiles, inflammatory cytokines, and genes and proteins related to lipid metabolism (Lemus-Conejo et al., 2020). In addition, a recent study has shown that lupin protein hydrolysate improves weight management,

adiposopathy, and gut dysbiosis in diet-induced obese mice (Ponce-España et al., 2024).

5.1.4 | Nuts

Bioactive peptides derived from nuts, especially walnut and hazelnut, demonstrate significant potential in combating obesity-related metabolic disorders. Walnut meal peptides derived from the enzymatic hydrolysate exhibited superior efficacy in hindering cholesterol solubilization in mixed micelles and inhibiting pancreatic lipase compared to alternative enzymes. The study highlights the antihyperlipidemic effect of walnut meal peptides in hyperlipidemic rats, emphasizing its role in preventing hyperlipidemia and regulating lipid metabolism. Notably, the administration of walnut meal peptides was proven to be beneficial in ameliorating liver damage and restoring abnormal levels of transaminases, specifically aspartate aminotransferase and alanine aminotransferase, suggesting its potential therapeutic impact (Yang et al., 2021). On the other hand, hazelnut-derived RLLPH exhibited a mitigating effect on adipogenesis by downregulating mRNA expression levels associated with adipogenesis-related factors and enzymes. Additionally, RILPH upregulated phosphorylated AMPK and its substrate ACC in 3T3-L1 adipocytes, indicating its anti-obesity potential as a promising nutraceutical or clinical drug for metabolic diseases (Wang et al., 2020). These findings underscore the promising role of bioactive peptides in nuts, particularly walnut and hazelnut, in addressing obesity-related health concerns. However, despite the promising anti-obesity properties of peptides from plant sources like cereals, nuts, seeds, and legumes, their effectiveness may be limited by low bioavailability and compositional variations influenced by processing conditions. Unlike animal protein sources, plant cells have walls that can entrap intracellular proteins, making them harder to access. This may require additional steps to break down the cell walls, increasing operational costs and potentially altering the effectiveness of the resulting peptides.

5.1.5 | Fruit

Peptides derived from sacha inchi meal were shown to be potent agents with lipid-lowering properties. Peptides NV-7 and FK-9, known for their pancreatic lipase inhibitory properties, effectively reduced lipid accumulation in HepG2 cells, influencing lipid metabolism (Wang, Liu, et al., 2023). Similarly, Saskatoon Berry cultivars 'Smoky' and 'Thiessen' contain elevated levels of key free amino acids and monophosphate nucleotides, particularly in the peel, which significantly inhibited pancreatic lipase enzymes associated with obesity. These contents showed a correlation with inhibitory activity toward α-amylase and α-glucosidase. Additionally, the fruit flesh exhibited a substantial content of total free amino acids with antihyperglycemic effects (Lachowicz et al., 2019). Nonetheless, bioactive peptides are generally found in lower concentrations in fruits compared to seeds, cereals, legumes, or nuts. Their composition varies with factors such as ripeness, processing, and fruit type. Therefore, more targeted research is needed to consider these factors and assess the feasibility of peptide production from fruits to confirm their effectiveness in managing obesity.

5.1.6 | Algae

Algae have been found to be a potential source of bioactive compounds with anti-obesity properties (Holdt & Kraan, 2011). Recent studies have emphasized the development of anti-obesity agents from algae protein. Zhang et al. (2019) purified and identified bioactive peptide fractions from enzymatic hydrolysates of *Chlorella pyrenoidosa*, demonstrating their ability to inhibit pancreatic lipase activity and suppress fat accumulation in 3T3-L1 cells (Zhang et al., 2019). Furthermore, the differentiation-inhibitory effects of peptides NPVWKRK and CANPHELPNK, extracted from blue-green algae (*Spirulina platensis*), showcased significant reductions in triglyceride accumulation, indicating impressive inhibition of triglyceride production compared to the control (Fan et al., 2018). The extraction of bioactive peptides, particularly those with anti-obesity properties, from microalgae and seaweeds is still relatively new. Like plants, protein extraction and hydrolysis from algae are hindered by their cell walls. Additionally, some algae species have low protein content, which presents feasibility issues for obtaining solely peptides from them. Furthermore, the potential interactions of the resulting peptides with other molecules in the algae matrix, such as carbohydrates and polyphenols, especially those forming irreversible covalent bonds, make it challenging to obtain pure fractions of anti-obesity peptides. Therefore, while algae peptides are promising as anti-obesity agents, the feasibility of their extraction and purification must be considered.

5.2 | Aquatic and terrestrial animals

5.2.1 | Aquatic animals

Seafood, renowned for its high protein content, offers numerous health benefits (Hyung et al., 2018). Modulating the gut microbiota with collagen peptides from fish skin has the potential to induce weight loss in mice by

reducing the Firmicutes/Bacteroidetes ratio (Baek et al., 2023). In mice treated with monkfish peptides, metabolic pathways were altered: AMPK pathway activation led to increased lipid oxidation and decreased FFA accumulation (Ye et al., 2022). Blue mussel hydrolysate has emerged as a promising candidate in the fight against obesity. Studies indicate that blue mussel hydrolysate increases lipolysis while simultaneously downregulating key adipogenic transcription factors such as PPARγ, C/EBPα, and SREBP1. These findings suggest that blue mussel hydrolysate may play a significant role in preventing the formation of fat cells and promoting the breakdown of existing fat stores, making it a potential therapeutic option for combating obesity (Oh et al., 2020).

5.2.2 | Terrestrial animals

Proteins and endogenous peptides occurring naturally in insects have demonstrated anti-obesity potential. Specifically, the novel peptides from *Allomyrina dichotoma* larvae, EIA10 and ALG9, have shown efficacy in reducing body weight gain. EIA10 has been notably effective in alleviating fatty liver symptoms and reducing lipid accumulation (Bae et al., 2020). Furthermore, ALG9 has been shown to activate AMPK/Nrf2 signaling, regulating lipid levels and suppressing lipid metabolism and oxidative stress in a nonalcoholic fatty liver disease mouse model (Fan et al., 2021). These findings underscore the potential of insect-derived peptides as therapeutic agents for obesity and related metabolic disorders (Quah et al., 2023). Moreover, porcine collagen peptide demonstrated significant efficacy in reducing white adipose tissue weight and size in HFD-fed mice. The anti-obesity effects are attributed to the modulation of transcription factors such as PPARγ and C/EBPα, along with changes in adipokine levels. Notably, the leptin level in the porcine collagen peptide-treated group was significantly decreased. These findings highlight the potential of porcine collagen peptide as a promising functional food ingredient, dietary supplement, or therapeutic agent for combating obesity (Lee, Bang, et al., 2022). Although anti-obesity peptides from terrestrial animals appear promising, sustainability concerns and their unsuitability for vegans and vegetarians should be considered.

5.3 | Milk and egg

Whey peptides have been observed to enhance lipolysis, increase mitochondrial content, and promote fat oxidation in adipocytes, suggesting a potential positive impact on fat cell energy metabolism. Additionally, whey peptides have shown promising effects in reducing organ weight, particularly for the liver, inguinal white adipose tissue, and epididymal white adipose tissue. They also contribute to lowering serum lipid levels, reducing circulating inflammation cytokines, and mitigating hepatic lipid accumulation, inflammation, and oxidative stress (D'Souza et al., 2020; Wang, Fu, et al., 2022). Assessment of pancreatic lipase inhibitory activity was conducted across all milk. The fermented milk exhibiting the most noteworthy pancreatic lipase inhibitory activity was selected for peptide identification using HPLC/tandem mass spectrometry, alongside adipogenesis studies. Furthermore, fermented milk J20 demonstrated effective inhibition of lipid accumulation in a 3T3-L1 cell line. In this investigation, peptides from the fraction with the lowest IC_{50} value underwent in silico analysis using the Peptide 2 database (PepSite 2) to predict their potential for pancreatic lipase inhibition. Among these peptides, 15 amino acid sequences were recognized within the peptides from fraction F5 (Manzanarez-Quin et al., 2023). Utilizing in silico analysis to determine protease types is essential for verifying their hypoglycemic activity. To confirm this activity, systematic exploration of various hydrolysates is required. Moreover, this method can also be applied to predict other activities (Du et al., 2022). Alcalase and papain hydrolysates from goat milk effectively inhibited α-glucosidase and α-amylase activities, indicating strong hypoglycemic potential in goat milk whey protein preparations (Du et al., 2022). Hydrolyzing camel milk proteins with alcalase and bromelain enhanced pancreatic α-amylase inhibitory activity, underscoring the importance of controlled hydrolysis using specific enzymes (Mudgil et al., 2018). This study highlights camel milk proteins as a promising source of peptides that inhibit key metabolic enzymes associated with disorders like cholesterol assimilation. Enzymatic hydrolysis revealed potent hypocholesterolemic peptides, with the type of enzyme and duration of hydrolysis significantly influencing their antihypercholesterolemic activities (Mudgil et al., 2019). Guha et al. (2021) highlighted findings concerning milk and milk products from minor dairy species. Their study clarified that the hydrolysate resulting from papain digestion displayed the utmost degree of hydrolysis. Furthermore, peptide identification unveiled three specific sequences, KFQWGY, SQDWSFY, and YWYPPQ, each showcasing a substantial affinity for the binding site of cholesterol esterase (Guha et al., 2021).

5.4 | Synthetic peptides

Synthetic peptides offer a promising avenue for the development of anti-obesity agents. For instance, a synthetic

peptide called D3 with nine amino acids has been instrumental in regulating body weight in model animals through ameliorating leptin resistance and upregulating the expression of uroguanylin to suppress appetite and *A. muciniphila* increase (Li, Zhang, et al., 2023). *Akkermansia muciniphila* is recognized for its contribution to preserving the integrity of the gut barrier and its association with a healthy gut microbiome. Functioning as a mucin-degrading bacterium, *A. muciniphila* feeds on the mucous layer of the gut. It has demonstrated anti-inflammatory effects and potential involvement in regulating glucose and lipid metabolism. Notably, individuals with obesity and type 2 diabetes often exhibit a depletion of *A. muciniphila* (Abbasi et al., 2023). The potential therapeutic utility of peptides emulating the vasculo-protective attributes of apolipoproteins A-I and E, present in HDL, is noteworthy. Notably, a synthetic peptide with 28 amino acid residues (Ac-hE18A-NHz) exhibited a remarkable capacity to significantly lower plasma cholesterol levels and inhibit atherosclerosis. Additionally, the analog of apolipoproteins A-I mimetic peptide (called 4F) demonstrated efficacy in reducing adiposity in obese mice (Wolkowicz et al., 2021). Moreover, hemp seed peptides (YNLPILRF, NQANQLDQF, and YNLPILSF) synthesized through the solid-phase method exhibited inhibitory activity against pancreatic lipase (Zhang et al., 2025). The multifunctional properties of nine novel peptides identified in an alcalase hydrolysate from *Erythrina edulis* (pajuro) seed proteins were investigated by Rodríguez-Arana et al. (2022). Synthesized peptides demonstrated in vitro antidiabetic effects by inhibiting α-amylase and α-glucosidase and/or modulating obesity through pancreatic lipase inhibition. These results elucidate the structural basis for multifunctional antidiabetic/anti-obesity activity and advocate for the potential utilization of pajuro protein hydrolysates as innovative ingredients in the development of anti-obesity agents (Rodríguez-Arana et al., 2022). Four artificially synthesized peptides, which were identified based on the analysis of the mass spectrum data of enzymatic hydrolysates of sacha inchi, exhibited significantly higher inhibitory effects on pancreatic lipase activity compared to those naturally occurring in enzymatic hydrolysates (Wang, Liu, et al., 2023). Additionally, a synthetic peptide (LLVVYPWTQR), designed according to the analysis of peptides derived from *C. pyrenoidosa*, exhibited anti-obesity properties by inhibiting fatty acid synthesis and reducing fat accumulation in adipocytes (Zhang et al., 2019). Li et al. (2021) discovered and synthesized a novel peptide named AOPDM1 derived from breast milk. This peptide exhibited promising potential in alleviating obesity and enhancing glucose metabolism (Li et al., 2021). Bioactive peptides found in natural health products showed potential in managing conditions associated with metabolic syndrome, including insulin resistance and obesity. Peptide 2, a synthetic peptide mimicking those from egg white, demonstrated improved insulin sensitivity and reduced hepatic steatosis upon supplementation, highlighting its distinct effects compared to the insulin-sensitizing drug rosiglitazone (de Campos Zani et al., 2023). Supplementing with IRW and IQW peptides from egg protein showed potential for slowing down the progression of obesity by reducing lipid deposition, regulating energy balance, and influencing gut microbiota composition (Liu et al., 2022). The hydrolysate of egg white underwent LC–MS/MS analysis, revealing the identification of 42 peptides. Among these, the two most active fractions were subjected to further analysis. Eleven peptides were generated, including WEKAFKDED, AMPFRVTEE, ERYPIL, and VEKGL from ovalbumin. These peptides were then validated for their PPARγ stimulatory activity in adipocytes. The study suggests that residues Asp (E), Leu (L), and Lys (K) within the sequences may contribute to the enhanced expression of PPARγ in pre-adipocytes (Jahandideh et al., 2018). Overall, the field of anti-obesity synthetic peptides is a burgeoning area of scientific inquiry, with ongoing research continually uncovering new potentials and applications for these innovative compounds in the fight against obesity.

6 | COMMERCIALIZATION OF ANTI-OBESITY PEPTIDES FROM NATURAL SOURCES—STATUS QUO AND OUTLOOK

When evaluating commercially available peptide products with anti-obesity properties, it is crucial to consider the origin of the peptides. In general, polypeptides are categorized as either endogenous or exogenous depending on their source. Endogenous polypeptides, which come from internal proteolysis or noncoding RNA, play roles in regulating biological processes such as energy metabolism and insulin resistance in the human body. On the other hand, exogenous polypeptides, which are found in nature (e.g., in plants and animals), include bioactive peptides that influence various biological functions. Endogenous peptides that play a role in regulating obesity in humans include leptin, a hormone produced by adipose tissue; neuropeptide Y from the nervous system; GLP-1, secreted by endocrine cells in the ileum; ghrelin, primarily produced by gastric X/A-like cells and also found in the small intestine and hypothalamus; adrenomedullin 2, which is involved in vascular and metabolic regulation; irisin, a peptide expressed in adipose tissue and muscle and encoded by the fibronectin type III domain-containing protein 5

gene; adropin, a peptide that regulates energy homeostasis and cardiovascular function; and preptin, secreted by pancreatic islet β cells (Gao et al., 2020). Most peptide-based anti-obesity products target GLP-1 receptor agonists, which are synthetic agents that replicate the activity of the natural hormone GLP-1, demonstrating significant potential in managing obesity and promoting weight loss (Deng et al., 2024). Examples include Victoza and Wegovy (which contain liraglutide and semaglutide, respectively) by Novo Nordisk, Trulicity (which contains dulaglutide) by Eli Lilly, and Bydureon, an extended-release form of exenatide, by AstraZeneca. Additionally, Pfizer is developing an investigational GLP-1 receptor agonist known as Danuglipron (PF-06882961). However, given that the primary focus of this review is on exogenous peptides with anti-obesity properties, this section will concentrate on commercially available products derived from these peptides.

As previously noted, most peptide-based products for managing obesity are derived from endogenous peptides. In contrast, those based on naturally produced or purified peptides intended for use as functional foods or dietary supplements are still in their early stages. The benefit of the latter is that consumers perceive them as food or dietary supplements rather than as drugs or drug-like products, making them more appealing. However, despite extensive research endeavors in this field, the market introduction of anti-obesity peptides sourced from food sources encounters numerous challenges. These challenges may include (i) the complexity of peptide production, (ii) issues with bioavailability and delivery, (iii) regulatory obstacles, (iv) market viability and costs, (v) intellectual property and competition concerns, (vi) lack of standardization, and (vii) difficulties in scaling up production (Figure 5).

Peptide production faces challenges with extraction, purification, instability, and synthetic production. Anti-obesity peptides are found in low concentrations in natural sources, making extraction and purification complex, time-consuming, and expensive (Purohit et al., 2024). Furthermore, peptides are prone to degradation during extraction, storage, and processing, complicating stability maintenance (Musaimi et al., 2022). Synthetic production, while addressing extraction issues, is costly and may not replicate natural peptides' structure and function, also facing sustainability concerns (Isidro-Llobet et al., 2019).

Peptides often have poor gastrointestinal absorption and are easily degraded by digestive enzymes. Developing effective delivery systems to protect and enhance peptide absorption is complex and costly (Vora et al., 2022) and requires advanced technologies like encapsulation or conjugation (Mahto et al., 2023). Also, commercializing anti-obesity peptides involves regulatory challenges. Extensive testing is needed to ensure safety and efficacy, involving preclinical studies, clinical trials, and toxicological assessments, which are time-consuming and costly (Chopra & Raynaud, 2020). Besides, securing approval from regulatory bodies such as the Food and Drug Administration or the European Food Safety Authority is a stringent process requiring extensive documentation and adherence to strict guidelines. This process is especially complex for biologically derived products, which often undergo additional scrutiny.

High production costs can limit marketability, especially when more affordable alternatives exist. Consumer reluctance toward peptide-based products from animal or unconventional sources stems from safety, efficacy, and naturalness concerns, as well as potential allergens and ethical considerations (Hajfathalian et al., 2018). In addition, patenting natural peptides can be challenging because they are often not deemed novel. This limitation can hinder the protection of intellectual property, thereby reducing the incentive for companies to invest in their commercialization. Moreover, anti-obesity peptides must contend with well-established pharmaceutical treatments that often possess more robust clinical data and a stronger market presence.

Peptide composition and concentration vary based on source, environmental conditions, and processing techniques (Maqsood et al., 2021). This complicates standardization and results in inconsistent efficacy and safety. Maintaining consistent quality and potency across batches is challenging with natural sources. Scaling up production from laboratory to industrial scale presents technical challenges, including preserving bioactivity, managing costs, and ensuring sustainability, along with process optimization, quality control, and regulatory compliance.

Table 2 showcases examples of commercially available products that utilize bioactive peptides from exogenous sources with potential anti-obesity properties. Our research indicates that while there are commercially available products containing peptides that may have anti-obesity effects, the number of products that specifically claim to be anti-obesity products and are primarily based on bioactive peptides from natural sources is limited. Several factors, as explained above, could hinder the production of these products and the translation of research findings on the anti-obesity properties of peptides from exogenous sources, such as food, animals, and plants, into commercial products. Despite these challenges, the use of bioactive peptides from natural sources with strong anti-obesity properties in commercial weight management products remains a promising field. For example, milk-derived peptides, such as those found in Pep2Dia, are claimed to regulate blood sugar by inhibiting α-glucosidase. Furthermore, fish peptides in Slimpro are marketed as contributing to weight management and

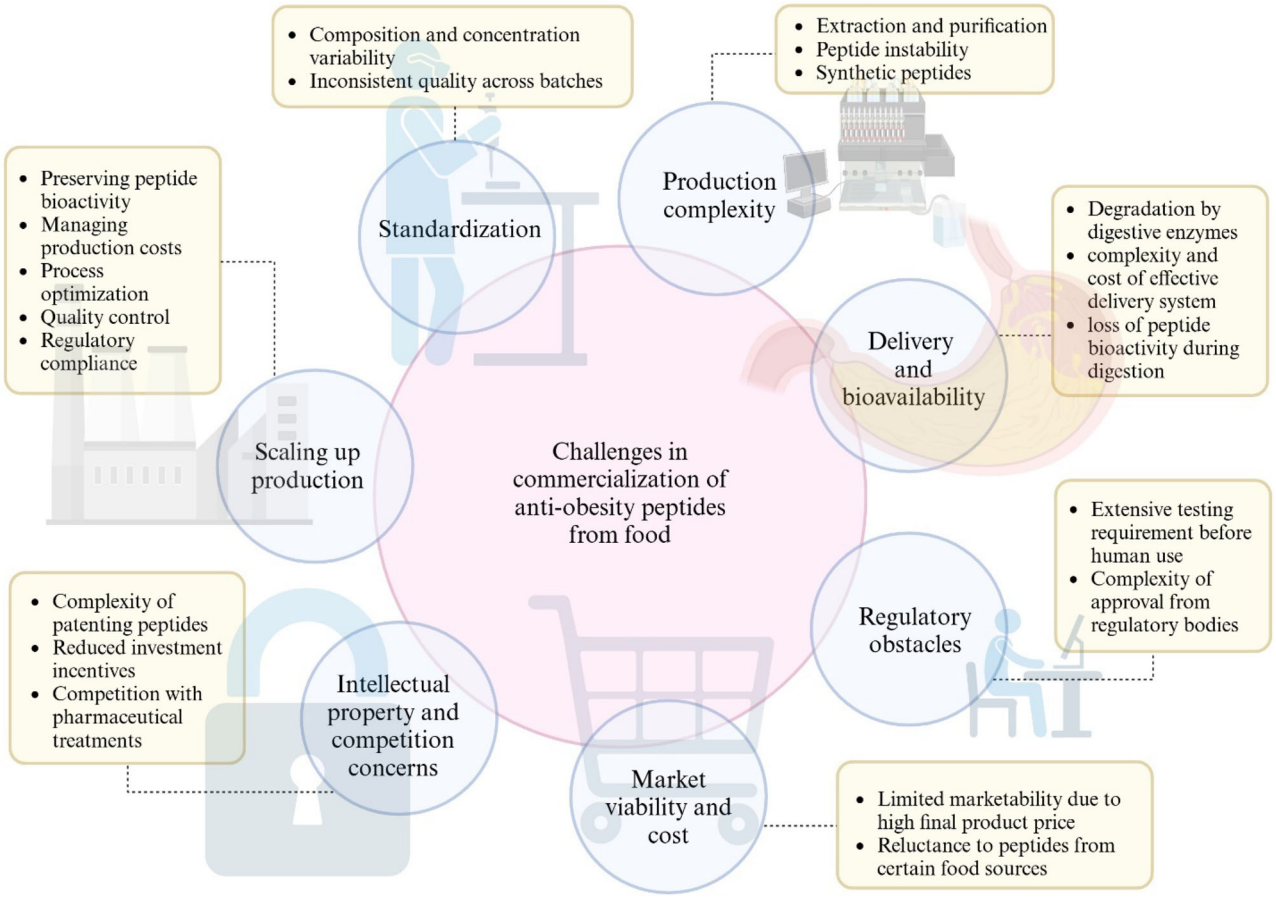

FIGURE 5 Challenges in commercialization of anti-obesity peptides from food.

TABLE 2 Examples of commercially available products based on anti-obesity peptides from natural sources.

Product	Source	Manufacturer	Claimed properties
BodyBalance	Collagen	Gelita AG	Increasing lean body mass and fat metabolism
Prolibra	Whey	Glanbia Nutritionals	Increasing fat loss and maintaining lean muscle
Pep2Dia	Milk protein	Ingredia Nutritional	Regulating blood sugar by inhibiting α-glucosidase
Seishou-sabou	Bovine and porcine blood	Moringa & Co.	Weight management
Slimpro	Fish peptide enriched with BCAAs	Nutraceuticals International Group	Weight management and satiety by increasing CCK and GLP-1
Fabenol	Kidney bean (*Phaseolus vulgaris*) protein	Sabinsa Corporation	Inhibiting α-amylase and preventing starch metabolizing activity
Numetra	Whey protein isolate, sodium caseinate, micellar casein	AmBari Nutrition	Weight loss
ProtiDiet	Whey protein concentrate, egg white, soy protein	ProtiFOODS	Weight management
Nutripeptin	Fish protein	Copalis Sea Solutions	Reducing glycemic index of foods and fat storage
Amizate	Atlantic salmon (*Salmo salar*) protein	Zymtech Production AS	Enhancing satiety and lean body mass

satiety by promoting the release of CCK and GLP-1. These examples reflect the broader trend of using food sources to develop functional products aimed at managing obesity. It is important to note that many commercially available products claim to support weight management and combat obesity, but they often rely on plant extracts containing other anti-obesity macromolecules rather than bioactive peptides. Given the potent obesity management properties of natural peptides, along with their additional benefits, it is reasonable to predict an increase in the number of anti-obesity products based on natural peptides in the future.

7 | CONCLUSIVE REMARKS AND FUTURE PERSPECTIVES

The increasing global incidence of obesity highlights the urgent need for innovative, safe, and effective interventions. Among the promising avenues of research are bioactive peptides derived from natural sources. These anti-obesity peptides, sourced from marine organisms, plants, and animal proteins, have demonstrated potential in regulating lipid metabolism, reducing fat accumulation, and influencing appetite-related pathways. Anti-obesity peptides can be obtained through conventional methods like microbial production and enzymatic hydrolysis with green extraction techniques, or methods like subcritical water processing. Alternatively, de novo strategies involve designing and chemically synthesizing peptides to enhance their specificity and functionality. These peptides are evaluated using in vitro techniques to measure the inhibition of digestive enzymes or cell-based assays to assess their effects on lipid accumulation and adipogenesis. In vivo methods, typically involving animal models, further evaluate their efficacy and safety. Emerging technologies like in silico modeling and docking studies predict peptide–target interactions, streamline screening, and optimize development. However, the journey from discovery to the development of functional products involves several critical challenges. These include ensuring the stability and bioavailability of the peptides, optimizing their extraction and purification processes, and conducting comprehensive clinical trials to confirm their efficacy and safety. Additionally, regulatory approval and consumer acceptance are pivotal for the successful commercialization of these peptide-based solutions.

A key consideration in developing anti-obesity peptides from food is understanding their mechanisms of action, including their influence on metabolic pathways and interaction with fat metabolism enzymes. Future research should prioritize bioinformatics tools and advanced peptide screening technologies to identify and design peptides with enhanced efficacy. Tailoring peptides for specific metabolic functions can create more effective interventions with minimal side effects, potentially leading to breakthroughs in obesity treatment. Integrating multidisciplinary efforts from molecular biology, pharmacology, and nutrition science can further enhance peptide development and application.

Another critical challenge in the application of anti-obesity peptides is their bioavailability and bioaccessibility after ingestion. Enzymatic degradation in the gastrointestinal tract can significantly limit the effectiveness of these peptides. To overcome these challenges, advanced delivery systems, including nano-encapsulation and peptide modifications, could be explored. These technologies can improve stability, absorption, and targeted release of peptides, ensuring that the bioactive compounds reach their sites of action in sufficient quantities. Additionally, research into optimizing the formulation and delivery of these peptides can further enhance their therapeutic potential, making them more viable as anti-obesity interventions. However, it is crucial to acknowledge the methodological limitations of current studies. Many are constrained by small sample sizes, short intervention periods, and inconsistencies across experimental models. These factors can impede our ability to draw definitive conclusions about the effectiveness of these peptides. Given these limitations, further research is necessary to better understand the mechanisms through which dietary peptides influence obesity. Future studies should aim to refine experimental designs, enhance model consistency, and consider factors such as age, gender, metabolic health, and dietary patterns. Clinical trials in diverse human populations are particularly important for confirming the efficacy and safety of these peptides as therapeutic agents for obesity management.

Moreover, the feasibility of producing anti-obesity peptides from natural sources compared with synthetic production remains a critical consideration. Research has highlighted that consumers perceive natural peptides as safer and more sustainable, enhancing their acceptance and marketability (Suryaningtyas & Je, 2023). However, this perception often conflicts with production challenges like seasonal availability, variability in raw material composition, and high extraction costs, which earlier research has identified as key barriers to scaling up natural peptide production (Henchion et al., 2017). In contrast, synthetic peptides offer advantages such as consistent quality, scalability, and ease of production (Lalani et al., 2024). These benefits can streamline manufacturing processes and meet regulatory requirements for consistency. However, synthetic products may face regulatory hurdles and consumer skepticism, as many prefer natural alternatives for perceived health benefits (Possidónio et al., 2025). This

mismatch between consumer preferences and production realities reflects a critical gap in the field. To address this, it is essential to build on earlier research to develop efficient and cost-effective extraction and purification methods for natural anti-obesity peptides while simultaneously exploring innovative and sustainable methods for synthetic peptide production. By addressing these gaps and bridging the challenges of production and consumer perception, researchers can optimize the development of anti-obesity peptides.

Additionally, sustainability is crucial when obtaining anti-obesity peptides from food. Overexploitation of sources like marine organisms can cause environmental concerns. To mitigate this, exploring sustainable alternatives is essential. One promising approach is using byproducts from the food industry, such as fish skin or whey, to minimize waste and contribute to a circular economy. Moreover, the environmental footprint of peptide extraction processes should be minimized by adopting green technologies. Techniques such as enzymatic hydrolysis and solvent-free extraction methods can reduce the environmental impact and enhance the sustainability of peptide production. Additionally, integrating renewable energy sources and optimizing resource use throughout the extraction and purification processes can further support environmental sustainability.

Another crucial consideration in the development of anti-obesity products is whether to use isolated peptides or the whole untreated raw material. Isolated peptides offer the advantage of targeted therapeutic actions and precise dosing, which can be critical for achieving specific health outcomes. However, using whole raw materials may provide synergistic effects from other bioactive compounds present, potentially enhancing the overall benefits. In addition, they might retain a broader spectrum of nutrients and bioactive compounds, which could contribute to a more comprehensive health benefit profile. Future research should focus on comparing the efficacy of products containing isolated anti-obesity peptides versus whole materials in clinical settings.

While anti-obesity peptides from food show promise for weight management, ensuring their safety and obtaining regulatory approval are crucial. Comprehensive toxicological studies are needed to confirm their safety, particularly regarding potential allergenicity and adverse effects. Regulatory frameworks are essential for consumer protection, but challenges such as harmonizing standards, validating health claims, and addressing long-term safety persist. Overcoming these challenges is vital for the successful integration of anti-obesity peptides into the food industry.

The future of anti-obesity peptide research hinges on overcoming these challenges through interdisciplinary collaboration, integrating advances in biotechnology, nutrition, food science, and sustainable development. Research should focus on enhancing the bioaccessibility and bioavailability of peptides, developing scalable and eco-friendly production methods, and ensuring the responsible use of natural peptide sources. In addition, future research should investigate the motivations and policy frameworks necessary for the successful commercialization of anti-obesity peptides from food, focusing on overcoming barriers and seizing opportunities to bring these innovations to market.

AUTHOR CONTRIBUTIONS

Mona Hajfathalian: Conceptualization; methodology; writing—original draft; investigation; visualization. **Sakhi Ghelichi**: Conceptualization; writing—original draft; methodology; investigation; visualization. **Charlotte Jacobsen**: Conceptualization; writing—review and editing; supervision.

CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

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