Clinical trials for NASH treatment: dual agonist drugs as potential treatment methods

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Abstract

Non-alcoholic steatohepatitis is a liver disease with no approved treatment methods. Because of the severity of this disease, it is of uttermost importance to develop drugs that tackle this disease. The severity of NASH is let by permanent scarring of the liver, with hepatocellular carcinoma and liver failure as a consequence. Today, NASH screening is very difficult and mostly occurs at a later stage of the disease. Current research is focussing on various types of targets to treat this disease. Potential targets focus either on a single target, a double target or a triple target. Fibroblast growth factors are described as key players to prevent the development of NASH because they have a key role in metabolic processes that are heavily disturbed in NASH patients. Analogues of FGF19 and FGF21 have been developed for the current clinical trials which are being performed on human patients diagnosed with NASH. These FGFs have been extensively studied in mice and humans and can be used for drug development. Multiple single agonist drugs have been described, resulting in body weight reduction, impairment of liver fibrosis and a decrease in overall liver fat. Besides single agonist drugs, current clinical trials focus more on treatments with dual or triple agonist drugs. However, they have mostly been tested on mouse models, but they could make a promising option for treatment in human patients with NASH because they target more than a single pathway. In that manner, dual or triple agonist drugs can impair NASH better than single agonist drugs would. This thesis describes the drug GLP-1-Fc-FGF21 with the most potential to impair NASH. It still needs to be tested in human patients as more dual agonist drugs described in this thesis, but dual agonism looks like the most potent method in treating patients with NASH.

Introduction

Metabolic diseases play a prominent role in western societies. Individuals with obesity are growing every year and is estimated to keep on growing in the future at this rate. Most recent numbers from the World Health Organisation show that in 2016, 39% of individuals worldwide over the age of 18 were overweight. 13% of these individuals were obese[23]. Obesity increases the chance of type 2 diabetes (T2D), non-alcoholic fatty liver disease (NAFLD), non-alcoholic steatohepatitis (NASH), cardiovascular disease and certain types of cancer. NAFLD is a liver disease where fattening of the liver occurs driven by a high calorie diet without the influence of alcohol consumption. NAFLD on itself is considered to be benign but more advanced stages of NAFLD become more serious and can lead to the development of NASH, in which the liver is an inflammatory state. NASH can cause fibrosis of the liver resulting in liver damage which could lead to permanent scarring of the liver (cirrhosis)[30]. Cirrhosis of the liver can eventually lead to hepatocellular carcinoma, liver failure and death if left untreated[26]. To put NASH relevance in perspective, data from a previous study has shown that out of a group of 1620 people with morbid obesity, 91% were diagnosed with NAFLD and 37% with NASH[18].

Unfortunately NASH is mostly identified at a later stage of the disease, which could have already led to severe consequences. Because cirrhosis of the liver is an irreversible process and earlier stages of NAFLD/NASH are reversible, the most obvious treatment for NASH is weight loss. As most patients diagnosed with NASH are (morbidly) obese, reducing calorie intake, more exercise, and healthy food options help to tackle the pathology of NASH very well. By reducing weight in an individual with NASH, overall fat in the liver can be reduced which is of uttermost importance to sustain a healthy liver. Unfortunately there are no FDA-approved medicines for NASH treatment yet, but there are various studies which are putting their efforts in finding a potential treatment for NASH. There are multiple targets for drug development on trial, affecting many processes including lipid metabolism, inflammation, fibrosis and weight loss.

Therefore a potential therapeutic target for NASH treatment are fibroblast growth factor receptors (FGFRs). To target FGFRs, analogues for the corresponding fibroblast growth factors (FGFs) need to be developed. FGFs are proteins that regulate processes like cell proliferation and differentiation[22]. FGFs play an important role in lipid, bile acid and carbohydrate metabolism. Because these processes can be severely disturbed during metabolic diseases, drugs which are based on the function of FGFs are interesting molecules for the development of a treatment for NASH. Researchers have found three specific FGFs which play an important role during these processes, namely FGF1, FGF19 and FGF21. Current research has shown that drugs based on FGF19 and FGF21, contribute to an improvement in hepatic steatosis. Multiple medicines that are analogues based on FGF19 and FGF21 are currently on trial for the treatment of NASH patients, but has not led to an FDA-approved medicine. How these drugs work and why they have not been approved (yet), will be handled in this thesis. A different therapeutic approach for NASH treatment are dual (or triple) agonist drugs. These dual agonistic drugs show a lot of potential as they work on multiple targets. Therefore they can act on different pathways at the same time instead of a more specific pathway. In this manner they could impede more consequences that NASH has on the liver and thus provide a better approach for the treatment of NASH. Current promising dual agonist drugs target various different molecules like GLP-1, GLP-2, PPAR and FGF21 which will be clearly described in this thesis.

Therefore this thesis aims to make an overview of the most promising treatments for NASH and to get a clear insight in how these drugs specifically work. To understand how these drugs work, the mechanisms behind the above mentioned molecules which they are based on will be discussed. An insight in the mechanisms of FGFs will be given along with the targets of the dual agonist drugs.

Function and mechanisms of FGFs

The entire family of FGFs consists of 18 distinctive fibroblast growth factors[22]. FGFs bind and activate fibroblast growth factor receptors (FGFRs) through autocrine, paracrine or endocrine stimulation. Depending on the type of targeting, FGFs rely on co-receptors to activate FGFRs. Autocrine and paracrine FGFs are mostly associated with the co-receptor heparan sulphate whereas endocrine secreted FGFs depend on the co-receptor β-Klotho for activating a specific FGFR. The affinity of endocrine FGFs for β-Klotho instead of heparan sulphate makes diffusion out of the secreted cell possible. There are 4 different FGFRs to which FGFs can bind[7,22]. Stimulation of an FGFR results in cell signalling through the RAS/MAP kinase pathway, PI3 kinase/Akt pathway, the PLCγ pathway[35] and the STAT pathway. When an FGF binds to an FGFR, a cascade of reactions occurs in the cytoplasm of a cell. The cascade begins with the phosphorylation of tyrosine residues of the docking protein FRS2α. After phosphorylation of FRS2α, it provides new binding sites for the complex consisting of the following substances: GRB2, SOS, SHP2 and GAB1[24,27]. This complex can activate RAS/MAP kinase, which regulates cell growth and cell differentiation. The formed complex by FRS2α can also activate the PI3 kinase/Akt pathway, which is implicated in cell survival and cell fate determination[24]. The third pathway that is associated with FGF cell signalling is the PLCγ pathway. Phosphorylation of the tyrosine residues leads to activation of PLCγ which hydrolyses phosphatidylinositol into IP3 and DAG. IP3 is responsible for the release of calcium molecules from the endoplasmic reticulum. Increased levels of calcium and the presence of DAG can activate PKC together[24]. PKC is an important factor in regulating the expression of immune genes[15]. The final pathway which can be activated by FGF stimulation is the STAT pathway. STAT1, STAT3 and STAT5 regulate gene expression in the nucleus of a cell[24]. Because FGFs are vulnerable for degradation by proteases, they possess a relatively short half-life of 0,5 – 2 hours[16,25,34].

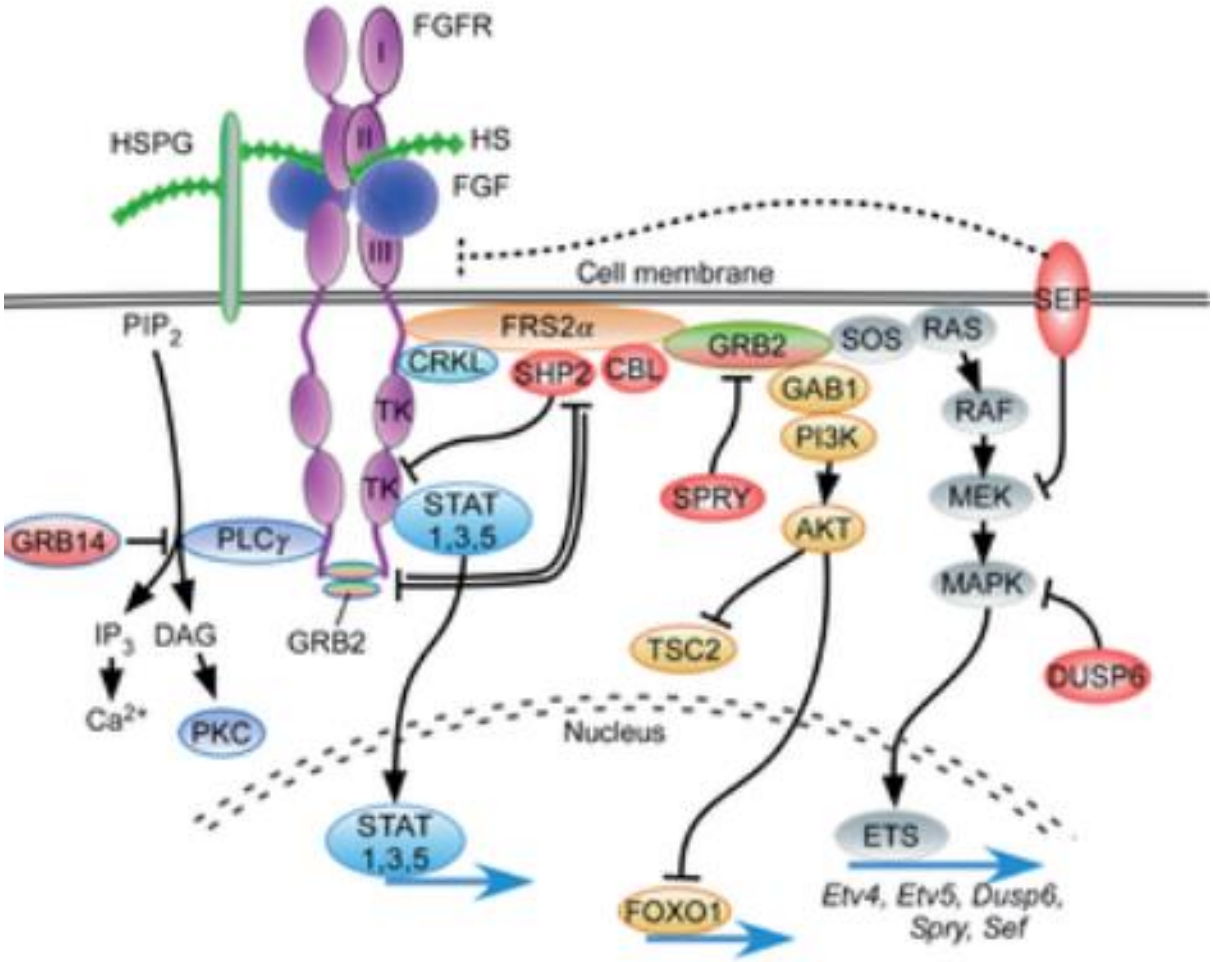

Figure 1: Overview of the four pathways which FGFs are involved in. FGF signalling occurs via the RAS/MAP kinase, PI3 kinase/Akt, PLCγ and STAT pathways[24].

The most prevalent FGFs associated with metabolism are FGF1, FGF19, and FGF21. FGF1 can bind and activate all four different FGFRs. FGF1 is regulated by PPARγ and is mostly expressed in white adipose tissue (WAT)[22]. Previous study has shown that FGF1 knockout mice cannot expand or reduce their WAT as a reaction to a fed or fasting state[36]. Additionally, it was found that FGF1 administration resulted in a decrease in blood glucose levels in diabetic mice. This mechanism was dependent on the numbers of FGFR1 in adipose tissue, further emphasizing the importance of adipose tissue for FGF1[31].

For the research of FGF19, researchers have used mouse models for the identification of the function of FGF 19. In mice the orthologue for human FGF19, is FGF15[16,22]. Mice FGF15 has shown that it is a key regulator for bile acid synthesis in the liver. This was initially discovered in FGFR4 knockout mice which showed an elevated level of bile acids[39]. As shown in figure 2, an increase in bile acids detected by FXR receptors leads to the production of FGF15 in the intestine (ileum). The produced

FGF15 travels to the liver where it has an inhibitory function on cholesterol 7-α-hydroxylase (CYP7A1) which is the rate-limiting enzyme during bile acid synthesis. Additionally to FGF15 production, increased levels of bile acids leads to the production of SHP in the liver which also has an inhibitory function on bile acid synthesis by inhibiting CYP7A1 expression. Together they regulate bile acid homeostasis under the influence of FXR[12,16,22]. In humans, FGF19 has a similar function as FGF15 in mice. A study has shown that FXR induces FGF19 in human hepatocytes. In situations where FGF15/19 was deleted, bile acid levels were elevated. Elevated bile acid levels can lead to enterohepatic damage, which emphasizes why bile acid synthesis must be a tightly regulated process[12].

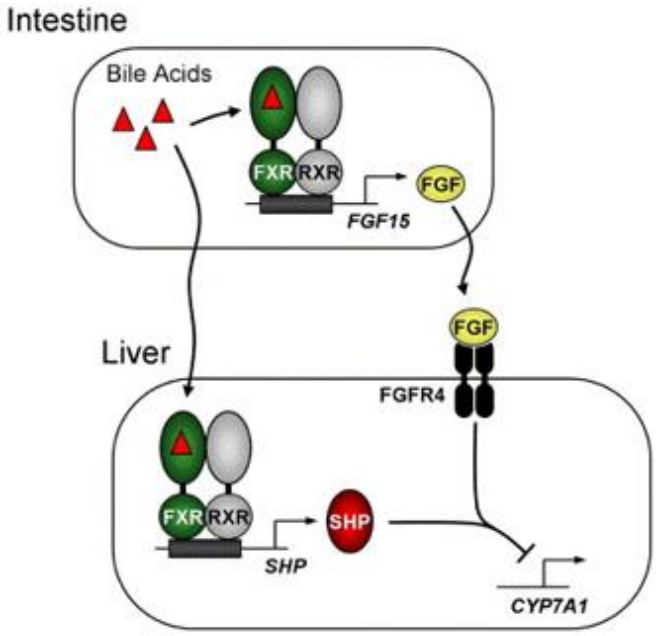

Figure 2: Mechanisms behind bile acid homeostasis. Increased levels of bile acids results in an increase in SHP (small heterodimer partner) in the liver and FGF15 in the intestine. Both factors have an inhibitory function on the rate-limiting enzyme for bile acid synthesis, CYP7A1[12].

FGF21 in humans is expressed in multiple tissues including the liver, brain, pancreas, and adipose tissue[4]. FGF21 is mainly produced in the liver and acts as a metabolic regulator. Findings have shown that FGF21 promotes the oxidation of fatty acids, inhibits lipogenesis, and stimulates the production of ketone bodies. Therefore, FGF21 can control energy expenditure which makes it an interesting therapeutic factor for drug development for metabolic diseases[32]. Research has shown that FGF21 does not completely overlap in function between humans and mice. In mice, FGF21 shows a clear indication that its levels are increased by fasting and ketogenic diets[19]. Multiple studies tried to investigate whether a response in FGF21 levels to fasting and ketogenic diets was present in humans, but there was no correlation found[31]. However, a clear correlation was found between increased FGF21 levels and an increased BMI. In particular, obesity-related diseases like T2D, NAFLD and NASH, tend to show an increase in FGF21[4].

Mimetics of dual agonist targets

This thesis will cover 3 different dual agonist drugs that all target a different combination of molecules. GLP-1 is one of the peptide hormones that dual agonist drugs base their treatments on. GLP-1 is secreted from the intestines and mainly functions to lower blood glucose levels. GLP-1

achieves this by promoting glucose-dependent insulin secretion, and inhibition of glucagon secretion. GLP-1 secretion is stimulated in response to food intake[25]. A different molecule that is used for NASH treatment is GLP-2. GLP-2 is secreted by the intestines and reduces gut permeability. It functions as an anti-inflammatory factor to maintain the mucosal immune barrier[13]. GLP-2 can stimulate crypt cell proliferation and inhibition of apoptosis to enhance the surface area of the epithelium of the mucosa. GLP-2 has also shown to regulate nutrient absorption in humans and rodents[13].

A final target for drug development are the so called peroxisome proliferator-activated receptors (PPARs). There are three subtypes of PPARs namely: PPARα, PPARγ, and PPARβ/δ[38]. All three subtypes possess their own specific functions and are highly associated with energy metabolism. PPARα is mostly found in hepatocytes where it is mainly involved in energy homeostasis. PPARα is directly involved in fatty acid oxidation, to provide energy for other surrounding tissues. PPARγ is an already, well studied receptor to which drugs have already been developed. PPARγ is involved in adipocyte differentiation, fatty acid storage and glucose metabolism[38]. Administration of Thiazolidinediones, a PPARγ agonist, resulted in an improvement in insulin resistance in diabetic patients, suggesting the potential of PPARγ agonism in metabolic diseases[37]. The final PPAR is PPARβ/δ, which is expressed in multiple tissues like adipocytes, the brain and skeletal muscle cells. PPAR-β/δ mainly functions to promote fatty acid metabolism and macrophage derived inflammation suppression[38].

FGF based drugs in clinical trials

A lack of approved therapies for the treatment of NASH has led to more studies researching therapeutic agents for NASH. Because of the severity of NASH as a disease, finding a cure to impede the complications is of uttermost importance. The main downside of NASH is that it is very hard to diagnose because the liver is not accessible for direct examination. Therefore the only possible way to diagnose NASH is to take a liver biopsy[20]. As mentioned before, FGFs play a prominent role in various metabolic pathways, therefore they are interesting targets for drug development. There are multiple types of drugs for the treatment of NASH. Trials with treatments for NASH focus on a single target or multiple targets. This section will cover single agonist and dual agonist drugs.

NGM282, also known as Aldafermin, which is currently in phase 2b of its clinical trial, is an FGF19 analogue that has an inhibitory effect on bile acid synthesis. The levels of C4, which is a marker for the enzyme CYP7A1, were significantly lowered during different trials with Aldafermin which indicates the inhibition of bile acid synthesis. This was paired with an eventual decrease in total liver fat in patients with NASH[8,9,10]. Results show a decrease in liver fat up to 81% with an administration of Aldafermin of 3 mg/day[8,9]. Besides decreasing liver fat, Aldafermin treatment also showed that 51% of

patients that received a dose of 3 mg/day of Aldafermin, had an improvement in liver fibrosis[8]. Alanine aminotransferase (ALT) and aspartate aminotransferase (AST) levels, which are common markers used for liver injury, were significantly reduced. Results showed a decrease of 30% of AST levels in NASH patients that received Aldafermin in contrast to patients that received a placebo which had a decrease of 1% of AST. Alt levels decreased with 49% in NASH patients that received Aldafermin in contrast to a decrease of 6% in NASH patients that received a placebo[9]. Histological analysis showed that 38% of NASH patients that received Aldafermin, had an improvement of fibrosis. The negative side-effects which were found during trials with Aldafermin were gastrointestinal symptoms. This mainly resulted in diarrhea, which was consistent for all trials[8, 9, 10]. The downside of CYP7A1 inhibition was an elevation in blood low-density lipoprotein (LDL). To tackle this problem, co-administration with the drug Rosuvastatin was done to control cholesterol levels. This resulted in positive outcomes with a significant decrease in LDL cholesterol in the blood[8, 9, 10].

The first FGF21-based drug which was developed is LY2405319. LY2405319 is an engineered variant of the classic FGF21 and was developed to improve the stability and productivity of FGF21. Trials with LY2405319 have been performed in mice with NASH. Results showed that treatment with LY2405319 decreased liver injury with almost 50% in both ALT and AST levels respectively. Liver fibrosis was significantly decreased with LY2405319 treatment as well. Finally, mitochondrial dysfunction is one of the consequences of NASH, but treatment with LY2405319 improved mitochondrial function significantly by measuring oxygen consumption rate (OCR) and beta-oxidation. The results show that mitochondrial function was improved due to an increase in in oxidative capacity and fatty acid oxidation[14]. The first test of LY2405319 in humans was in patients with T2D[6]. Results provided a decrease in total lipid concentrations and a reduction in body weight due to an increase in metabolic rate but not a decrease in food intake. Adiponectin levels, which is an important homeostatic factor for regulating glucose homeostasis, lipid metabolism and insulin sensitivity[1], were also increased. A decrease in overall adiponectin levels is associated with obesity and insulin resistance. The main negative side-effects which occurred during trials with LY2405319 were relatively mild. However, there were three subjects that discontinued the trial because of either: hypersensitivity, liver enzyme elevation or reactions at the injection site, headache, and urticaria. There were also three very serious cases in which patients showed more intense reactions to LY2405319 administration. Two of these three cases were related to already present diseases in these patients (cholecystitis and optic neuropathy), but one of these cases had a severe reaction to a dose of 20 mg of LY2405319 that resulted in a significant drop in blood pressure, urticaria, and pruritis[6].

Another promising drug for NASH treatment with FGF21 as a target, is BMS-986036 also known as Pegbelfermin. Pegbelfermin is an FGF21 human analogue that has, in contrast to LY2405319, a

prolonged half-life to expand its duration of action[34]. Clinical trials, which are currently in phase 2a, have shown that Pegbelfermin decreases bile acid concentrations in patients with NASH. Patients who received either a daily dose or weekly dose of Pegbelfermin, all showed a significant improvement in decreasing bile acid concentrations in contrast to patients who received a placebo[17,29]. Patients administered with Pegbelfermin, also showed a decrease of up to 30% in liver fibrosis by measurements of the biomarker PRO-C3[29]. They also showed massive improvements in adiponectin concentrations as these concentrations can be 50% less in patients with NASH[29]. This would suggest that adiponectin molecules have an important effect that provide protection against NASH. The reported adverse side effects of Pegbelfermin were mostly mild, resulting in patients with diarrhea (16%) and nausea (14%)[17, 29].

A third FGF21 analogue that has been used for research is Efruxifermin. Not many trials have been performed yet with patients diagnosed with NASH, but a study examining the performance of Efruxifermin has been done on NASH patients. Efruxifermin is a so-called fusion protein of IgG Fc linked to a modified FGF21 (Fc-FGF21). Fc-FGF21 has a higher receptor affinity than its human FGF21 analogue. Efruxifermin also has an extended half-life, like Pegbelfermin, in contrast to normal FGF21[33]. A clinical trial in phase 2a performed by Harrison et al. including patients diagnosed with NASH, showed that 88% of the patients treated with Efruxifermin saw a general reduction of ≥50% in liver fat. In accordance, the liver injury marker ALT showed a decrease up to 69%[11]. Levels of liver fibrosis marker pro-C3 also showed a decrease in patients administered with Efruxifermin. Treatment with Efruxifermin mostly had mild to moderate adverse effects but 89% of all patients experienced at least on adverse effect that was related to Efruxifermin treatment. As more of the current described drugs in this thesis, Efruxifermin treatment resulted in symptoms like diarrhea (27 cases), nausea (26 cases) and/or vomiting (15 cases). A more severe case let to the development of acute pancreatitis subsequently with diabetic ketoacidosis related to Efruxifermin administration[11].

Dual agonist drugs in clinical trials

Multiple drugs have been defined with dual agonistic targets. The advantage of dual agonist analogues, is that they can target multiple pathways at the same time. In that way, they can tackle multiple sites which have been heavily disturbed by a metabolic disease like NASH. First of all, a GLP-1

and FGF21 dual agonist (GLP-1-Fc-FGF21)[25]. Because GLP-1 can reduce body weight by lowering energy intake and FGF21 by promoting energy expenditure, as mentioned before, they make a promising couple for drug development[25]. A study performed by Pan et al optimized the properties of FGF21 by extending the half-life and increasing the affinity of FGF21 to β-Klotho. As previous mentioned drugs have also shown, extending the short half-life of FGF21 analogues is a main site of improvement. This eventually led to the development of GLP-1-Fc-FGF21 D1[25]. The study was performed on mice and rats and has not been tested in human patients yet. Results from the dual agonist GLP-1-Fc-FGF21 D1 showed improved results in mice compared to their respective solo analogs (Dulaglutide, a GLP-1 agonist and FGF21 S1). Figure 3 (E & F) show that the high-fat induced (HFD) mouse models treated with GLP-1-Fc-FGF21 mainly reduced their body weight by reducing their food intake. Liver function was measured by ALT and AST levels. Both markers showed lowered levels in comparison to Dulaglutide and FGF21 S1 suggesting an improvement in liver function. Liver fibrosis measurements could not be performed due to the fact that the mouse models used for research had not developed fibrosis of the liver yet[25].

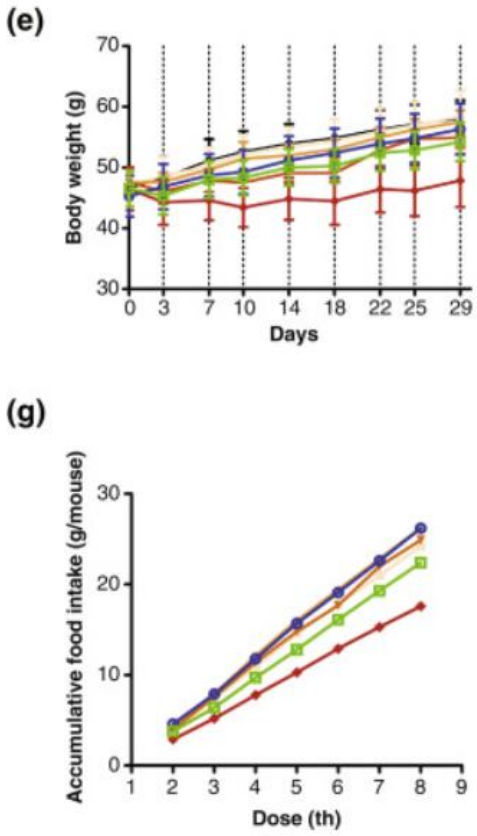

Figure 3: *Body weight loss in HFD-mice*[25]. Figure 3E & 3F show the reduction in body weight and the accumulative food intake of HFD mouse models who received GLP-1-Fc-FGF21 D1 (20nmol/kg), depicted by the red line[25].

Secondly, a GLP-1 and GLP-2 receptors dual agonist. A study performed by El-Jamal et al. showed that treatment with GLP-2 promotes liver regeneration in mice[2]. Because of these properties, combined with the properties of GLP-1, the dual agonist ''GLP1/2-Fc'' was evaluated as a therapeutic agent for the treatment of NASH[13]. The main results show that GLP1/2-Fc treated mice had a significant improvement on liver fibrosis. By Sirius red staining, liver cells were examined and the lowest concentrations of hepatic fibrosis was found in the group that was treated with GLP1/2-Fc. The solo analogs, GLP1-Fc and GLP2-Fc did not show a decrease in liver fibrosis. Hepatic fat accumulation was examined by fecal microbiota transplantation and showed a decrease when treated with GLP1/2-Fc. Body weight was reduced in mice treated with GLP1/2-Fc and GLP1-Fc, but not in mice treated with GLP2-Fc. As the properties of GLP-1 have already shown, GLP-1 administration reduces body weight, perfectly mimicking the results of this research. GLP1/2-Fc treatment also positively changed the gut

microbiome by improving the structures of the intestine[13]. Studies to this date, with GLP1/2-Fc in human patients diagnosed with NASH, have not been performed yet.

The third dual agonist drug which will be looked at is a GLP-1 - Glucagon receptor-based drug named ALT-801. In an obese mouse model, this drug was tested along with two single agonist drugs Semaglutide and Elafibranor[21]. Semaglutide is a GLP-1 receptor agonist and Elafibranor is a PPAR-α and PPAR-δ agonist. Mice received either one of the three drugs, and results were compared amongst each other together with a vehicle control group. Administration of ALT-801 directly resulted in a significant reduction in body weight. This was also observed in mice administered with Elafibranor and Semaglutide, but this weight loss was around 3 grams less than ALT-801[21]. Total liver cholesterol levels were significantly from 62 mg/liver to 13 mg/liver with ALT-801 administration. Semaglutide administration showed a decrease of 62 mg/liver to 27 mg/liver. Elafibranor showed no differences in total liver cholesterol levels[21]. To measure an improvement in liver fibrosis, the marker Col1A1 was used. Total Col1A1 lowered from 475 mg to 125 mg with ALT-801 treatment. Elafibranor and Semaglutide administration also showed a decrease in Col1A1 but not as significant as ALT-801[21]. A different study, which researched the effect of a GLP-1 and glucagon receptor dual agonist on mice and monkeys, showed similar results in which body weight was significantly reduced in comparison to a different drug called Liraglutide, which is a GLP-1R single agonist[3] .

The last drug that will be discussed in this thesis is the triple agonist Lanifibranor which is a PAN – PPAR agonist. Lanifibranor targets the three different PPAR isotypes: PPARα, PPARγ, and PPARβ/δ. A clinical trial in phase 2b has been performed on human patients diagnosed with NASH and received either Lanifibranor or a placebo[5]. The main results showed a very distinct difference between the two groups. 49% of the patients administered with a dose of 1200mg of Lanifibranor had an impairment of hepatic fibrosis in comparison with the placebo group (22%). Body weight was not positively influenced by Lanifibranor. Patients saw an increase in body weight possibly by the increase in adiponectin levels which improves adipose tissue functioning. The main adverse effects of Lanifibranor were gastrointestinal related consequences, peripheral edema (4 cases), anemia (6 cases) and a reduction of 5 to 6% in blood hemoglobin levels in the patients that received Lanifibranor [5].

Discussion

NASH is a very dangerous disease that is getting more prevalent in current societies. Because of the rising cases of NASH, efforts are being put in finding possible treatments. The main consequences of patients diagnosed with NASH are cirrhosis of the liver. Because of the fact that cirrhosis is a permanent consequence of NASH, drugs which are in trial focus a lot on preventing liver fibrosis and thus eventual liver cirrhosis. There are still no FDA-approved drugs for NASH, but multiple

clinical trials are being performed on human NASH patients. Trials are done with single agonist, dual agonist, and even a triple agonist drug.

This thesis aimed to visualize various types of drugs, currently being developed to treat patients with NASH. The focus lies on testing the efficacy and safety of these drugs. Targets for these drugs, that have been described in this thesis, mostly had a single or dual target. One of the evaluated drugs was a triple agonist. The single agonist drugs which have been described target the fibroblast growth factors FGF19 and FGF21. The drugs Aldafermin, LY2405319, Pegbelfermin and Efruxifermin all indicated that they improve liver fibrosis significantly, which is one of the important targets for NASH improvement. Because later stages of liver fibrosis can lead into liver cirrhosis, preventing/inhibiting liver fibrosis is very important. Accordingly, these drugs showed the same type of side-effects during the trial with the exception of a few more severe cases. An interesting method for impairing a side-effect of the drug Aldafermin was the combination of the drug together with Rosuvastatin. Because administration of Aldafermin increased blood LDL cholesterol, Rosuvastatin was administered to decrease the elevated levels of LDL cholesterol. Aldafermin administration together with Rosuvastatin administration, resulted very positively and treatment with Aldafermin was, therefore, more effective with co-administration of Rosuvastatin. The full potential of LY2405319 still needs to be fulfilled as this drug has not yet been tested on human patients with NASH. Patients with T2D have been treated with LY2405319 but liver fibrosis was not measured. Therefore trials in patients with NASH should take liver fibrosis measurements into account, as already has been done in mice. Because obesity is one of the main predictors of liver diseases, some trials for NASH were directed at lowering liver fat and reducing body weight. Liver fat was decreased during Aldafermin, Pegbelfermin and Efruxifermin treatment and body weight was reduced during trials with Aldafermin and LY2405319 (T2D).

Three dual agonist drug that have been described in this thesis have yet to be tested in human patients diagnosed with NASH. The current state of various dual agonist drugs in mouse models show the potential for success in human patients. The dual agonist GLP-1-Fc-FGF21 D1 used the properties of GLP-1 and FGF21 to optimize the efficacy of NASH treatment. Treatment with GLP-1-Fc-FGF21 D1 mainly resulted in a considerable decrease in body weight paired with improved liver function. The next step for preclinical trials with GLP-1-Fc-FGF21 D1 is to measure its effect on liver fibrosis development. As described before, targeting FGF21 can result in a decrease in liver fibrosis. This has been hypothesized by the researchers who performed this preclinical trial as well[25]. GLP1/2-Fc and ALT-801 both targeted GLP-1 for the treatment of mice with NASH, like GLP-1-Fc-FGF21 D1. Because GLP-1 functions to regulate body weight by regulating energy intake, GLP-1 has shown to be a good target to reduce body weight significantly. But only targeting GLP-1 as a single agonist for example, Semaglutide, shows that it does not improve liver fibrosis on its own[21]. The combined target of GLP1/2-

Fc, however did improve liver fibrosis which led to the hypothesis that GLP-2 possibly has a role in improving liver fibrosis. The triple agonist, Lanifibranor has a completely different target in comparison to all drugs described in this thesis. Lanifibranor showed that it impairs liver fibrosis in comparison with the placebo group. A consequence of Lanifibranor administration was that patients who underwent a trial with Lanifibranor, saw an increase in body weight. This could be traced back to the increase in adiponectin molecules.

When analysing the properties of all the different clinical trials, it shows that only reducing body weight does not improve liver fibrosis. However, body weight should be one of the main targets for NASH treatment. Ideally, patients with NASH would decrease their body weight by exercising more and eating healthier food. This transition in lifestyle is not that simple, or not achievable, which why it is important to develop drugs to help people that show difficulty in exercising or a change in diet. Fortunately, as shown in this thesis, there are a lot of potential drugs which positively influence body weight. Liver fibrosis has also been one of the main targets for NASH treatment. Most clinical trials saw a positive improvement in liver fibrosis as markers for liver fibrosis were significantly decreased. This is a very positive development for NASH treatment as previous trials with less effective drugs did not impair liver fibrosis[28]. All treatments performed on human patients with NASH showed a similar pattern in their adverse effects. Most studies depicted adverse effects like diarrhea and nausea as mild. Yet, there has not been a single drug that has been FDA-approved. All current drugs in trial have a few more severe cases, paired with the abundance of ''mild'' adverse effects, which questions the safety of these drugs. Therefore it seems very difficult, to for example, develop an FGF-agonist that shows less adverse effects. The benefits are still not outweighing the risks of the clinical trials. Therefore, if the adverse effects are not ameliorating in future NASH treatments, we should reconsider if the adverse effects are worth it, considering that the pathology of NASH is far worse than the adverse effects of these drugs. As emphasized in the introduction, NASH can lead to hepatocellular carcinoma with the consequence that liver transplantation is the only viable last resort.

Dual agonism is an upcoming manner for NASH treatment combining two different targets that play a key role in tackling NASH development. Until this date, there have not been dual agonists which target FGF19. As treatment with Aldafermin has shown, targeting FGF19 can ameliorate NASH. However, Aldafermin did need the help of Rosuvastatin administration to impair its increase in LDL cholesterol levels. This co-administration is not classified as a ''dual agonist'', but it does use the properties of two different types of drugs which together show the optimal improvement in NASH impairment. Dual agonism has also shown in the case of GLP1/2-Fc that targeting two different sites can lead to an improvement in liver fibrosis, that single agonism is not able to handle. The dual agonist GLP-1-Fc-FGF21 looks like one of the best drugs displayed in this thesis. It tackles two major factors

which have shown to be of great value in treating NASH. FGF21 agonists have already shown that targeting FGF21 ameliorates NASH progression to a great extent. GLP-1 function has also been clearly described and why it makes for an important target in NASH treatment especially for weight loss. The next step is to clinically trial GLP-1-Fc-FGF21 in human patients.

The current challenge for tackling NASH does not lie in how to impair the severe consequences that NASH has on patients, but how to develop treatments which both show efficacy and safety. All drugs described in this thesis, that have been clinically trialled on humans, resulted in positive improvements of NASH impairment. The most promising approach for therapeutic targets for NASH treatment are dual or triple agonist. In particular, an FGF-based target shows potential for drug development. When developing a treatment that uses the properties of for example FGF21, efforts should be put in extending the half-life of FGF21. In that way, the amount of administration needed, could be minimized as it will function for a longer time period. Future research should definitely focus on treating NASH by targeting more than one specific molecule as it shows a lot of potential, by targeting multiple pathways that are involved in NASH development, in the eventual prevention of the cirrhotic stage of the liver.

References

1. Diep Nguyen, T. (2020). Adiponectin: Role in physiology and pathophysiology. *International Journal of Preventive Medicine*, *11*(1), 136. https://doi.org/10.4103/ijpvm.ijpvm_193_20
2. El-Jamal, N., Erdual, E., Neunlist, M., Koriche, D., Dubuquoy, C., Maggiotto, F., Chevalier, J., Berrebi, D., Dubuquoy, L., Boulanger, E., Cortot, A., & Desreumaux, P. (2014). Glugacon-like peptide-2: broad receptor expression, limited therapeutic effect on intestinal inflammation and novel role in liver regeneration. American Journal of Physiology-Gastrointestinal and Liver Physiology, 307(3), G274–G285. https://doi.org/10.1152/ajpgi.00389.2012
3. Elvert, R., Herling, A. W., Bossart, M., Weiss, T., Zhang, B., Wenski, P., Wandschneider, J., Kleutsch, S., Butty, U., Kannt, A., Wagner, M., Haack, T., Evers, A., Dudda, A., Lorenz, M., Keil, S., & Larsen, P. J. (2018). Running on mixed fuel-dual agonistic approach of GLP-1 and GCG receptors leads to beneficial impact on body weight and blood glucose control: A comparative study between mice and non-human primates. Diabetes, Obesity and Metabolism, 20(8), 1836–1851. https://doi.org/10.1111/dom.13212
4. Fisher, F. M., & Maratos-Flier, E. (2016). Understanding the Physiology of FGF21. *Annual Review of Physiology*, *78*(1), 223–241. https://doi.org/10.1146/annurev-physiol-021115-105339
5. Francque, S. M., Bedossa, P., Ratziu, V., Anstee, Q. M., Bugianesi, E., Sanyal, A. J., Loomba, R., Harrison, S. A., Balabanska, R., Mateva, L., Lanthier, N., Alkhouri, N., Moreno, C., Schattenberg, J. M., Stefanova-Petrova, D., Vonghia, L., Rouzier, R., Guillaume, M., Hodge, A., . . . Abdelmalek, M. F.

(2021). A Randomized, Controlled Trial of the Pan-PPAR Agonist Lanifibranor in NASH. New England Journal of Medicine, 385(17), 1547–1558. https://doi.org/10.1056/nejmoa2036205

6. Gaich, G., Chien, J., Fu, H., Glass, L., Deeg, M., Holland, W., Kharitonenkov, A., Bumol, T., Schilske, H., & Moller, D. (2013). The Effects of LY2405319, an FGF21 Analog, in Obese Human Subjects with Type 2 Diabetes. *Cell Metabolism*, *18*(3), 333–340. https://doi.org/10.1016/j.cmet.2013.08.005
7. Goetz, R., & Mohammadi, M. (2013). Exploring mechanisms of FGF signalling through the lens of structural biology. *Nature Reviews Molecular Cell Biology*, *14*(3), 166–180. https://doi.org/10.1038/nrm3528
8. Harrison, S. A., Abdelmalek, M. F., Neff, G., Gunn, N., Guy, C. D., Alkhouri, N., Bashir, M. R., Freilich, B., Kohli, A., Khazanchi, A., Sheikh, M. Y., Leibowitz, M., Rinella, M. E., Siddiqui, M. S., Kipnes, M., Moussa, S. E., Younes, Z. H., Bansal, M., Baum, S. J., . . . Lieu, H. D. (2022). Aldafermin in patients with non-alcoholic steatohepatitis (ALPINE 2/3): a randomised, double-blind, placebo-controlled, phase 2b trial. *The Lancet Gastroenterology & Hepatology*, *7*(7), 603–616. https://doi.org/10.1016/s2468-1253(22)00017-6
9. Harrison, S. A., Neff, G., Guy, C. D., Bashir, M. R., Paredes, A. H., Frias, J. P., Younes, Z., Trotter, J. F., Gunn, N. T., Moussa, S. E., Kohli, A., Nelson, K., Gottwald, M., Chang, W. C., Yan, A. Z., DePaoli, A. M., Ling, L., & Lieu, H. D. (2021). Efficacy and Safety of Aldafermin, an Engineered FGF19 Analog, in a Randomized, Double-Blind, Placebo-Controlled Trial of Patients With Nonalcoholic Steatohepatitis. *Gastroenterology*, *160*(1), 219–231.e1. https://doi.org/10.1053/j.gastro.2020.08.004
10. Harrison, S. A., Rossi, S. J., Paredes, A. H., Trotter, J. F., Bashir, M. R., Guy, C. D., Banerjee, R., Jaros, M. J., Owers, S., Baxter, B. A., Ling, L., & DePaoli, A. M. (2019). NGM282 Improves Liver Fibrosis and Histology in 12 Weeks in Patients With Nonalcoholic Steatohepatitis. *Hepatology*, *71*(4), 1198–1212. https://doi.org/10.1002/hep.30590
11. Harrison, S. A., Ruane, P. J., Freilich, B. L., Neff, G., Patil, R., Behling, C. A., Hu, C., Fong, E., De Temple, B., Tillman, E. J., Rolph, T. P., Cheng, A., & Yale, K. (2021). Efruxifermin in non-alcoholic steatohepatitis: a randomized, double-blind, placebo-controlled, phase 2a trial. *Nature Medicine*, *27*(7), 1262–1271. https://doi.org/10.1038/s41591-021-01425-3
12. Inagaki, T., Choi, M., Moschetta, A., Peng, L., Cummins, C. L., McDonald, J. G., Luo, G., Jones, S. A., Goodwin, B., Richardson, J. A., Gerard, R. D., Repa, J. J., Mangelsdorf, D. J., & Kliewer, S. A. (2005). Fibroblast growth factor 15 functions as an enterohepatic signal to regulate bile acid homeostasis. *Cell Metabolism*, *2*(4), 217–225. https://doi.org/10.1016/j.cmet.2005.09.001
13. Kim, E. R., Park, J. S., Kim, J. H., Oh, J. Y., Oh, I., Choi, D. H., Lee, Y. S., Park, I. S., Kim, S., Lee, D. H., Cheon, J. H., Bae, J., Lee, M., Cho, J. W., An, I. B., Nam, E. J., Yang, S., Lee, M., Bae, S. H., & Lee, Y.

(2021). A GLP-1/GLP-2 receptor dual agonist to treat NASH: Targeting the gut-liver axis and microbiome. Hepatology, 75(6), 1523–1538. https://doi.org/10.1002/hep.32235

14. Lee, J. H., Kang, Y. E., Chang, J. Y., Park, K. C., Kim, H. W., Kim, J. T., Kim, H. J., Yi, H. S., Shong, M., Chung, H. K., & Kim, K. S. (2016). An engineered FGF21 variant, LY2405319, can prevent non-alcoholic steatohepatitis by enhancing hepatic mitochondrial function. *American Journal of Translational Research*. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5126319/
15. Lim, P. S., Sutton, C. R., & Rao, S. (2015). Protein kinase C in the immune system: from signalling to chromatin regulation. *Immunology*, *146*(4), 508–522. https://doi.org/10.1111/imm.12510
16. Lin, B. C., Wang, M., Blackmore, C., & Desnoyers, L. R. (2007). Liver-specific Activities of FGF19 Require Klotho beta. *Journal of Biological Chemistry*, *282*(37), 27277–27284. https://doi.org/10.1074/jbc.m704244200
17. Luo, Y., Decato, B. E., Charles, E. D., Shevell, D. E., McNaney, C., Shipkova, P., Apfel, A., Tirucherai, G. S., & Sanyal, A. J. (2022). Pegbelfermin selectively reduces secondary bile acid concentrations in patients with non-alcoholic steatohepatitis. *JHEP Reports*, *4*(1), 100392. https://doi.org/10.1016/j.jhepr.2021.100392
18. Machado, M., Marques-Vidal, P., & Cortez-Pinto, H. (2006). Hepatic histology in obese patients undergoing bariatric surgery. Journal of Hepatology, 45(4), 600–606. https://doi.org/10.1016/j.jhep.2006.06.013
19. Markan, K. R., Naber, M. C., Ameka, M. K., Anderegg, M. D., Mangelsdorf, D. J., Kliewer, S. A., Mohammadi, M., & Potthoff, M. J. (2014). Circulating FGF21 Is Liver Derived and Enhances Glucose Uptake During Refeeding and Overfeeding. *Diabetes*, *63*(12), 4057–4063. https://doi.org/10.2337/db14-0595
20. Nalbantoglu, I., & Brunt, E. M. (2014). Role of liver biopsy in nonalcoholic fatty liver disease. World Journal of Gastroenterology. https://doi.org/10.3748/wjg.v20.i27.9026
21. Nestor, J. J., Parkes, D., Feigh, M., Suschak, J. J., & Harris, M. S. (2022). Effects of ALT-801, a GLP-1 and glucagon receptor dual agonist, in a translational mouse model of non-alcoholic steatohepatitis. Scientific Reports, 12(1). https://doi.org/10.1038/s41598-022-10577-2
22. Nies, V. J. M., Sancar, G., Liu, W., Van Zutphen, T., Struik, D., Yu, R. T., Atkins, A. R., Evans, R. M., Jonker, J. W., & Downes, M. R. (2016). Fibroblast Growth Factor Signaling in Metabolic Regulation. *Frontiers in Endocrinology*, *6*. https://doi.org/10.3389/fendo.2015.00193
23. *Obesity and overweight*. (2021, 9 juni). WHO.Int. Geraadpleegd op 21 juni 2022, van https://www.who.int/news-room/fact-sheets/detail/obesity-and-overweight
24. Ornitz, D. M., & Itoh, N. (2015). The Fibroblast Growth Factor signaling pathway. *WIREs Developmental Biology*, *4*(3), 215–266. https://doi.org/10.1002/wdev.176

25. Pan, Q., Lin, S., Li, Y., Liu, L., Li, X., Gao, X., Yan, J., Gu, B., Chen, X., Li, W., Tang, X., Chen, C., & Guo, L. (2021). A novel GLP-1 and FGF21 dual agonist has therapeutic potential for diabetes and non-alcoholic steatohepatitis. EBioMedicine, 63, 103202. https://doi.org/10.1016/j.ebiom.2020.103202
26. Pandyarajan, V. P., Gish, R. G. G., Alkhouri, N. A., & Noureddin, M. N. (2019). Screening for Nonalcoholic Fatty Liver Disease in the Primary Care Clinic. *Gastroenterology & Hepatology*, 357–365. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6676352/
27. Pearson, G., Robinson, F., Beers Gibson, T., Xu, B. E., Karandikar, M., Berman, K., & Cobb, M. H. (2001). Mitogen-Activated Protein (MAP) Kinase Pathways: Regulation and Physiological Functions*. *Endocrine Reviews*, *22*(2), 153–183. https://doi.org/10.1210/edrv.22.2.0428
28. Ratziu, V., Francque, S., & Sanyal, A. (2022). Breakthroughs in therapies for NASH and remaining challenges. Journal of Hepatology, 76(6), 1263–1278. https://doi.org/10.1016/j.jhep.2022.04.002
29. Sanyal, A., Charles, E. D., Neuschwander-Tetri, B. A., Loomba, R., Harrison, S. A., Abdelmalek, M. F., Lawitz, E. J., Halegoua-DeMarzio, D., Kundu, S., Noviello, S., Luo, Y., & Christian, R. (2018). Pegbelfermin (BMS-986036), a PEGylated fibroblast growth factor 21 analogue, in patients with non-alcoholic steatohepatitis: a randomised, double-blind, placebo-controlled, phase 2a trial. *The Lancet*, *392*(10165), 2705–2717. https://doi.org/10.1016/s0140-6736(18)31785-9
30. Seitz, T., & Hellerbrand, C. (2021). Role of fibroblast growth factor signalling in hepatic fibrosis. Liver International, 41(6), 1201–1215. https://doi.org/10.1111/liv.14863
31. Struik, D., Dommerholt, M. B., & Jonker, J. W. (2019). Fibroblast growth factors in control of lipid metabolism. *Current Opinion in Lipidology*, *30*(3), 235–243. https://doi.org/10.1097/mol.0000000000000599
32. Tezze, C., Romanello, V., & Sandri, M. (2019). FGF21 as Modulator of Metabolism in Health and Disease. *Frontiers in Physiology*, *10*. https://doi.org/10.3389/fphys.2019.00419
33. Tillman, E. J., Brock, W. J., & Rolph, T. (2022). Efruxifermin, a long-acting Fc-fusion FGF21 analogue, reduces body weight gain but does not increase sympathetic tone or urine volume in Sprague Dawley rats. *British Journal of Pharmacology*, *179*(7), 1384–1394. https://doi.org/10.1111/bph.15725
34. Verzijl, C. R., Van de Peppel, I. P., Struik, D., & Jonker, J. W. (2020). Pegbelfermin (BMS-986036): an investigational PEGylated fibroblast growth factor 21 analogue for the treatment of nonalcoholic steatohepatitis. *Expert Opinion on Investigational Drugs*, *29*(2), 125–133. https://doi.org/10.1080/13543784.2020.1708898
35. Yun, Y. R., Won, J. E., Jeon, E., Lee, S., Kang, W., Jo, H., Jang, J. H., Shin, U. S., & Kim, H. W. (2010). Fibroblast Growth Factors: Biology, Function, and Application for Tissue Regeneration. *Journal of Tissue Engineering*, *1*(1), 218142. https://doi.org/10.4061/2010/218142

36. Jonker, J. W., Suh, J. M., Atkins, A. R., Ahmadian, M., Li, P., Whyte, J., He, M., Juguilon, H., Yin, Y. Q., Phillips, C. T., Yu, R. T., Olefsky, J. M., Henry, R. R., Downes, M., & Evans, R. M. (2012). A PPARγ–FGF1 axis is required for adaptive adipose remodelling and metabolic homeostasis. Nature, 485(7398), 391–394. https://doi.org/10.1038/nature10998
37. Kubota, N., Terauchi, Y., Kubota, T., Kumagai, H., Itoh, S., Satoh, H., Yano, W., Ogata, H., Tokuyama, K., Takamoto, I., Mineyama, T., Ishikawa, M., Moroi, M., Sugi, K., Yamauchi, T., Ueki, K., Tobe, K., Noda, T., Nagai, R., & Kadowaki, T. (2006). Pioglitazone Ameliorates Insulin Resistance and Diabetes by Both Adiponectin-dependent and -independent Pathways. Journal of Biological Chemistry, 281(13), 8748–8755. https://doi.org/10.1074/jbc.m505649200
38. Tyagi, S., Sharma, S., Gupta, P., Saini, A., & Kaushal, C. (2011). The peroxisome proliferator-activated receptor: A family of nuclear receptors role in various diseases. Journal of Advanced Pharmaceutical Technology & Research, 2(4), 236. https://doi.org/10.4103/2231-4040.90879
39. Yu, C., Wang, F., Kan, M., Jin, C., Jones, R. B., Weinstein, M., Deng, C. X., & McKeehan, W. L. (2000). Elevated Cholesterol Metabolism and Bile Acid Synthesis in Mice Lacking Membrane Tyrosine Kinase Receptor FGFR4. Journal of Biological Chemistry, 275(20), 15482–15489. https://doi.org/10.1074/jbc.275.20.15482