

Application of Glucagon and Glucagon-Like Peptide Agonists in Disease Treatment

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Abstract. With the continuous advancement of medical technology, glucagon has been confirmed to be closely related to many diseases, such as diabetes, glucagonoma cell hyperplasia and neoplasm (GCHN), hepatitis and neurological diseases, which take numerous life each year. The identification of several diseases, like Mahvash syndrome as an autosomal recessive inherited pancreatic neuroendocrine tumor syndrome associated with GCGR inactivation mutations deepens our understanding of GCG signal pathway. As disease mechanisms are better understood, more novel drugs are being developed to treat these diseases. Glucagon-like peptide agonists, such as cotadutide, are shown to possess neuroprotective properties in the context of neurodegenerative diseases, also exhibits potential in ameliorating blood glucose control, weight management, and liver fat reduction, offering a promising avenue for developing novel treatments. The linkage between GCHN and GCGR gene inactivation highlights the potential role of GCGR in tumorigenesis, emphasizing its relevance in oncological research. In summary, these findings provide comprehensive insights into neuroprotection, metabolic regulation, genetic disorders, and oncology, offering a holistic view of the potential implications of these entities in various medical contexts.

Keywords: Glucagon; GLP-1 agonists; Disease; Treatment.

1. Introduction

Glucagon, an essential pancreatic islet hormone, is comprised of a linear polypeptide chain [1]. It was discovered in 1922, but until 1950 scientists finally determined its amino acid sequence and in 1970s its role in physiology and disease become more fully understood because the development of a new, specific method of radioimmunoassay for the detection of glucagon. The functions of glucagon are to increase sugar level in blood by promote glycogenolysis and increase the process of lipolysis by reducing the amount of fatty acid produced in adipose tissue and liver. Its pivotal roles in maintaining glucose homeostasis are evident in its functions, primarily centered around elevating blood sugar levels. This is achieved through the promotion of glycogenolysis, which involves breaking down stored glycogen into glucose, making it available for immediate energy needs. Glucagon has a great influence in the lipid metabolism. It does so by stimulating lipolysis, a process that facilitates the breakdown of fats into fatty acids, thereby reducing fatty acid synthesis both in adipose tissue and the liver [2].

It was originally thought to be a simple-purpose hormone that can increase hepatic glucose production. Although it has fast tolerance characteristics, and it has an opposite synergistic effect with insulin in hyperglycemia and hypoglycemia states. But the current understanding of it has been expanded to be a peptide hormone that has exhibited several additional metabolic effects, related to satiety and energy expenditure, and thus has potential effects on obesity and dyslipidemia. These findings highlight the interplay of various pancreatic hormones in diabetes pathophysiology and offer potential avenues for therapeutic interventions. Furthermore, irregular glucagon production in pancreatic α cells is proposed as a contributing reason in the process of type 2 diabetes, highlights the need to comprehend the multifaceted roles of glucagon in diabetes research. In clinical practice, glucagon is employed as a treatment for severe hypoglycemia when administered alongside glucose solutions, further emphasizing its clinical relevance in diabetes care [3].



The production and secretion of glucagon is controlled by amylin. In recent years, there are more evidence that shows the unregular production of glucagon is one of the causes of T2D, another founding is that glucagon-like peptide-1 (GLP-1) can dramatically reduce glucagon concentration in type I diabetics. With the fast development in biology and medicine, glucagon-like peptide-1 receptor (GLP1R) can be act as a potential therapy for type 2 diabetes and non-alcoholic steatohepatitis (NASH) due to its special features in weight and fat reduction [4]. Glucagon shows a significant correlation with various illnesses. Glucagon, which is secreted by the α cells, have a considerable influence in maintaining glucose homeostasis. Glucagon released from α -cells communicates through the glucagon receptor (GCGR) located on β -cells, leading to an elevation in insulin secretion. Nevertheless, glucagon exhibits a preference for signaling through the GLP1R situated on β -cells, lead to a more pronounced insulinotropic affect than the GCGR signal pathway [5]. Glucagon is also a center factor in diabetes. In type 1 or type 2 diabetes, patients' concentration of glucagon in blood shows much higher than normal people. Glucagon also emerges as a central factor in diabetes. Individuals with type 1 and type 2 diabetes exhibit markedly elevated concentrations of glucagon in their bloodstream compared to individuals without the condition. Hyperglucagonemia is closely linked to illnesses such as obesity and fatty liver. This connection primarily stems from hyperglucagonemia leading to reduced glucose clearance alongside increased endogenous glucose levels [6]. Moreover, hyperglucagonemia and insufficient glucagon levels in the presence of low endogenous glucose can potentially lead to damage of the sympathetic nerves [7].

However, further exploration is necessary to unveil additional mechanisms of associated with glucagon. On a positive note, numerous therapeutic approaches have been uncovered. For instance, there are methods involving glucagon agonism in conjunction with GLP1R agonists centered around glucagon. GLP1R agonists, established pharmaceutical treatments for managing type 2 diabetes and overweight, mirror the influence of GLP-1, effectively reducing glucose levels by promoting insulin secretion and restraining glucagon secretion. This mechanism underscores their efficacy in diabetes and weight management. On the other hand, the glucagon receptor (GCGR) assumes a great necessity in control the concentration of glucose, amino acid, and fat metabolism. Blocking GCGR, as evidenced by various physiological changes including hypoglycemia, elevated amino acid levels, heightened glucagon concentrations, and reduced adipose tissue and liver fat accumulation, reveals the receptor's substantial influence on multiple metabolic processes. These findings reaffirm the importance of GLP1R agonists in diabetes and obesity treatment, while also highlighting the multifaceted role of GCGR in metabolic regulation, urging further exploration of its therapeutic potential and associated side effects [8]. This research will analyze the application of glucagon and glucagon-like peptide agonists for treatment of different diseases.

2. Application of glucagon and glucagon-like peptide agonists for disease treatment

2.1. Glucagon and GLP-1RAs

2.1.1. Glucagon

Glucagonoma cell hyperplasia and neoplasm (GCHN), a condition primarily affecting middle-aged individuals, manifests clinically with a range of nonspecific symptoms, like abdominal pain [9]. Though the presence of elevated pancreatic glucagon levels, patients harboring GCGR mutations do not display the typical glucagonoma syndrome, suggesting defects in receptor signaling pathways as a potential explanation. The genetic underpinning of GCHN in GCGR-mutated patients adheres to an autosomal recessive pattern. These findings collectively indicate that GCHN is characterized by hyperglucagonemia, pancreatic glucagon cell hyperplasia, and neuroendocrine tumor formation, shedding light on the intricate pathogenic mechanisms and genetic factors contributing to this condition [9].

Neuroendocrine tumors in the pancreas of patients with specific endocrine microadenomas exhibited substantial overexpression of pancreatic glucagon. The Cedars Sinai Medical Center identified the

inaugural case with distinct clinical characteristics and designated the condition as "Mahvash" syndrome. The diffuse pancreatic glucagon cell hyperplasia within the patient's islets of Langerhans can be also investigated, progressing subsequently to cellular microadenomas. A series of patients afflicted by this ailment and formally termed it GCHN were present, unraveling its etiological mechanisms and genetic underpinnings in a gradual manner. Significantly, by 2017, GCHN has been officially incorporated into the WHO's classification of tumors.

2.1.2. GLP-1RAs

As a neuropeptide, GLP-1 participates in regulating cellular satiety, water intake, and stress responses. Moreover, GLP-1 plays a pivotal role in the modulation of neurological and cognitive functions, holding promise as a therapeutic avenue for addressing cognitive impairments associated with diabetes. Notably, glucagon-like peptide-1 receptor agonists (GLP-1RAs), containing medications like exenatide, liraglutide, and semaglutide, have emerged as effective antihyperglycemic treatments. Recombinant GLP-1 can enhance neural function in diabetic rats by mitigating oxidative stress and cellular apoptosis, suggesting potential neuroprotective properties. These findings open up exciting possibilities for the use of GLP-1RAs in managing cognitive decline associated with diabetes and even exploring their therapeutic potential in neurodegenerative diseases, for instance, Parkinson's Disease (PD) and Alzheimer's Disease (AD). Such avenues of research could significantly impact the management and treatment of these challenging conditions. In diverse preclinical PD models, GLP-1RAs have exhibited notable neuroprotective capabilities, influencing brain motor functions, dopaminergic neurons, cortical activities, and energy utilization. AD stands as a chronic ailment that profoundly affects memory, cognitive faculties, and behavior. Its core pathological hallmarks encompass the entanglement of neurofibrillary fibers and the aggregation of hyperphosphorylated tau protein into oligomers, along with the formation of amyloid plaques composed of aggregated β -amyloid (Ab) peptides. As shown in Fig 1, through a range of preclinical AD investigations, GLP-1RAs have demonstrated their capacity to enhance nearly all neuropathological features and sensory cognition. Additionally, these compounds exhibit promise in enhancing memory function and safeguarding hippocampal neuron loss [10].

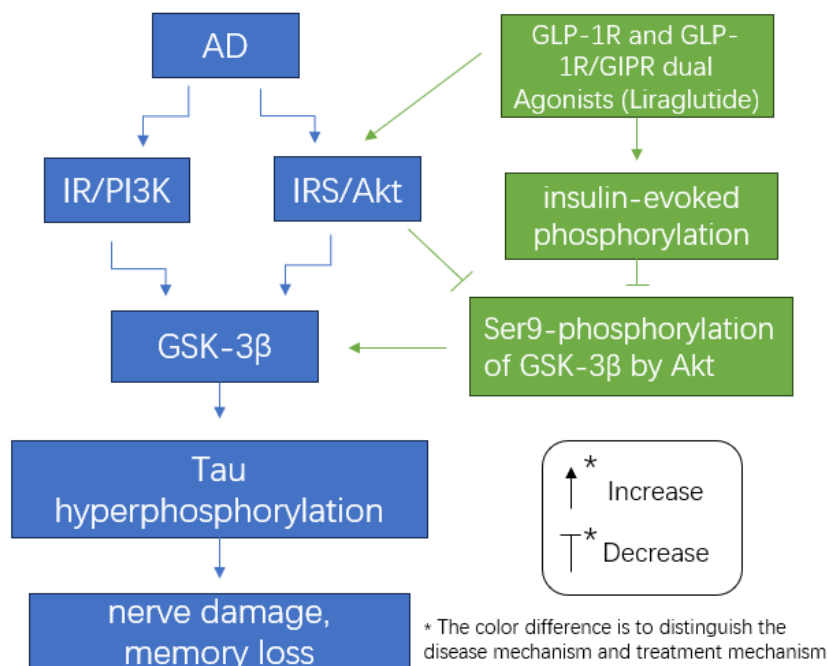


Figure 1. GLP-1 receptor mimetics suppress Tau hyperphosphorylation and aggregation during Alzheimer's disease. [10]

All in all, GLP-1RAs exhibit a promising avenue to potentially address long-deemed incurable neurological disorders in the times ahead. Nevertheless, the clinical investigation targeting human

subjects remains inadequate, calling for a more comprehensive exploration through human in vivo experiments.

2.2. Mahvash disease treatment

Mahvash disease is defined by reactive pancreatic α cell hyperplasia and is linked to hereditary biallelic inactivating mutations within the glucagon receptor gene. Notably, the mutant GCGR variant known as P86S exhibits abnormal cellular trafficking, primarily localizing to the endoplasmic reticulum [5]. This cellular mislocalization has profound consequences on cellular function and signaling pathways. Cells expressing the P86S mutant display a significant reduction in cAMP production and lower calcium levels in response to glucagon stimulation when compared to cells expressing the wild-type GCGR. These data underscore the genetic and cellular intricacies of Mahvash disease, shedding light on how glucagon receptor mutations can disrupt normal cellular processes and signaling cascades.

The defective glucagon signaling in the liver, leading to hyperaminoacidemia, emerges as a potent driver of α cell proliferation. This proliferation, in turn, is closely associated with the development of pancreatic neuroendocrine tumors (PNETs), shedding light on the intricate relationship between glucagon signaling, amino acid metabolism, and tumorigenesis. Mahvash disease, although rare with an estimated prevalence of approximately 4 per million, presents with nonspecific symptoms, with abdominal pain being the most prevalent complaint. Anatomical imaging often reveals pancreatomegaly, sometimes accompanied by distinct masses. These gross PNETs, which can vary in size from 1 to 8 cm and occur throughout the pancreas, are predominantly glucagonomas, with occasional instances of truly nonfunctioning tumors.

Moreover, in Mahvash disease, there seems to be a genotype-phenotype correlation. Patients with biallelic mutations resulting in premature termination of GCGR translation are typically diagnosed at a younger age compared to those with biallelic point mutations that lead to improperly trafficked GCGR with some residual activity. This comprehensive information offers insights into the clinical presentation, imaging findings, and genotype-phenotype relationships within the context of Mahvash disease, an uncommon condition intricately linked to defective glucagon signaling.

Clinical experience with Mahvash disease remains limited, and treatment may follow guidelines for other hereditary PNET syndromes. It stands out as a unique condition among known hereditary PNET syndromes, contributing to our understanding of glucagon's physiological function. Given its connection with overweight and type 2 diabetes mellitus, the exploration of treatments for NASH is crucial. Cotadutide, acting as a GLP-1R and GCGR agonist, shows promise in NASH treatment by reducing blood glucose, body weight, and hepatic steatosis. Ongoing efforts are focused on utilizing pharmacological chaperones to facilitate the proper trafficking of mutant GCGR to the plasma membrane, providing potential avenues for addressing Mahvash disease's underlying genetic defects.

Treatment with cotadutide in wild-type (WT) mice yields weight loss, but this weight-lowering effect lacking GLP-1R while cotadutide is administered, is not observed in them, indicating its dependence on GLP-1R. Cotadutide's influence on body weight and others is GLP-1R dependent. Cotadutide has been shown to decrease hepatic lipid content and modify carbohydrate metabolism in diet-induced obese (DIO) mice. Cotadutide induces changes in hepatic phosphoproteomics, revealing previously undiscovered targets of hepatic GcgR signaling. Furthermore, it enhances mitochondrial turnover and directly give an increase to mitochondrial oxidative capacity induced by GCGR signaling. These findings elucidate the multifaceted metabolic effects of cotadutide, underscore the significance of GLP-1R in its action, and highlight its potential in addressing issues related to NASH and hepatic mitochondrial function.

In vivo alleviating NASH, as shown in Fig 2, cotadutide indicates high efficacy. Terminal non-fasted blood glucose levels, as well as plasma insulin, triglycerides were elevated. However, the administration of cotadutide resulted in a reduction in both glucose and cholesterol concentrations. One of pivotal characteristics of NASH pathogenesis is Hepatic fibrosis, all treatments resulted in

varying degrees of reductions in fibrosis, but notably, cotadutide was the only treatment where no animals experienced worsening of fibrosis. Clinical data and versatile effects of cotadutide indicating that it's worth to treat T2DM11 patients' high liver fat symptom, which shows a feasible therapeutic choice to treat NAFLD and NASH [4].

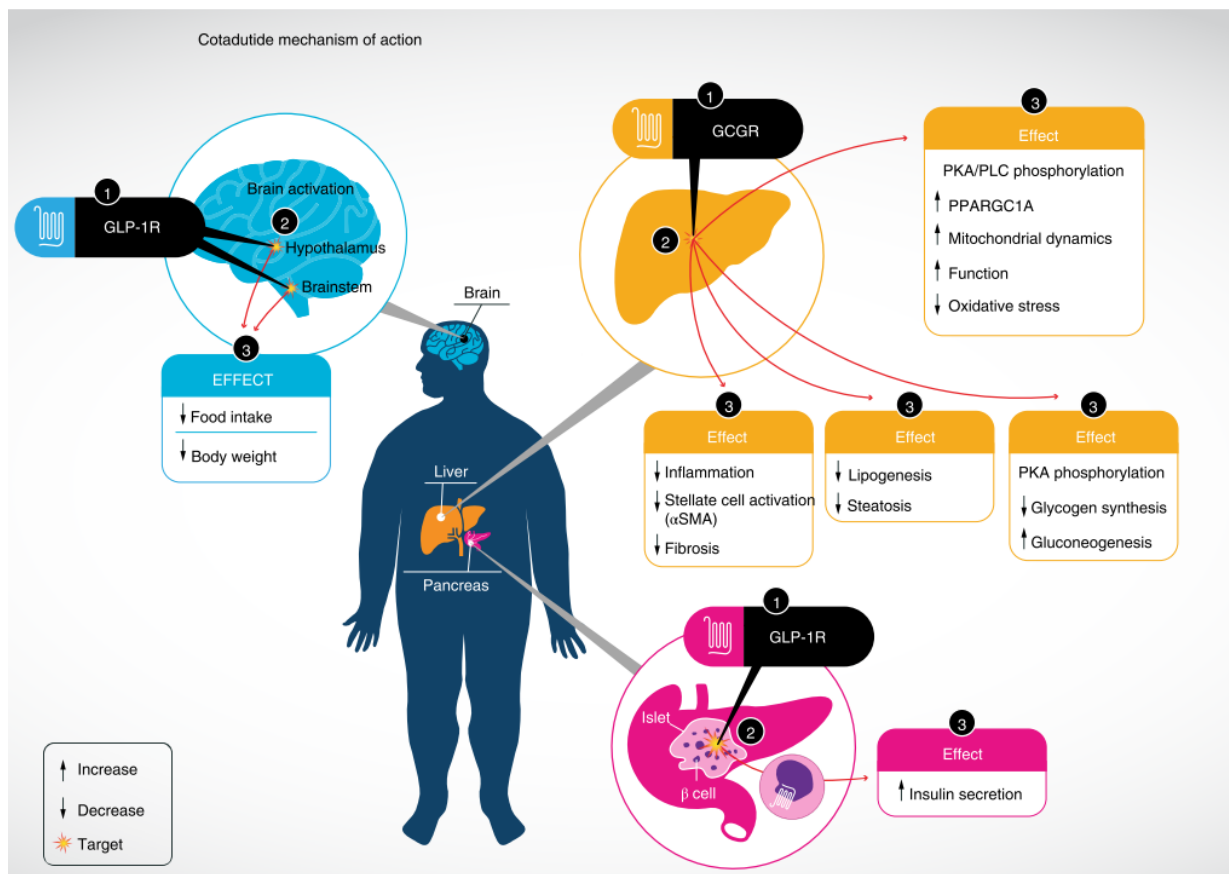


Figure 2. Cotadutide mechanism of action [4].

2.3. Type 2 diabetes treatment

GLP-1RAs are approved treatments for type 2 diabetes (T2D) and obesity, with proven effectiveness in enhancing glycemic control and facilitating weight reduction. These therapies offer support and protection to individuals at risk of or with pre-existing cardiovascular disease, demonstrating their broad utility in managing diabetes and related disorders. Different substances have been investigated, which are being used as therapies for T2D, these include glucagon, fibroblast growth factors (FGFs), amylin, cholecystokinin, and combining glucagon with glucose-dependent insulinotropic polypeptide (GIP). These substances are tested in preclinical trials and glucagon with GIP is also tested in clinical trials to determine their ability of reducing body weight, so scientists can develop drugs which are used to treat T2D, NASH or other disease which related to obesity in the future.

Some of the samples tested in the investigation are used as a monotherapy while other are combined with other substances. All the samples that are included in the investigation have shown promising effect in loss of body weight and fat in liver. For example, leptin only showed a little impact on body weight but when it used with pramlintide a bigger weight reduction is produced which is higher than the results that achieved by each individual agent. Many preclinical trials of co-agonists showed exciting findings but their clinical progress is slow and difficult. Some co-agonists give severe side effect when moved to clinical studies which was not shown or only has little effect in preclinical studies [11].

NAFLD and T2D often happens in patients' body at the same time, and they share a closely related pathophysiological thread of central obesity, insulin resistances, and these will also lead to liver inflammation. Deaths related to NAFLD are common in T2D. Weight reduction can bring positive

impact to patients with both T2D and NAFLD, usually this problem is solved by lifestyle changes. But in recent years the fast development of GLP1-RAs adds one more choice to the treatments, as GLP1-RAs can significantly reduce the weight and make improvements to metabolic control. There are now 5 GLP1-RAs have started their clinical trials. During it, liraglutide and dulaglutide have given very close effect and ability of weight reduction, better than lixisenatide and exenatide. While in ongoing clinical studies semaglutide is the most effective agent in terms of glycemic lowering and weight reduction. A newly published meta-analysis has showed that GLP1-RAs made the situation of patients' ALT, AST, GGT levels much better than before and significant reduction of liver fat, which means lower risk of liver inflammation. This new discovery also was improved by biopsy of liver.

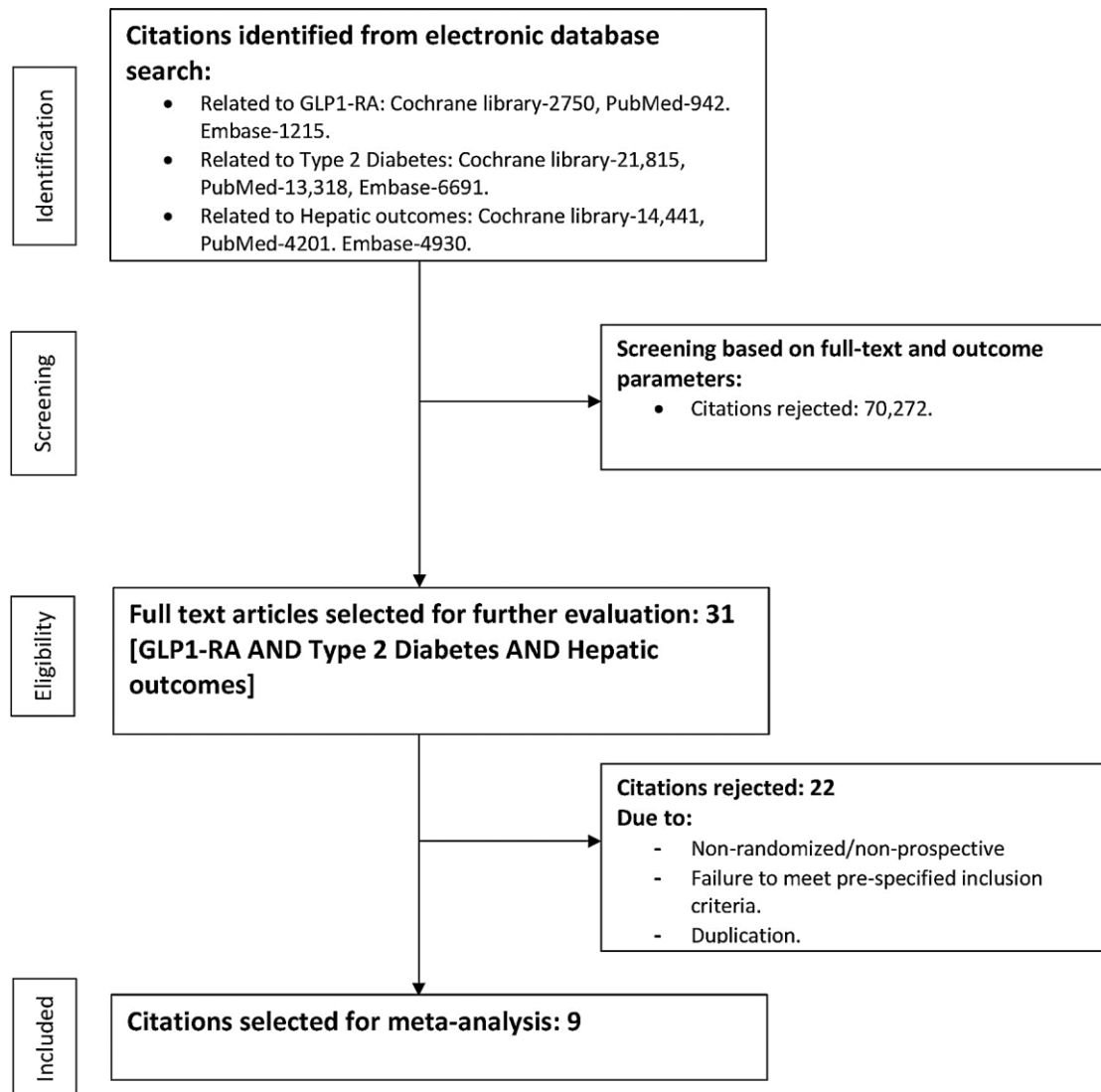


Figure 3. The selection process [12].

The limitations of this meta-analysis are also obvious. First, the imaging equipment used to assess improvements in liver is different, as some use CT scan while others use ultrasonography or MRI. Second, the number of liver biopsy is too small in terms of the large quantity of patients involved in this meta-analysis. Third, the improvements made by GLP1-RAs are not specific. On the flip side, it's worth noting that the article's extensive patient cohort did not encompass trials that featured agents such as pioglitazone within the control group, which can bring a positive impact to liver, contribute to a more valid analysis [12].

In addition, the efficacy of GLP1-RAs which acts as a newly developed therapy to treat patients with both NAFLD and T2D was analyzed, as shown in Fig. 3. The article included 8 studies and 1454 patients with both of the diseases are involved, of these 8 studies involved 3 are cohort studies and

other 5 are randomized controlled trials. This analysis was made after a broadly searching for evidence of the efficacy of GLP1-RAs in patients with both T2D and NAFLD and support.

NAFLD has a global incidence rate of about 25% and it is closely related to T2D. T2D and NAFLD share a bilateral pathogenic relationship, over 60% of patients with T2D also have NAFLD. There is not any medical treatment that is approved by FDA for NAFLD. The utilization of GLP-1RA in patients with NAFLD appears to offer potential benefits, as evidenced by a recent phase 2 trial that demonstrated notable improvements in steatohepatitis among NASH patients. However, prevailing recommendations continue to exercise caution regarding the use of GLP-1RAs in individuals with NAFLD. due to the small number of studies that give evidence on its efficacy.

In the 1454 patients that involved in this analysis, 669 were treated by GLP-1RA. The time of these treatments ranged between 12 to 52 weeks. In this analysis, it has shown that GLP1-RAs treatment can make improvements in hepatic steatosis and many other aspects. This result proves the efficacy in bringing positive effects to the situation of patients with NAFLD and T2D. GLP-1RAs also gives better efficacy of treatment in lowering hepatic fat content than metformin or insulin-based therapies, this can be explained by the weight loss which is caused by GLP-1RAs. As insulin and Sulfonylureas treatment is known to bring increasing body weight. Decreasing body weight is the most effective therapy for NAFLD. GLP-1RAs therapy can make significant improvements in obesity measurement such as BMI, waist and hip circumference and body weight. The analysis shows that after being treated by GLP-1RAs, there are some improvements in the FIB-4 index and APRI. Which can be used to indicate how bad hepatic fibrosis is and is proved in prognosis of liver fibrosis. The liver function markers such as AST and GGT are also being proved and reduction of inflammatory markers is shown in patients after being treated by GLP-1RAs. Depends on this we can say that GLP1-RAs therapy may give some benefits to patients with NASLD.

This analysis also has its limitations. First, none of the included studies use liver biopsy to assess hepatic steatosis which is required for phase II and III of clinical trials for NAFLD. Instead, they use other methods such as magnetic resonance spectroscopy and ultrasonography to assess hepatic steatosis. Secondly, the scarcity of trials providing substantial evidence of biopsy-proven efficacy for GLP-1RAs may be a contributing factor to why they have not yet been recommended as a readily available therapy for NAFLD. These involving studies are lack of placebo-control group, which means no comparing data has been made from the studies. Lastly, 4 studies reported the side effect of GLP-1RAs [11].

3. Conclusion

This research investigates the application of glucagon and glucagon-like peptide agonists in the field of disease treatment, including Mahvash disease and T2D. By now, in the treatment of NAFLD and T2D change lifestyle and diet play a major part to control the body weight, but if GLA1-RAs get passed in clinical trials and appears in the market the situation will completely being changed. NAFLD is currently the most prevalent disease in our world with an estimated global incidence of 35% and males have a higher risk than females. The introduction of GALP1-RAs as a treatment for NAFLD will benefit millions of people with broad prospects. Among the patients included in the studies, therapies that use GLP1-RAs give improvements in factors such as body weight, liver fat and glycemic control. Overall, the utilization of GLP-1RAs as a treatment for individuals with both NAFLD and T2D has shown promising results in current clinical trials. However, further studies are warranted to conclusively confirm the efficacy and safety of GLP-1RAs in mitigating liver fibrosis in patients with both NAFLD and T2D, even though GLP-1RA has shown promising effect in current studies.

Authors Contribution

All the authors contributed equally and their names were listed in alphabetical order.

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