Biomedicine & Pharmacotherapy 128 (2020) 110314

Contents lists available at ScienceDirect

Biomedicine & Pharmacotherapy

journal homepage: www.elsevier.com/locate/biopha

Review

Lipase Inhibitors for Obesity: A Review

Tian-Tian Liu[1], Xiao-Tian Liu[1], Qing-Xi Chen, Yan Shi*

Key Laboratory of the Ministry of Education for Coastal and Wetland Ecosystems, School of Life Sciences, Xiamen University, Xiamen 361005, China

ARTICLE INFO

Keywords:
obesity
lipid metabolism
lipase inhibitor
natural product

ABSTRACT

With the rapid increase in the population of obese individuals, obesity has become a global problem. Many kinds of chronic metabolic diseases easily caused by obesity have received increasing attention from researchers. People are also striving to find various safe and effective treatment methods as well as anti-obesity medicines. Pancreatic lipase (PL) inhibitors have received substantial attention from researchers in recent years, and PL inhibitors from natural products have attracted much attention due to their structural diversity, low toxicity and wide range of sources. They have been used in the intestinal tract, blood, and the central nervous system with no side effects, and these advantages could lead to a new generation of diet pills or health care products with great development potential. This article is mainly aimed at discussing the research of obesity drug treatment with PL inhibitors and offers a brief review of related properties and the use of PL inhibitors in the field of weight loss.

1. Introduction

Obesity is a long-term problem that has persisted for many years. Obesity not only results in aesthetic problems but also causes abnormal physiological metabolism, which causes a series of physiological, psychological and social problems. Obesity is an important risk factor for diseases such as cardiovascular disease [1], hypertension, hyperlipemia [2], diabetes [3] and even cancer [4], and it is closely related to the emergence of many chronic diseases [5,6]. Obese individuals suffer greater relative risks from type 2 diabetes, gallbladder disease and syndrome than do individuals with a normal weight [7,8]. Diseases such as coronary heart disease, hypertension, osteoarthritis and gout increase the risk of obesity, and there are some reproduction effects as well [9,10]. The incidence of systemic and gastrointestinal tumours, such as endometrial cancer, breast cancer, colon cancer, polycystic ovary syndrome, infertility, is also significantly elevated in obese patients [4]. Therefore, prevention and treatment of obesity itself is the key to reducing the prevalence and mortality of chronic metabolic diseases. In addition to long-term dietary regulation and exercise intervention, the treatment of obesity includes a common short-term drug treatment and surgery (liposuction). However, liposuction sometimes makes the fat distribution uneven. Scientists have also found that liposuction has little effect on subcutaneous fat and even has no effect at all while exposing patients to certain risks. Most of the current treatments use safer short-term drug treatment combined with long-term diet plus exercise to achieve weight loss. Pancreatic lipase (PL) inhibitors plays a key role in the metabolism of human fat. It breaks down the oil in the food source into small molecules of glycerol and fatty acids that the body can absorb and participate in metabolism [11]. PL inhibitors can make PL lose part of the decomposition ability, and can control the fat entering the blood from the source to achieve the effect of lipid-lowering. In this paper, PL inhibitors are mainly used as drug treatments for research subjects, and the related properties and progress of the field of weight loss for PL inhibitors is briefly reviewed.

2. Metabolic mechanisms of obesity and Pancreatic lipase (PL) inhibitors

2.1. Fat-rich diet and function of lipase in obesity

Obesity in modern society is often caused by excessive fat intake and lack of exercise due to unhealthy lifestyles. Excessive fat absorption and accumulation leads to the appearance of obesity [1,12,13]. The liver and white adipose tissue are the main organs for lipid storage. Excessive lipid accumulation often causes excessive hypertrophy of non-alcoholic fatty liver and white adipose tissue [14]. Obesity and hyperlipidaemia are medical conditions associated with a series of risk factors such as insulin resistance, aggravated degree of liver fibrosis and injury, impaired glucose tolerance and hypertension, leading to an increased rate of mortality [5,6]. Studies in type 2 diabetes patients suggest that intramyocellular lipid accumulation and muscle insulin resistance precede the development of hepatic insulin resistance and

* Corresponding author.
E-mail address: yshi@xmu.edu.cn (Y. Shi).
[1] These authors contributed equally to this work.

https://doi.org/10.1016/j.biopha.2020.110314
Received 14 November 2019; Received in revised form 18 May 2020; Accepted 19 May 2020

type 2 diabetes [15]. The development of obesity is closely related to the metabolism of body fat. The main diet (90%) is composed of mixed triglycerides. Exogenous fat cannot be directly used by the human body and must be hydrolysed for absorption. Lipases present in the digestive system include tongue lipase, gastric lipase and pancreatic lipase. Gastric lipase is often considered to be an important factor that regulates pancreatic lipase secretion and plays an auxiliary role in lipolysis [16,17]. The most important of these is pancreatic lipase, which directly affects the absorption of fatty acids in the intestine. Pancreatic lipase (PL) is the primary lipase secreted from the pancreas, that hydrolysed dietary lipids in the digestive system, converting triacylglycerol substrates found in ingested oils to monoglycerides and free fatty acids. In the intestine, monoglycerides and free fatty acids are subsequently moved to enterocytes, cells lining the intestines and then absorbed [18,19].After the fat-containing food is ingested by the human body, the triglyceride-based lipid is first hydrolysed by lipase to monoglyceride, glyceryl ester and free fatty acid, and the content of 1,2-glycolide and fatty acids in the product is higher. The degradation of fat by the lingual lipase is very small, but it can degrade 50% to 70% of the fat intake of infants and young children. Next, it is hydrolysed into free fatty acids and monoacylglycerol by gastric lipase (10%～30% decomposition) and pancreatic lipase (50%～70% decomposition) in the gastrointestinal tract and small intestine, and then cholesterol and lipoprotein are formed in the body. Lipid mixed particles, such as bile acid, are absorbed by the small intestine, and re-synthesis of triacylglycerol stores energy in the form of adipose tissue.

2.2. Metabolic mechanisms of Pancreatic lipase (PL) inhibitors inhibitors

Fig. 1 shows the lipid metabolic pathway in the human body [20]. The lipase inhibitors, by combining with the active lipase part of the stomach and small intestine, changes the conformation of the stomach/trypsin, inhibits catalytic activity, and thus reduces lipids such as triglycerides. The hydrolysis reduces the digestion and absorption of lipids in food as well as the accumulation of adipose tissue and achieves the effect of controlling and treating obesity [21]. After the lipase inhibitor acts, it is usually excreted along with the lipase to which it is bound. Therefore, it does not cause long-term effects in the human body.

Currently, commonly used weight loss medicines include peripheral lipase inhibitors and central appetite suppressants, which are mainly divided into two categories: (1) medicines that inhibit intestinal absorption of fat, such as orlistat. (2) An appetite suppressant acting on the central nervous system that is mainly composed of fenfluramine and sibutramine, but a several clinical studies have found that taking these medicines makes patients more prone to adverse reactions, such as headaches, dizziness, dry mouth, and bitterness, constipation and insomnia. More seriously, these medications may cause various mental or cardiovascular adverse reactions, which greatly limits the scope of clinical applications, and has even led to medications being withdrawn. As the safety of central medicines has not yet been fully clarified, the advantages of lipase inhibitors acting on peripheral elements in the development of new medicines are based on the fact that they do not enter human blood vessels or the nervous system, and they have no effect on the balance of body's minerals and bone circulation. Therefore, lipase inhibitors have been proven to be relatively safe.

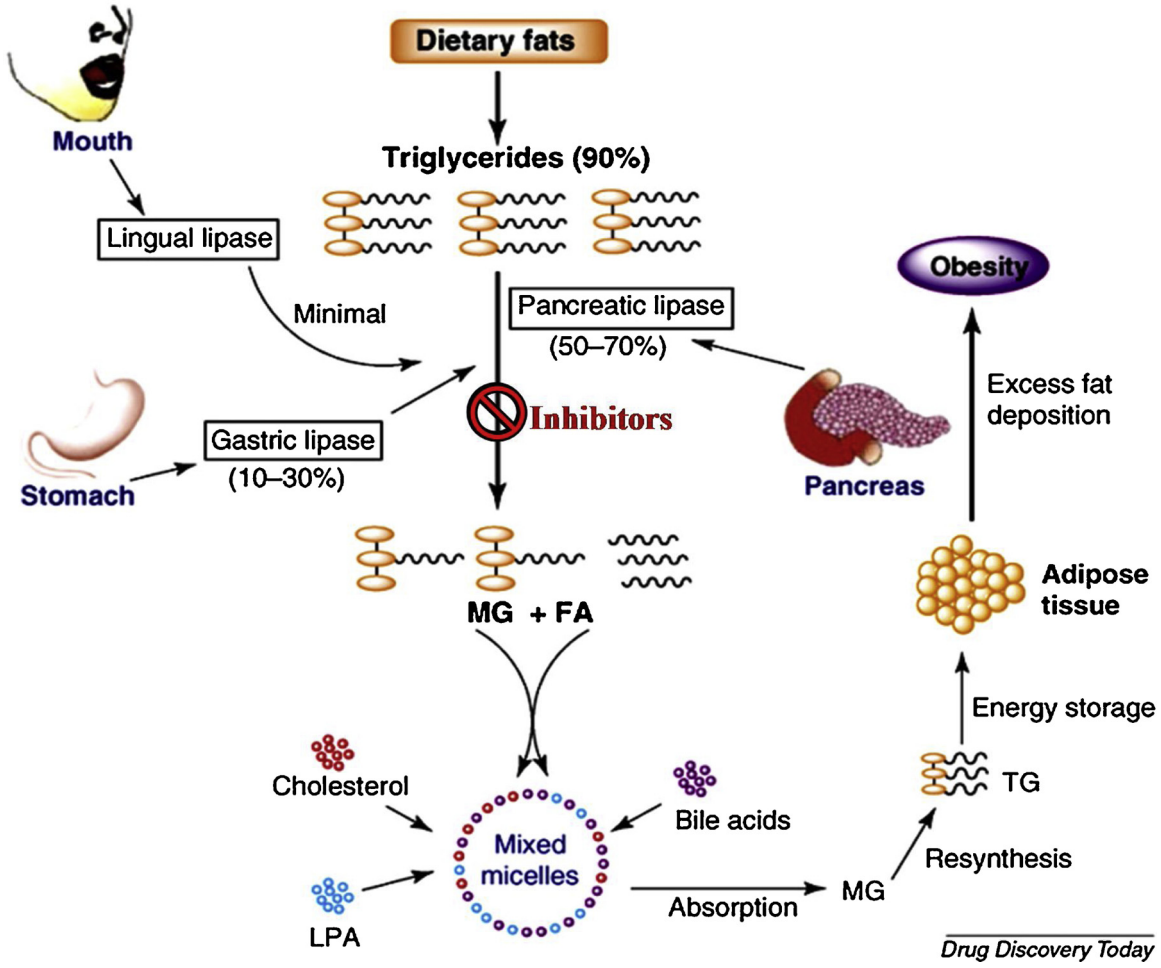

Fig. 1. Lipid metabolic pathways in the human body.

Table 1
Orlistat targeting sites and related reports.

Interaction sites	Interaction types	Sources	PMIDs	Score
PNLIP (Pancreatic triacylglycerol lipase)	inhibitor	Tdg Clinical Trial/ Guide To Pharmacology Interactions/ ChemblInteractions/ TEND/DrugBank	10730683|10095983|18200802| 15382615|12007529|10338682| 16956313|16259579|10932681| 11752352|9711448|11728255| 11054601	18
LIPF (Gastric triacylglycerol lipase)	inhibitor	ChemblInteractions/ TEND/DrugBank	10730683|15563254|18987274| 18200802|12007529|16956313| 16259579|11728255	11
FASN (Fatty Acid Synthase)	inhibitor	TdgClinicalTrial/ DrugBank	16749788|15138278|15870086 |11752352|16091048|15026345	8
SERPINE1(Endothelial plasminogen activator inhibitor)	None	NCI	11410822	2
LPL (Lipase)	None	TdgClinicalTrial	None	1

A number of clinical trials have confirmed that orlistat as a pancreatic lipase inhibitor can reduce obesity caused by a high-fat diet, and that taking orlistat can lead to oily stool [22–24]. Lipase inhibitors have become an important means of clinical obesity treatment. In Table 1, we list that orlistat has been widely used in clinical practice as an inhibitor on pancreatic triacylglycerol lipase and gastric triacylglycerol lipase.

2.3. Effect of lipase inhibitor on lipid metabolism of obesity in vivo

Lipase inhibitors have been shown in numerous animal and clinical trials to improve lipid metabolism in obese individuals. By inhibiting the absorption of fatty acids and thereby reducing the accumulation of fatty acids in the body, meanwhile, reducing the level of LDL in the serum and increasing the level of HDL. In the study of Nam-Hee Kang et al., lipase inhibitors named *protamine* and *chitooligosaccharide* reduced the levels of triglycerides, LDL content and increased HDL contentin the liver or serum [25]. Previous study from Huanhuan Chen et al has shown that Soluble Dietary Fibre (SDF from micronized powders of lotus root nodes) significantly reduce the Pancreatic lipase activity and the LDL content, improved the HDL content, prevented the growth of adipocyte and regulated the lipid metabolism in a nutritional mice model of non-alcoholic fatty liver disease (NAFLD) [26]. In our previous studies, we found that *Theophylline* extracted from Fu Brick Tea as a lipase inhibitor can improve the lipid transport environment in the body. For example, the types of phosphatidylcholine (PC) and phosphatidyl ethanolamine (PE) in the liver are increased, and the accumulation of diacylglycerol (DAG), fatty acid (FA) and acetylated fatty acid (FAHFA) in the liver is reduced, because the hydrolysis of triacylglycerol in the intestine is reduced due to pancreatic lipase inhibitor activity intestinal tract. Research from Meng Xu et al, *Theophylline* extracted from coffee has also been shown to have an effect on relieving LDL concentrations in serum and increasing HDL concentrations [27]. Biochemical sequelae of orlistat pharmacological intervention include significant reductions in serum total cholesterol and LDL cholesterol concentrations in orlistat-treated patients [28]. Other experiments from D. O'Donovan1 et al showed that lipase inhibitors were also found in clinical studies to promote the emptying of food in the stomach and relieve blood pressure after meals in patients with type 2 diabetes [29].

3. Lipases and inhibitors

Lipase (EC 3.1.1.3) is a special ester bond for hydrolysing fat. It can catalyse the hydrolysis of natural substrate oil (triglyceride) on the oil-water interface, and hydrolyse triglyceride to glycerol esters, monoglycerides, glycerol and fatty acids gradually. It has a high degree of chemical and corresponding selectivity. The first study of lipase was reported in 1834, and the activity of rabbit pancreatic lipase has been investigated for hundreds of years [30].

3.1. Classification of lipase

Lipase substrate lipids can be roughly classified into neutral lipids, phospholipids, sphingolipids, ether lipids and oxidized lipids. According to this classification, we can classify lipase into neutral esterase, amidase and phospholipase. There are many neutral enzymes: fatty triglyceride lipase, hormone sensitive lipase, carboxyl esterase, triacylglycerol esterase (pancreatic lipase, gastric lipase), hepatic lipase, endothelial esterase, lipid protein lipase, lysosomal acid lipase, monoacylglycerol lipase (MAGL), diacylglycerol lipase, cholesterol lipolytic enzyme, etc. Amidase is mainly fatty acid amide hydrolase (FAAH). Phospholipase mainly has PLA (TF34), PLA_2, PLC and PLD [31].

This paper focuses on pancreatic lipase and gastric lipase inhibitors as well as two special enzymes, MAGL and FAAH. Drug therapy with inhibitors for obesity can be divided into two categories. The first category is to prevent the absorption of fat or carbohydrates and reduce the energy-absorbing substances that absorb energy from food. The second category is to suppress appetite and increase satiety, which reduces body energy intake. Pancreatic lipase and gastric lipase inhibitors are mainly involved in fat metabolism, which prevents the hydrolysis of macromolecular lipids, reduces the absorption of small molecules of fat and reduces fat accumulation. The two special lipases that function as appetite suppressants, MAGL and FAAH, are mainly involved in the degradation of endogenous cannabis. There are two endogenous cannabinoids (EC) that have been confirmed so far: N-arachidonic acid amino glycol (anandamine) and 2-arachidonic acid glycerol (2-AG), which are potentially useful for treating some conditions, including pain, inflammation and anxiety [32]. American researcher Kenny found [33] that the fat in food stimulates a naturally occurring marijuana-like chemical, the endogenous cannabinoid. From an evolutionary point of view, there are few opportunities to obtain natural fat from nature, but fat is essential for maintaining the normal function of cells, so when animals are exposed to fatty foods, they instinctively produce a reflex signal to the brain. The brain passes the information through vagus nerve transfer to the intestines. Transmitted to the stomach, the signal stimulates endogenous cannabinoids to be produced in large quantities, which causes people to suddenly want to eat more high fat foods. However, for humans, fatty foods are not so rare now, but the instinct to secrete the hormones that lead to a desire to eat high-oil and high-fat foods still exists, which may lead to dangerous diseases, such as obesity, diabetes and cancer. The results of this research may help people find ways to solve this problem, such as blocking the transmission of addictive signals by controlling the reception of endocannabinoids through medicines (inhibitors). If endogenous cannabinoids are blocked in the brain, people will feel anxiety and sorrow, but the drug actually works outside the brain, so it does not cause the side effects of the central inhibitors mentioned above. Afzal.[34] guided molecular modelling and structure-based methods to obtain novel MAGL inhibitors. Their work also suggested that monoacylglycerol lipase plays a

key role in the endocannabinoid nervous system, and its inhibitors have other medical therapeutic effects in addition to fat loss, such as treatment of pain, inflammation, cancer and central nervous system diseases.

3.2. Sources of lipases

Lipases are widely available and are ubiquitous in animal and plant tissues as well as a variety of microorganisms. Most of the lipases used in research are derived from microorganisms, and there are many types of microbial lipases. There is a larger variety of microbial lipases than the plant and animal lipases in terms of functional pH, temperature and the specificity of the substrate for action, which is convenient for industrial production and generating enzymes with high purity. These factors have promoted the research of basic and practical applications of lipases in various fields. There are many types of microorganisms that produce lipase, and 65 genera have been reported so far, including 28 genera of bacteria, 10 genera of yeast, 4 genera of actinomycetes, and 23 genera of other fungi [35]. Currently, the research of microbial lipase mainly focuses on Rhizopus, Aspergillus, Penicillium, Mucor, Candida, Pseudomonas. (Pseudomonas), Burkholderia and other strains. Table 2 lists some basic enzymatic properties of lipases from different microbial sources. The optimum temperature of most lipases is between 30 and 50 °C, the optimum pH is alkaline, and the activity is affected by some metal ions and organic solvents.

3.3. Mechanism of action for lipases and inhibitors

To know how the lipase inhibitors work, first it is necessary to understand the mechanism of the interaction between a normal lipase and substrate. A study by Winkler [50] found that although different sources of lipases have different amino acid compositions (residues, molecular weights, three-dimensional structures, etc.), due to biological homology and conservation of evolutionary processes, their catalytic centres have similar or identical characteristic regions, including His-XY-Gly-Z-Ser-W-Gly or Y-Gly-His-Ser-W-Gly (W, X, Y, Z refers to non-specific amino acids). This conserved region provides a useful reference to scholars who study the properties of lipase inhibitors through animal experiments. Human pancreatic lipase consists of 449 amino acids with a coated catalytic centre of the N-terminus, including Ser-152, His-263 and Asp-176. Typically, the conformation of the lipase begins to change, and the "lid" is opened, which exposes the hydrophobic part of the active site. This opening increases the binding capacity of the substrate and lipase. Simultaneously, the substrate can easily enter the hydrophobic channel and bind to the active site [51]. After chemical modification of the Ser-152, which constitutes the ternary active centre of the C-terminal edge located in the N-terminal domain close to the β-layer of the double helix structure, it was found that the enzyme activity was significantly reduced, that is, Ser-152 is an essential factor for enzyme catalytic activity [50]. Pancreatic lipase activity depends on another pancreatic exocrine protein, colipase. The colipase is formed by the division of the pro-lipase-producing pro-colloid (Procolipase) secreted by the pancreas. The procolipase can specifically bind to the C-terminal region of pancreatic lipase and does not induce any changes in its conformation [52]. Delorme [53] synthesized a phosphate lipase inhibitor by a carrier chemical functionalization technique that monitors the active site of the enzyme. It is immobilized on a carrier through extensive covalent immobilization, which forms a functional orientation capture tool for serine hydrolase for capture and physical adsorption. Furthermore, the selective mass spectrometry analysis of captured lipases from some complex biological extracts confirmed that the lipase inhibitor supporting covalent capture has an inhibitory effect. This "fishing enzyme" tool, which was studied by examining the active site of the enzyme structure, provides a new perspective on the applications of lipase inhibitors and proteomics.

Table 2
Lipase properties from different sources of microbial lipases.

Source of bacteria	Optimal Temperature	Optimum pH	Affecting active ions		References
			Promote	Inhibition	
Root enzyme R*hizopus ZM - 10*	35 ℃	8.0	Na^{+}, Cu^{2+}, Fe^{3+}, SDS, EDTA	Zn^{2+}, alcohol organic solvent, Tween-80	[36]
Rice root enzyme R*hizopus oryzae*	35 ℃	7.5	Mg^{2+}	TritonX100、SD, Fe^{3+}, Fe^{2+}, Cu^{2+}, Hg^{2+}	[37]
Huagen enzyme *R hizopus chinensis saito*SSLl	40 ℃	8.0	K^{+}	Cu^{2+}, Hg^{2+}	[38]
Aspergillus niger	30 ℃	7.0	Triton X-100	Fe^{2+}, Zn^{2+}	[39]
Aspergillus *terreus*	50 ℃	4.0	Ca^{2+}, Co^{2+}, Mn^{2+}, Ni^{2+}	Hg^{2+}	[40]
Genus *flavigena* UNP3	30 ℃	7.0	Mg^{2+}	Hexane, benzene, ethanol, Fe^{2+}, Hg^{2+}, Ag^{2+}, Zn^{2+}	[41]
Propionibacterium *P. shermanii*	47 ℃	7.2		AsO_4^{3-}, Hg^{2+}	[42]
Penicillium	35 ℃	7.0	Ca^{2+}, Mn^{2+}, Cr^{2+}		[43]
Penicillium Chrysogenum J23	33℃	7.5	Ca^{2+}, Mg^{2+}, K^{+}	Fe^{2+}, Mn^{2+}, Cu^{2+}, Pb^{2+}, Li^{2+}	[44]
Hair enzyme *Mucor*	28℃	6.0	Tween -80		[45]
Candida *Candida* 99-125	40℃	8.0	Ca^{2+}, Mg^{2+}, Triton X - 100, Tween80	Cu^{2+}, Zn^{2+}, Ni^{2+}, Fe^{2+}, SDS	[46]
Pseudomonas	40℃	9 . 0	Li^{+}, Mg^{2+}, Ba^{2+}, Ca^{2+}, Tween 80	EDTA, acetic acid Ethyl ester	[47]
Pseudomonas aeruginosa C7828-5	37℃	8.0	Mg^{2+}, Mn^{2+},Fe^{2+}	Cu^{2+}, K^{+}	[48]
Klebsiella *Klebsiella sp* A2	45℃	10.0	Triton X - 100, Tween 80, K^{+}	Pb^{2+}, Ba^{+}	[49]

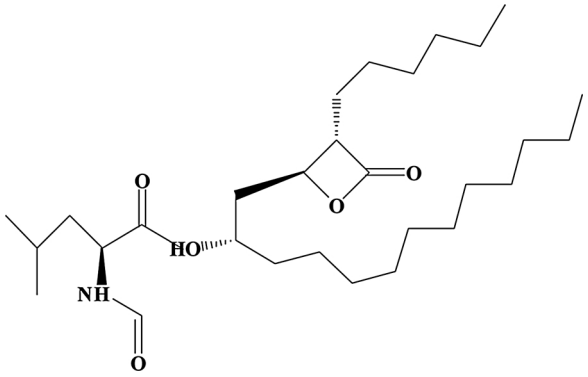

Fig. 2. The molecular structure of orlistat.

4. Lipase inhibitor anti-obesity status

4.1. Clinical listing of lipase inhibitors

Orlistat is the only lipase inhibitor diet drug currently in clinical use and is the only anti-obesity medicine that does not act on the central nervous system or enter the bloodstream. Lipstatin, which is found from St. streptococci by Ballinger, is a potent irreversible inhibitor of pancreatic lipase. Roche Corp. succeeded in hydrogenating lipstatin to a more stable inhibitor called orlistat, which is a tetrahydro derivative of lipstatin and was approved by the FDA as an anti-obesity drug in 1997. The chemical structure of orlistat is shown in Fig. 2. It exerts an inhibitory effect by covalently binding to a serine residue at the active site of the lipase and mildly improves the concentration of total cholesterol, low density lipoprotein, blood pressure, fasting blood glucose, and insulin [54]. In addition, clinical studies have shown that orlistat has a beneficial effect on cirrhosis, fatty liver, disorders of glucose metabolism, insulin resistance, blood pressure, and hyperlipidaemia leading to atherosclerosis. A typical clinical dose is 120 mg every time, 3 times a day, and this dose can reduce the fat absorption inhibition rate by approximately 30% with a significant anti-obesity effect. Currently, experimental chemical synthesis or natural product screening basically uses the inhibitory activity of orlistat as a reference for comparative study.

The latest research shows that orlistat does not affect the secretion and appetite of incretin, but it reduces the body's absorption of certain lipid nutrients, which affects the body's absorption of certain fat-soluble vitamins such as A, D, and E. Therefore, it is necessary to supplement standard multivitamins during daily use to prevent abnormal vitamin serum concentrations. Orlistat also affects the absorption of essential fatty acids, such as arachidonic acid (ARA), eicosapentaenoic acid (EPA), and docosahexaenoic acid (DHA). Therefore, it needs to be supplemented with fish oil or a particular diet to improve the lack these fatty acids [55]. It is worth noting that excessive supplementation of DHA and EPA can also cause adverse reactions, such as dizziness and nausea, and can even change the original antihypertensive effect of lowering blood pressure and become hypertensive and proinflammatory. The main adverse side effects of this lipase inhibitor occur in the gastrointestinal tract, which often occurs early in treatment, and the adverse reactions tend to decrease with continued treatment. Potentially severe gastrointestinal discomfort limits its clinical effects to some extent. The safety and effectiveness of this drug for long-term weight maintenance, cost-effective treatment, overall fat-related morbidity and mortality have yet to be determined. Therefore, the above problems are also problems that need to be solved in the development of lipase inhibitor medicines.

4.2. Chemical syntheses of lipase inhibitors

Currently, there are many chemically synthesized lipase inhibitors, but most of them are applied to endogenous cannabinoids, such as MAGL and FAAH inhibitors. Chupak [56] studied the structure-activity relationship, profile characteristics and physicochemical properties of a series of diglyceride lipase (DAGL) inhibitors for the first time. N-benzyl-substituted glycine sulfonamides reversibly inhibit diglyceride (DAG) lipase. The most effective inhibitors obtained included those that are highly lipophilic, lipophilic, and that do not accept alpha-carboxy acids and homologous reactions. Alanine and β-alanine derivatives have no inhibitory activity. The inhibitory effect of 2-(N-(1-(30,50-dichloro-[1,10-biphenyl]-3-yl) cyclobutyl)-2, 2-dimethylchro man-6-sulfonamido) acetic acid was found to be ideal. There is no linkage to the enzyme supply and the type of inhibition is reversible. Matthias [31] selected eight FAAH and MAGL potent inhibitors for in vitro comparison experiments. The most active compound found in this study was N,N-dimethyl- 5-(4-phenoxy) 2H-tetrazole-2-carboxamide (IC_{50} FAAH: 0.012 μM; IC_{50} MAGL: 0.028 μM). This inhibitor is modified in an orderly manner in the lipophilic 4-phenoxy region. The cleavage between the tetrazole ring of the FAAH/MAGL inhibitor and its amide substituent is easily deactivated. In recent years, studies have shown that MAGL inhibitors can function as an analgesic, anxiolytic, antiemetic, etc., and they can address the addiction paradigm through the enhanced performance of nerve signals, which leads to attenuated pain. MAGL inhibitors have also been shown to exert anti-inflammatory effects in the brain, which prevents neurodegeneration by reducing the production of eicosanoids. In terms of tumours, MAGL inhibitors have been shown to have anticancer properties, not only for regulating the endocannabinoid-eicosanoid system network but also for controlling the release of pre-synthesis tumour signalling lipid fatty acids. In summary, MAGL acts as a key point in the human physiology and disease environment while coordinating multiple lipid signalling pathways. MAGL inhibitors will also have great potential to address the treatment of a large number of complex diseases in humans [57].

Zhang [58] conducted a total synthesis study of a natural pancreatic lipase inhibitor. Norartocarpin was generated for the first time through 14 steps with inexpensive and readily available trimethoxybenzene, and the total yield was 0.41%. In the course of the synthetic research, the complete synthesis of another active natural product, Artocarpin, was also completed for the first time. Moreover, it was found that the flavonoid nucleus is a structure necessary for pancreatic lipase inhibitory activity, and the intermediate without a flavonoid nucleus has no inhibitory activity. The methyl, butenyl and phenolic hydroxyl groups were also found to be important activity-determining groups. These works have great value for the development and utilization of natural prenyl flavonoids.

4.3. Natural product sources

China has rich natural medicine resources and a long history of application of Chinese herbal medicines, and people have accumulated various valuable local medicinal materials in their struggle against diseases, which have become important sources for medicines and leading compounds. Plant-derived natural compounds are characterized by structural diversity, low toxicity, and a wide range of sources. From a safety point of view, natural chemical-derived inhibitors are more applicable than chemically synthesized medicines, so screening new lipase inhibitors from plants with fewer side effects has been a hot topic in research. Common natural sources of lipase inhibitors contain active substances, including polyphenols, flavonoids, saponins, terpenoids, alkaloids and other active substances. We have listed some of the active ingredients and inhibitory activities of inhibitors from different natural sources from the past five years in Table 3. It has been shown that there is great potential for the extraction of lipase inhibitors from plants and fruits and vegetables, and some researchers have obtained known single products, whereas some have only studied the inhibitory activity of extracts on lipase. Thus, there is an inevitable research difficulty because it difficult to separate and extract natural product

Table 3
Natural sources of lipase inhibitors.

Material	Effective active ingredient	Inhibitory activity/mechanism	References
Pu'er tea	Tea polyphenols, Tea polysaccharides	Significantly reduces body weight, triglyceride and total cholesterol concentrations	[59]
Brick tea	Polyphenols, flavonoids	Significantly reduces triacylglycerol, cholesterol and low-density lipoprotein	[60]
Kuding tea	Ergoside	Non-competitive inhibition, $K_I = 1.88 \times 10^4\ M^{-1}$	[61]
Black tea	Polyphenols, caffeine, theaflavins	Non-competitive inhibition, caffeine IC_{50} = 1.12 mg/mL, Theaflavin IC_{50} = 0.83 mg/mL	[62]
Chrysanthemum morifolium	10a-hydroxy-1a, 4a-endoperoxy-guaia-2-en-12, 6a-olide(flavonoid glycosides)	IC_{50} = 161.0 μM	[63]
Rose	Piper phenol	Major inhibition of large intestinal lipase	[64]
Mushroom	Methanol extract	Inhibition rate 79.07%	[65]
Turmeric	Ethanol Extract (FTE) Gingerol and diaryl heptane	Mouse fat weight loss The presence of thioesterase	[66,67]
Dried ginger powder	Ginger alcohol, ginger phenol	IC_{50} = 1.29 mg/mL	[68]
Adzuki bean	polyphenols	IC_{50} = 12.5 μg/mL	[69]
Buckwheat	Buckwheat flavonoids, buckwheat alcohol	IC_{50} = 1.94 mg/mL	[70]
Apple pomace	Pectin	Inhibition rate 94.3%	[71]
Green pepper	Ethanol extract (capsaicin)	Inhibition rate 46.15%	[72]
Lotus leaf	Alkaloid	Inhibition of adipocyte proliferation	[73]
Grape seed	Ethanol extract (polyphenols)	Inhibition rate > 61.53%	[74]
Ginseng / American ginseng	Saponin	Has an effect on blood lipids, liver fat	[75]
Platycladus	Ethyl acetate extract	IC_{50} = 26. 09 μg/mL	[76]
Chickpea	Saponin	IC_{50} = 9.74 μg/mL	[77]
Brown algae	Brown algae polyphenol	IC_{50} = 37.2 μM	[78]
Hebridean brown algae	Algae polyphenol	IC_{50} = 0.119 mg /mL	[79]
Alginate bread	Alginate	Will not decompose and deactivate at high temperatures	[80]
Houttuynia	Water extract (WEH)	Inhibits absorption of unesterified fatty acids and glycerol	[81]
Tortoiseshell (Sponge)	Brominated unsaturated lipid	IC_{50} = 3.11 μM	[82]
Mosquito (Japan)	Flavonoid glycoside	IC_{50} = 26 μM	[83]
Horned squash	Second	IC_{50} = 9.47 μg/mL	[84]
Salacia reticulata	Proanthocyanidin	IC_{50} = 10.9 μM	[85]
Luo Zizi (legume)	Methanol extract	IC_{50} = 152.0 μg/mL	[86]
White birch	Birch acid	IC_{50} = 21.10 mM	[87]
Monascus pigment	Aromatic and non-polar aliphatic L-, D-amino acids	L-Leu-OEt IC_{50} = 12.2 μM L-Tyr-OEt IC_{50} = 13.8 μM	[88]
Kanzinoki	Shushu Ning A (phenolic)	IC_{50} = 28.4 μM	[89]
Ural licorice	Licorice Chalcone A	IC_{50} = 35 μg/mL	[90]
Nepeta japonica Maximowicz	Ethanol extract	Inhibition rate = 37.3%	[91]
Araucaria	Condensed tannin	K_I =332.7 μg/mL	[92]
Origanum vulgare	Phenolic Resin	IC_{50} = 7.26 μg /mL	[93]
Resveratrol (Plumbago zeylanica)	Naphthoquinone (Plumbagin)	IC_{50} = 82.08 μM	[94]
Cocoa	polyphenols	IC_{50} = 8.5 μg/mL	[95]
Mentha viridis	Flavonoids, condensed tannins	IC_{50} = 0.43 mg/mL	[96]
Mangosteen (Garcinia)	α-mangostin (1)	IC_{50} = 5.0 μM	[97]

components. However, it can be concluded that most of the extracts contain polyphenols and flavonoids as effective active substances to inhibit lipase catalytic activity.

Polyphenols are a general term for polyhydroxyphenolic compounds. They are widely found in vegetables, fruits and various medicinal plants. They have antioxidant, antiviral, hypolipidaemic and other biological activities, and they have inhibitory effects on various enzymes. The most common ones are tea polyphenols in tea, black tea and green tea, which contain different levels of polyphenols and various active lipase inhibitor active substances, such as catechins, theaflavins, and caffeine. Green tea catechins can alter the synthesis of enzymes by inhibiting lipid metabolism of fatty acids and increase fat oxidation by stimulating the sympathetic nervous system. Zhen [98] explored acteoside, which is a rich active ingredient in Kudingcha, and developed its anti-obesity effect by studying its effect on lipase. It was shown that acteoside binds to the lipase Lys271, Leu272, and Thr68 hydrogen bonds. This non-covalent combination alters the molecular conformation of the lipase, which reduces lipase activity. Hu [99] separated Ligupurpuroside A, which is an active substance also from Kudingcha. The results show that it can bind to the hydrophobic cavity amino acid residue at the lipase catalytic site, which results in decreased enzyme activity. This study contributes to the design and application of Ligupurpuroside A in the food industry for weight loss medicines.

Mucor miehei lipase (EC3.1.1.3) was used as a lipase, and p-nitrophenol palmitic acid (p-NPC) was used as a substrate in our laboratory. The effective substances in Fu Brick tea were selected. Phenols, such as epigallocatechin gallate (EGCG), epigallocatechin (EGC), and epicatechin (EC), as well as organic acids, such as salicylic acid, glycolic acid, oxalic acid, citric acid, and benzoic acid, were analysed. The results showed that EGCG, EGC, EC, oxalic acid, glycolic acid, p-hydroxybenzoic acid, citric acid and salicylic acid have good inhibitory effects on lipase. The IC_{50} values were 0.016, 0.027, 0.177, 13.039, 15.469, 17.297, 18.297, and 26.219 mmol/L, respectively. All of the effectors had reversible inhibition, and all of them had mixed inhibition types, except for salicylic acid, which has a non-competitive inhibition type. Further endogenous fluorescence quenching analysis showed that EGC and EGCG changed the structure of the enzyme. Among them, EGCG had the largest number of phenolic hydroxyl groups and galloyl groups in the molecular structure, and the binding constant and binding site number for lipases were the largest. In this study, after screening high-activity pancreatic lipase inhibitors from natural compounds, the mechanism and structure-activity relationship

of pancreatic lipase were studied in depth. The natural products that were screened were used as leading compounds and molecular modification was performed chemically or in microbes to obtain a pancreatic lipase inhibitor with a higher activity and yield.

Flavonoids are the main active ingredients in many medicinal plants and have a wide range of pharmacological effects. Saponins are widely distributed among plants, and they are composed of sapogenins and sugars, uronic acids or other organic acids. Saponins have various biological activities, such as sterilization, anti-inflammation and antitumour activity. In recent years, saponin compounds that inhibit pancreatic lipase have been isolated from different plants. Studies have found that flavonoids and saponins not only have lipase inhibition but also α-glucosidase inhibitory activity, which can prevent hyperglycaemia, alleviate hyperinsulinaemia, increase glucose tolerance, and prevent and treat obesity. Ercan [100] used a static in vitro digestion method to determine the total saponin content [101] and in vitro bioaffinity of the tribulus and chickpea. At the same time, the in vitro inhibitory effects of lipase, α-amylase and α-glucosidase on the selected food samples were evaluated.

Terpenoids are hydrocarbons with molecular formulas containing multiples of isoprene and their oxygen-containing derivatives. They are widely found in nature and have certain physiological activities. Patil extracted a pancreatic lipase inhibitor from the root of squash (Rosaceae) and performed kinetic parameter analysis to explore its hypolipidaemic activity. The results showed that the purified diterpene moiety showed the strongest inhibitory activity IC_{50} of 9.47 μg/mL and the standard inhibitor orlistat IC_{50} was 0.15 μM as a control. For the purified diterpenoid moiety, the K_m value is increasing while V_{max} remains unchanged. The inhibition constant of the purified diterpenoids was significantly lower compared to the forward control (orlistat), and in addition, the purity specificity of the purified substance (K_{cat} /K_m) was also found to be greatly reduced. According to kinetic analysis, the inhibitors have a competitive inhibition type.

Alkaloids are a class of nitrogen-containing basic organic compounds found in nature. They are also important active constituents of many Chinese herbal medicines and have various biological activities. The alkaloid component extracted from lotus leaf [73] has an inhibitory effect on pancreatic lipase. Liu [102] characterized the pancreatic lipase activity through determining the release rate of oleic acid from triolein and comparing the weight loss effects of 30 kinds of fruits and vegetables ethanol extracts. The results showed that the ethanol extract from green pepper had the strongest inhibitory effect on pancreatic lipase, and the inhibition rate was 46.15%. Capsaicin can promote hormone secretion, accelerate metabolism to achieve the effect of burning body fat, while also inducing heat production and dissipation, which prevents fat accumulation and achieves weight loss.

4.4. Lipase inhibitor screening and detection methods

The screening of pancreatic lipase inhibitors in plants has been a hot topic in the research of diet pills at home and abroad. Although a large number of natural compounds that inhibit the activity of pancreatic lipase have been screened from plants, the mechanism of action of most isolated compounds is not known. Therefore, screening and detection methods for lipase have become an indispensable area of research.

Liao [103] developed a method for rapid screening and identification of lipase inhibitors of oolong tea by using lipase-functionalized magnetic nanoparticles as a stationary phase for high-performance liquid chromatography-mass spectrometry extraction. Three pancreatic fat ligands were screened and identified: (-)-epigallocatechin-3-3-gallic acid (EGCG), (-)-catechin-3-O-gallic acid (GCG), (-)- epicatechin-3- O-gallic acid (ECG). Their inhibitory activity is significantly higher than those that do not have a ligand. The structure-activity analysis showed that the substance capable of adsorption in the stationary phase required the presence of a galloyl group, which had a strong inhibitory effect on the lipase. Fan [104] designed a method for rapidly screening lipase inhibitors from traditional Chinese medicine decoctions. The study of lipase inhibitors was performed by HPLC-MS ultrafiltration and separately applied to four Chinese herbal medicines (Wu-Ling-San, Ze-Xie, Xiao-Xian-Xiong and Xiao-Chai-Hu) to screen, compare and analyse the. As a result, 16 natural lipase inhibitors were obtained and identified through high-level analysis and multi-MS analysis. The inhibitory activities of the two compounds have been confirmed by lipase functional analysis, which verifies the reliability of the method. Molecular docking simulations were conducted to explore the potential mechanisms of action for these compounds. Bayineni [105] developed a proprietary method based on thin-layer chromatography to detect lipase inhibitors from a chemical system consisting of p-butyric acid p-nitrophenyl ester and bromothymol blue. Through this method, the inhibition zone for lipase can be directly observed, which appears as a blue spot on a green-yellow background. The concentration of orlistat can reach 1 ng, which is better than other methods. Tao [106] selects an immobilized enzyme (HF-AS) based on the affinity with hollow fibre, that is, the surface of the polypropylene aerial fibre has a stable matrix ligand assembly to adsorb porcine pancreatic esterase. A new LC/MS method for affinity screening of medicinal plant lipase inhibitors was developed. Three lipase inhibitors were screened and identified in lotus leaves: quercetin-3-Od-arabinose-(1→2)-d-galactoside, quercetin-3-Od - Glucuronide and kaempferol-3-Od-glucuronide. Savinainen [107] describes a general fluorescence-based method for screening lipase inhibitors. Monoglycerol lipase (MGL) is a serine hydrolase that is primarily responsible for signal termination of the main neuron 2-AG. The authors have been able to identify time- and dose-dependent MGL inhibitors with one or more binding sites by improving fluorescence-based techniques, whereas inhibitors that exhibit reversible inhibition types have a simple kinetic analysis format. Known reference materials and novel inhibitors, such as JZL184 and CAY10499, have been evaluated for their MGL binding properties and energy efficiency.

5. Summary

The incidence of obesity-related diseases has been rising, and various research institutions and biopharmaceutical companies have been studying weight-loss drugs. The regulation of the weight-loss drug market and the need for further development of weight-loss products are becoming more and more urgent. Lipase inhibitors have also received more and more attention. Lipase inhibitors derived from natural products have become potential research hotspots. Compared with chemically synthesized lipase inhibitors, plant-derived lipase inhibitors are widely available, relatively cheap, relatively safe and reliable, although they have inhibition grade and are difficult to determine active components. These natural inhibitors play an important role in the development of slimming drugs or health products. There are many researches on lipase inhibition from natural products and some of them have obvious inhibitory effects, but few of them have been applied to clinical stage. This may be due to the low content of active ingredients, complicated extraction procedures and low recovery rate of lipase inhibitors derived from natural products, which cannot be produced in large quantities. This is also a major bottleneck for the industrialization of lipase inhibitors derived from medicinal and edible plants. If the mechanism of action and structure-activity relationship of natural compounds on pancreatic lipase are further studied while the high-activity pancreatic lipase inhibitors are continuously screened, the screened natural products are taken as lead compounds, and the chemical synthesis method is used to carry out molecular modification and microbial method to improve the yield, so as to obtain pancreatic lipase inhibitors with stronger activity and higher yield, or the microbial method is used for modification, so as to obtain pancreatic lipase inhibitors with higher activity, and finally the pancreatic lipase inhibitors are applied to clinical treatment of obesity, the field of traditional Chinese medicine will achieve a further development, which is also the direction of key research from this field in the future.

Disclosure statement

The authors report no declarations of interest.

Acknowledgements

This study was supported by the Natural Science Foundation of China (No. 31571896), Fujian Natural Science Foundation (Grant No. 2016J0101), Xiamen Science and Technology Project (Grant No. 3502Z20174059).

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