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Thème

L'action des beta 2 agonistes formotérol et salbutamol sur la stéatose hépatique non alcoolique induite par un régime riche en lipide HFD : Analyse histopathologique

The action of the beta 2 agonists formoterol and salbutamol on Non Alcoholic Fatty Liver Disease induced by a High Fat Diet : Histopathological analysis

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Dedication

To my dear family, precious friends and partner in crime 'K',

This moment marks the end of a significant chapter in my life, and I wouldn't be here without your unwavering support. You have been my pillars, my guides, and my sources of inspiration throughout this journey. Every success I've achieved is a result of your love, encouragement, and sacrifices.

I celebrate not only my accomplishments but also our collective success. We have grown together, learned together, and overcome obstacles together.

With all my love and sincere gratitude,

Massyl Amayes.

Dedication

I dedicate this achievement to my parents,

This thesis is dedicated to my dear parents, for their love, unconditional support, and constant encouragement. Their faith in me has given me the strength and motivation necessary to pursue my studies with determination. Mom, this thesis is especially for you. You have made countless sacrifices for my success. Your patience and perseverance have carried and guided me. I am infinitely grateful to you for everything you have done. Thank you for giving me the strength and determination needed to reach this goal.

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These dedications express my deep gratitude to those who have contributed to the accomplishment of this thesis.

Selma.

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Thank you to all the teachers who contributed to our university education.

List of abbreviations

NAFLD	**Non alcoolic fatty liver disease**
DNL	**De novo lipogenesis**
T2DM	**Type 2 diabetes**
FFAs	**Free Fatty Acids**
PNPLA3	**Patatin-like Phospholipase Domain-containing 3**
TM6SF2	**Transmembrane 6 superfamily member 2**
NASH	**Non Alcoolic Steato Hepatitis**
TNF-α	**Tumor necrosis factors**
IL-6	**Interleukin-6**
FIB-4	**Fibrosis Index Based on 4 Factors**
FDA	**Food and Drug Administration**
PPARα/δ	**Peroxisome proliferator-activated receptor α/δ**
THR-β	**Thyroid hormone receptor beta**
LDL	**Low density lipoprotein**
GLP-1	**Glucagon-like peptide 1**
HMG-CoA	**Hydroxymethylglutaryl-coenzyme A**
DNA	**Deoxyribonucleic nucleic acid**
ADRB2	**Adrenoceptor beta 2**
COPD	**Chronic Obstructive Pulmonary Disease**
HFD	**High fat diet**
CTL	**Control**
FOR	**Formoterol**

SAL	**Salbutamol**
BMI	**Body mass index**
IL-8	**Interleukin-8**
IL1β	**Interleukin-1 beta**
HDL	**High density lipoprotein**
VLDL	**Very-low-density lipoprotein**
IR	**Insulin resistance**
SREBP-1	**(Sterol regulatory element-binding protein 1)**
acetyl-CoA	**Acety- coenzyme A**
malonyl-CoA	**Malonyl- coenzyme A**
ROS	**Reactive oxygen spieces**
ASAT	**Aspartate aminotransferase**
ALAT	**Alanine aminotransferase**
GGT	**Gamma-glutamyl transferase**
TAG	**Triacylglycerol**
IKK	**Inhibitor of nuclear factor-κB (IκB) kinase**
JNK	**Jun N-terminal kinase**
TGF-β	**Transforming growth factor β receptor**
PDGF	**Platelet-derived growth factor receptor**
HSC	**Hepatic stellate cells**
TLR4	**Toll Like Receptor 4**
KC	**Kupffer cells**
PHT	**Portal hypertension**
HCC	**Hepatocellular carcinoma**

ADH	**Antidiuretic Hormone**
LPS	**Lipopolysaccharides**
TAMs	**Tumor-associated macrophages**
GPCRs	**G protein-coupled receptors**
TMD	**Transmembrane domains**
GRAFS	**System Glutamate, Rhodopsin, Adhesion, Frizzled/Taste2, Secretin**
CNS	**Central nervous system**
GTP	**Guanosine-5'-triphosphate**
GDP	**Guanosine diphosphate**
PKA	**Protein kinase A**
CAMP	**Cyclic adenosine monophosphate**
PKC	**Protein kinase A**
ERK	**Extracellular signal-regulated kinase**
GRK	**G protein-coupled receptor kinases**
PDE4	**Phosphodiesterase 4**
FEV	**Forced expiratory volume**
IC	**Inspiratory capacity**
Akt-Mtor	**Mammalian target of Rapamycin**
IFCC	**International Federation of Clinical Chemistry**
GPO	**Glycerol-3-phosphate oxidase**
POD	**Peroxidases**
GK	**Glucokinase**
CE	**Cholesterol ESTERASE**

CO	**Cholesterol OXY**
H&E	**Hematoxylin and Eosin**
ChREBP1	**Carbohydrate response element binding protein**
LXRα	**Liver X receptors**
AICAR	**5-aminoimidazole-4-carboxamide ribonucleotide**

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Introduction

Non-alcoholic fatty liver disease (NAFLD) is a disorder associated with obesity and metabolic syndrome, affecting 20 to 30% of individuals **(Rinaldi *et al.*, 2021)**. diet high in fat and fructose can lead to the development of NAFLD. Risk factors for fatty liver disease include obesity, metabolic syndrome (MS), type 2 diabetes mellitus (T2DM), dyslipidemia, and aging. High-fat diets increase energy intake, resulting in greater body fat, peripheral tissue insulin resistance, and metabolic syndrome. This leads to increased lipolysis, de novo lipogenesis (DNL), and the continuous absorption of high-energy nutrients, which collectively raise free fatty acids (FFAs) and ultimately cause hepatic steatosis. **(Juanola *et al.*, 2021)**.

Among these, 10% develop a more serious form called non-alcoholic steatohepatitis (NASH). Histologically, NASH is marked by fat accumulation, lobular inflammation, and liver cell damage known as ballooning **(Haldar *et al.*, 2019)**. This condition raises the likelihood of advancing to severe fibrosis and cirrhosis (Figure 1), which significantly increases overall mortality risk, including extra-hepatic issues, liver-related illness and death, and potentially necessitates a liver transplant **(Anstee *et al.*, 2022; Vilar-Gomez *et al.*, 2018)**. The disease's progression appears to follow a "three-hit" model: steatosis, lipotoxicity, and inflammation. The presence of steatosis, oxidative stress, and inflammatory mediators like TNF-α and IL-6 has been linked to changes in nuclear factors associated with NAFLD **(Cobbina and Akhlaghi, 2017)**. NASH independently increases the risk of developing complications beyond the liver, including cardiovascular conditions such as heart attacks and strokes, along with type 2 diabetes **(Francque *et al.*, 2021),** obstructive sleep apnea, chronic kidney disease, osteoporosis, and polycystic ovary syndrome **(Adams *et al.*, 2017)**. Recently, cumulative evidence has drawn attention to pathological correlations that are not strictly confined to metabolic diseases, this broader spectrum of systemic implications now includes hypothyroidism, psoriasis, male sexual dysfunction, periodontitis, and kidney stones **(Rosato *et al.*, 2019).**

Liver biopsy continues to be the definitive method for diagnosing NAFLD and NASH, despite its inconvenients like sampling variability, invasiveness, and high costs **(Sheridan *et al.*, 2017).** However, numerous non-invasive biomarkers, especially serum markers and imaging techniques, are being used to detect steatosis, NASH, and advanced fibrosis **(Pearce *et al.*, 2013).** Ultrasound is currently the preferred first-line screening method for identifying steatosis. Identifying advanced fibrosis is a critical step in managing NASH patients and can be reliably excluded using the NAFLD-Fibrosis score, the FIB-4 score, or transient elastography **(Papatheodoridi and Cholongitas, 2019)**.

To prevent the progression to cirrhosis and its associated complications, as well as other extra-hepatic complications of NASH, treatment is essential **(Tesfay *et al*., 2018)**. While some interventional drug studies have shown positive results **(Chen *et al*., 2019)** these remain modest and the evaluation criteria can be challenging to interpret **(Younossi *et al*., 2019)**. Currently there is no FDA-approved pharmacological treatment for NAFLD or NASH, Several therapeutic approaches are currently being developed to counteract the deleterious effects of this progressive disease **(Chaudhry *et al*., 2023)**. The intricate pathophysiology of the disease offers various potential therapeutic targets. For example, Elafibranor a Peroxisome proliferator-activated receptor α/δ (PPARα/δ) agonist, has shown improvements in insulin sensitivity and the normalization of blood lipid levels, which are principal contributors to the progression of NASH. However, it can cause increases in serum creatinine, potentially restricting its use in individuals with kidney disease **(McDonald and Ayala, 1978),** did not succeed in resolving NASH and worsened fibrosis **(Ratziu *et al*., 2016).**

Other medications target the liver directly, such as resmetirom, a selective thyroid hormone receptor (THR)-β agonist, which reduces liver fat content **(Karim *et al*., 2023)**, Obeticholic acid, a farnesoid X receptor ligand can resolve NASH and improve fibrosis without worsening it, though it increases LDL cholesterol with decreases in high-density lipoprotein cholesterol and triglycerides **(Chapman and Lynch, 2020; Loomba *et al*., 2020; Younossi *et al*., 2019)**. Other reported side effects encompass skin rash, throat pain, vertigo, bowel irregularities, joint pain, dyslipidemia, migraines, skin inflammation, mood disorders, allergic reactions, and thyroid dysfunction **(Markham and Keam, 2016)**. In addition, glucagon-like peptide-1 (GLP-1) receptor analogs, such as semaglutide, improve hepatic steatosis and hepatocyte ballooning but do not impact hepatic fibrosis **(Dufour *et al*., 2022)**. According to a large randomized controlled trial, vitamin E, known for its potent antioxidant effect, can improve steatosis, inflammation, and ballooning **(Cardoso *et al*., 2021)** and enhance liver transaminase levels in adults with NAFLD and type 2 diabetes **(Pacana and Sanyal, 2012).** Vitamin E promotes hepatic homeostasis by regulating macrophage polarization **(Nagashimada and Ota, 2019).** Statins, on the other side, reduce hepatic cholesterol synthesis by inhibiting the rate-limiting step catalyzed by the enzyme HMG-CoA reductase in patients with pre-existing liver disease such as NAFLD **(Mundi *et al*., 2020).** Muscle pain, liver toxicity, and potential drug interactions resulting from high doses of statins can limit their use **(Pougeois *et al*., 1979)**. β-Cryptoxanthin, a xanthophyll carotenoid, possesses antioxidant and

DNA repair properties that may also be beneficial in the treatment of NAFLD **(Sodum *et al*., 2021).**

Unfortunately, despite intensive treatment regimens, not all patients respond, and additional therapeutic interventions, such as endoscopic procedures and bariatric surgery, are often necessary **(Mundi *et al*., 2020)**. To achieve effective treatment for controlling NAFLD/NASH, it is essential to identify the signaling pathways involved in the pathogenesis of this liver disease **(Friedman *et al*., 2018).** Several studies have demonstrated over the last several years the significant role of beta-2 adrenergic agonists in cellular mediation within liver tissues related to lipid and carbohydrate metabolism **(Cero *et al*., 2021).** Several mechanisms might explain the anabolic effects of β2 agonists on the central nervous system **(Abosamak and Shahin, 2024).** These compounds stimulate lipolysis while reducing lipogenesis **(Peterla and Scanes, 1990)** and insulin activity **(Kalinovich *et al*., 2020)**. Another finding suggests that β2-adrenergic receptors are involved in energy expenditure and lipolysis, with ADRB2 polymorphisms potentially influencing weight loss **(Szendrei *et al*., 2016).**

More specifically, an activation of β2-adrenergic receptors increases glycerol levels in skeletal muscles and enhances tissue blood flow **(Lessard *et al*., 2009).** The effects of β-adrenergic receptor activation on astrocytes seem to include both harmful (amyloid plaque formation) and beneficial outcomes, such as reducing inflammation **(Laureys *et al*., 2010).** β2-adrenergic agonists are commonly used to treat respiratory conditions like bronchial asthma and chronic obstructive pulmonary disease (COPD) **(Abosamak and Shahin, 2024).** These drugs replicate the actions of catecholamines such as epinephrine, norepinephrine, and dopamine, triggering various autonomic responses in the body. Moreover, β2 agonists have significant effects on the smooth muscles of the airways, uterus, intestines, and systemic vascular system **(Hsu and Bajaj, 2024).** They are also frequently misused in doping **(Kindermann and Meyer, 2006)**. Therefore, apart from their performance-enhancing effects, β2-agonists may also possess anabolic and lipolytic properties **(Piribauer *et al*., 2023)** and contribute to glycemic control by enhancing glucose absorption **(Kalinovich *et al*., 2020).** To date, most of the studies focusing cardiovascular side effects associated with systemic β2-agonist therapy. Nonetheless, converging lines of evidence begun to show that an activation of β2-adrenergic receptors may prevent the progression of NAFLD to steatohepatitis by exerting varying effects on energy, lipid, and protein metabolism. However, direct actions on adipocyte, vascular system, and muscle β2-adrenergic receptors, along with indirect effects mediated through the neuroendocrine system or pancreatic beta cells, should be noted. Given that these

drugs are typically administered alongside diet, it's crucial to consider their primary impact in the digestive tract, affecting motility and nutrient absorption **(Lafontan *et al*., 1988).**

This study aims to demonstrate how beta2 agonists like salbutamol and formoterol affect tissues involved in the development of non-alcoholic fatty liver disease (NAFLD) induced by a high-fat diet (HFD) in rats. Given these facts and our initial observation described above, we set out in the study to define the development of hepatic steatosis before and after treatment with each of formoterol and salbutamol via a histological analysis of the liver, serum protein levels associated with liver failure as well as their impact on their organs.

Chapter I: Bibliographic review

I.1. Abstract

Non-alcoholic fatty liver disease (NAFLD) is defined as an excess of fat in the liver (≥ 5% of hepatocytes laden with lipid droplets upon histological analysis). Ten percent of individuals with NAFLD develop a more aggressive condition, non-alcoholic steatohepatitis (NASH), characterized histologically by steatosis (Figure 1), lobular inflammation, and hepatocellular ballooning **(Benedict and Zhang, 2017).** In individuals with NASH, the liver may also display Mallory-Denk bodies, marked by the buildup of damaged intermediate filaments within hepatocyte cytoplasm. This occurrence signifies hepatocyte distress **(Ribback *et al.*, 2024).** Insulin resistance, metabolic syndrome, or type 2 diabetes, along with genetic variants of Patatin-like Phospholipase Domain-containing 3 PNPLA3 (Figure 2), or transmembrane 6 superfamily member 2 TM6SF2, are believed to contribute to the development of non-alcoholic fatty liver disease (NAFLD) **(Barata *et al.*, 2019).** The course of NAFLD varies widely, with the potential for reversibility of disease stages, including steatohepatitis **(Grzych *et al.*, 2023).** Non-alcoholic steatohepatitis may advance to progressive fibrosis (Figure 1), ultimately culminating in cirrhosis. Complications of cirrhosis, such as decompensation and the development of hepatocellular carcinoma, are potential outcomes **(Bernsmeier and Heim, 2011).**

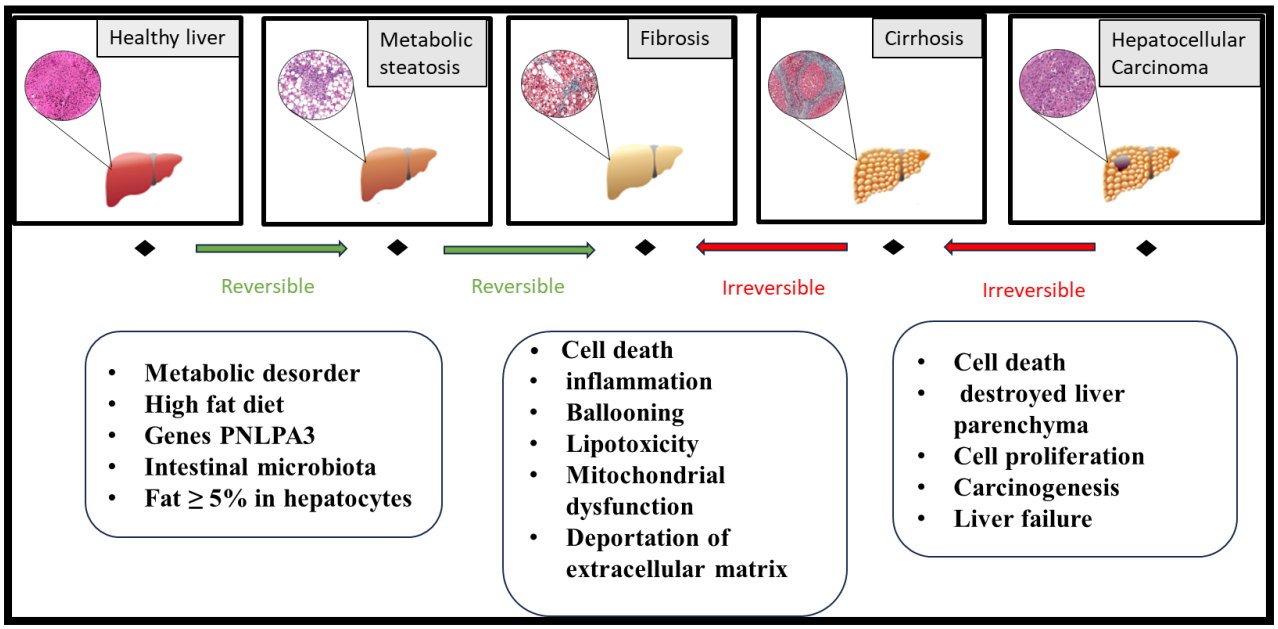


Figure 1. The disease spectrum of NAFLD **(Cohen *et al.*, 2011)**

I.2. Prevalence and incidence of NAFLD

Non-alcoholic fatty liver disease (NAFLD) has emerged as the predominant chronic liver condition, affecting over 25% of the world's population and showing a higher prevalence among men than women **(Pouwels *et al*., 2022).** Projections suggest a sustained escalation in NAFLD cases, with an estimated 27 million occurrences of non-alcoholic steatohepatitis anticipated by 2030, resulting in a significant rise in cirrhosis, hepatocellular carcinomas, and liver transplant procedures **(Grgurevic *et al*., 2021).** A meta-regression analysis of studies conducted worldwide has additionally shown an increase in the prevalence of NAFLD from 15% in 2005 to 25% in 2010 **(Perumpail *et al*., 2017).** However, there is a large discrepancy in estimation of NAFLD prevalence in general population. In fact, different techniques to diagnose NAFLD have been used such as imaging, liver biopsy and blood analysis **(Jang and Song, 2023).** The prevalence of NAFLD by using blood tests (liver enzymes) consistently yielded lower estimates than those studies that used imaging **(Younossi *et al*., 2016).** A Meta analysis of studies between 2000 -2014 and conducted in adults age 18 or older, revealed that the global prevalence of NAFLD diagnosed by imaging is around 25% **(Estes *et al*., 2018; Younossi *et al*., 2016).** The highest prevalence rates of NAFLD were found from Middle East and South America respectively with 32% and 31% **(Younossi *et al*., 2016).** Additionally, the prevalence rates of NAFLD in North America, Europe and in Asia ranges from 24% to 28%. Similar to other reports from industrialized societies, a third of population of Australia have NAFLD **(Younossi *et al*., 2019).** The lowest prevalence rate of NAFLD was reported from Africa with 14% ***(Younossi et al*., 2016).** This low rate may be explained by very few studies on the epidemiology of NAFLD from Africa **(Almobarak *et al*., 2014).** Most challenge is to conducted research programs to explore NAFLD in different regions in Africa.

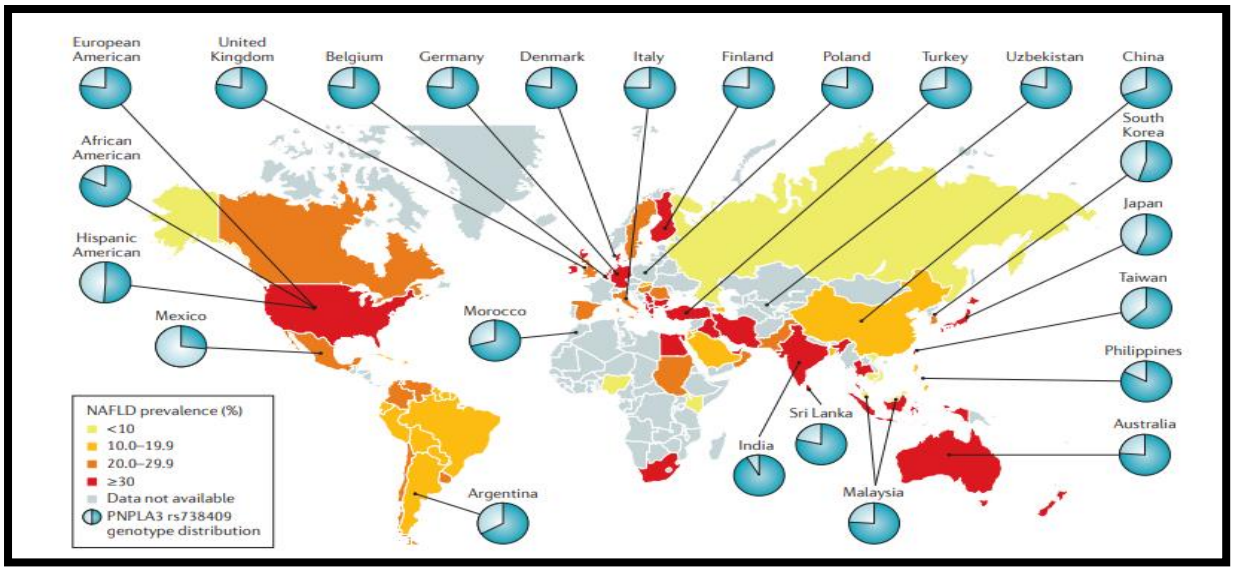


Figure 2. Estimated prevalence of NAFLD in the world and distribution of PNPLA3 genotypes. *PNPLA3 is presented as minor allele frequency (Light blue section of the pie chart)* **(Younossi *et al.*, 2018).**

I.3. Etiology of NAFLD

I.3.1. Body mass index (BMI) and Obesity

Body mass index (BMI) remains an important risk factor for NAFLD with adipose tissue dysfunction contributing to NAFLD pathogenesis **(Wang *et al.*, 2021).** Converging lines of evidence have demonstrated the high correlation between BMI and development or resolution of NAFLD **(Anderson *et al.*, 2015; Younes and Bugianesi, 2019; Younossi *et al.*, 2018).** Bedogni et al, have reported that every increase of 1 kg/m^2 of BMI at baseline further lowered the remission rate of fatty liver of 5% in subjects with suspected liver disease persistence **(Bedogni *et al.*, 2007).** Obesity stands as a primary risk factor for various chronic ailments largely attributed to chronic inflammation and oxidative stress **(Wellen and Hotamisligil, 2005).** NAFLD prevalence can reach 98%, with around 37% advancing to NASH in subjects with severe obesity, especially visceral obesity **(Bedogni *et al.*, 2007; Loomis *et al.*, 2016)**. Central adiposity, also known as abdominal obesity correlates with insulin resistance **(Westphal, 2008),** increased lipolytic activity **(Strawbridge *et al.*, 2016)** and the release of free fatty acids into the liver **(Frohnert *et al.*, 2013; Kojta *et al.*, 2020).** This cascade, combined with the over-expression of pro-inflammatory cytokines (TNFα, IL-6, IL-8, IL1β), sustains a chronic inflammatory state, contributing to NAFLD pathogenesis **(Dimitrov *et al.*, 2017).**

I.3.2. Type 2 Diabetes

In Hong Kong, Kwok et al reported that approximately 70% of individuals diagnosed with both diabetes or NAFLD **(Kwok *et al*., 2016).** Among those with NAFLD, diabetes is associated with more severe manifestations of the disease **(Bazick *et al*., 2015)**. Histological examinations in the United States, suggest that 80% of diabetic patients with NAFLD present with histological features of NASH such fat vacuoles within the hepatocytes, lobular inflammation and advanced fibrosis is observed in 17% to 40% of these cases **(Kleiner and Makhlouf, 2016)**.

I.3.3. Metabolic syndrome, hypertension and dyslipidemia

NAFLD is considered the hepatic component of Metabolic syndrome **(Paschos and Paletas, 2009).** Metabolic syndrome is characterized by a waist circumference, along with at least two of the following factors: fasting hyperglycemia, hypertension (HTN) and/or blood pressure, hypertriglyceridemia and decreased High-Density Lipoprotein (HDL) cholesterol **(Eckel *et al*., 2010).** This combination represents a constellation of risk factors for NAFLD, including diabetes, central obesity, dyslipidemia, and hypertension **(Hamaguchi *et al*., 2005).** Non-alcoholic fatty liver disease (NAFLD) is generally associated with elevated plasma levels of VLDL triglycerides and low concentrations of HDL cholesterol **(Lonardo *et al*., 2024).**

I.3.4. Insulin resistance

In states of insulin resistance, the activity of insulin on its target organs (muscle, liver, and adipocytes) **(Silva Rosa *et al*., 2020).** Visceral obesity, is associated with the development of insulin resistance, increased lipolytic activity in adipose tissues, and a massive release of free fatty acids that flow into the liver via portal drainage **(Jornayvaz *et al*., 2010; Kahn and Flier, 2000).** Adipokines, such as leptin, adiponectin, and resistin, produced primarily by adipose tissue, are involved in energy homeostasis. Resistin acts as a potent competitive antagonist of insulin by binding to insulin receptors (IR) on muscle **(Clemente-Suárez *et al*., 2023),** liver **(Han *et al*., 2021),** and adipocytes **(Shojima *et al*., 2002).** In the liver, the inhibition of insulin activity (central insulin resistance) is accompanied by the stimulation of de novo lipogenesis, leading to steatosis **(Bugianesi *et al*., 2005; Utzschneider and Kahn, 2006; Weiss and Caprio, 2005).**

I.3.5. Diet

Diet composition and energy intake are linked to NAFLD, with carbohydrates being more significant contributors than fats Diets high in saturated fats, refined sugars, sugary drinks

and fructose not only contribute to the expansion of adipose tissue, leading to insulin resistance and lipolysis, but also upregulate the hepatic enzyme SREBP-1 (Sterol regulatory element-binding protein 1) **(Aragno *et al.*, 2009).** Sterol regulatory element-binding proteins (SREBPs) are a family of transcription factors that play a crucial role in the biosynthesis of cholesterol, fatty acids, and triglycerides **(Moslehi and Hamidi-zad, 2018).** This results in increased de novo lipogenesis and pathogenesis of nonalcoholic fatty liver disease **(Barrera and George, 2014).**

I.3.6. Genetic factors

Interethnic variations and familial aggregation suggest a genetic role in the prevalence of NAFLD. Several genes have been identified as being associated with the development of NAFLD, particularly PNPLA3 and TM6SF2 **(Marchisello *et al.*, 2019).** PNPLA3 plays a role in hepatic lipogenesis by esterifying lysophosphatidic acid into phosphatidic acid **(Birkenfeld and Shulman, 2014).** The Transmembrane 6 Superfamily 2 gene (TM6SF2, variant E167K) is another genetic variant recently linked to NAFLD and liver cirrhosis **(Dongiovanni *et al.*, 2015; Smagris *et al.*, 2016).**

I.3.7. Intestinal microbiota

The gut microbiota plays a significant role in the pathophysiology of NAFLD **(Lau and Wong, 2018).** Dysbiosis can compromise the integrity of the intestinal barrier, leading to a disruption of the gut-liver axis and increased intestinal permeability. This hyperpermeability causes bacterial translocation, increased hepatic absorption of free fatty acids, metabolites from the fermentation of fibers and carbohydrates in the colon, and altered bile acid metabolism **(Bashiardes *et al.*, 2016; Cani *et al.*, 2007).** As a result, hepatic lipid metabolism is disrupted, and the liver is exposed to harmful substances, exacerbating inflammation and fibrosis **(Bashiardes *et al.*, 2016).**

I.3.8. Mitochondrial dysfunction and oxidative stress

Mitochondrial structural and functional alterations play a crucial role in the pathogenesis of NAFLD **(Zheng *et al.*, 2023).** During hyperglycemic states, acetyl-CoA is converted into citrate, some of which moves from the mitochondrial matrix to the cytosol. There, it regenerates acetyl-CoA, which is further converted into malonyl-CoA. High levels of malonyl-CoA promote hepatic fatty acid synthesis and inhibit their oxidation **(Wei *et al.*, 2008).** Mitochondrial dysfunction can lead to both fat accumulation and the production of reactive oxygen species (ROS) and cytokines, contributing to the progression of NAFLD by inducing liver inflammation and fibrosis **(Sanyal *et al.*, 2001; Wei *et al.*, 2008).**

I.3.9. Age et gender

The frequency of NAFLD rises with advancing age **(Frith *et al.*, 2009).** It exhibits a twofold higher occurrence in males compared to females **(Park *et al.*, 2004).** Research has unveiled instances of familial clustering of NAFLD **(Wagenknecht *et al.*, 2009).**

I.4. Diagnosis

Non-alcoholic fatty liver disease (NAFLD) often presents without symptoms **(Friedman *et al.*, 2018).** Diagnosis commonly occurs during routine health screenings **(Choudhury and Sanyal, 2004).** Typically involving liver function tests or abdominal ultrasound as part of the assessment. While liver biopsy remains the gold standard for assessing NAFLD severity, offering a precise histological diagnosis. **(Friedman *et al.*, 2018; Marchisello *et al.*, 2019).** Various invasive and non-invasive techniques are available to aid in its evaluation (Figure 3).

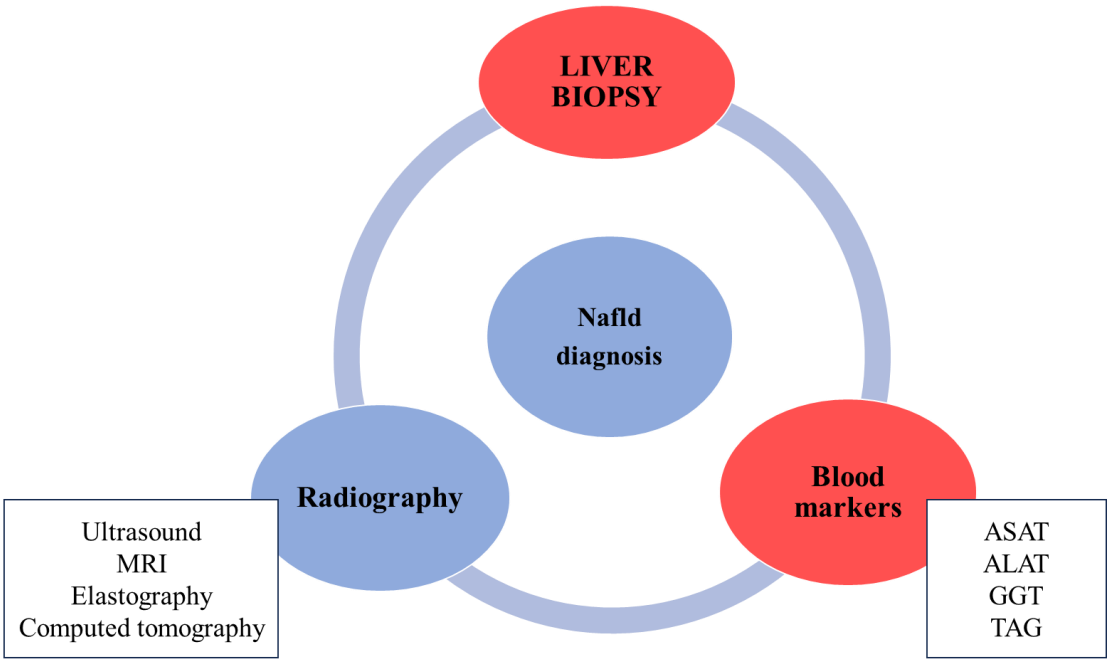


Figure 3. The different approaches used for NAFLD investigation. ***ASAT**: aspartate aminotransferase. **ALAT**: alanine aminotransferase. **GGT**: gamma-glutamyl transferase. **TAG**: Triacylglycerol. **MRI**: magnetic resonance imaging* **(National Guideline Centre (UK), 2016).**

I.5. Underlying diseases

Non-alcoholic fatty liver disease (NAFLD) should be viewed as part of a complex multisystemic disorder due to its elevated likelihood of leading to severe chronic conditions **(Hydes *et al.*, 2020).**

Table I. Underlying diseases of NASH/NAFLD.

NASH / NAFLD			
Type 2 Diabetes	**Chronic kidney disease**	**Cardiovascular disease**	**Extra hepatic cancers**
people with NAFLD often display heightened insulin resistance, which can advance to type 2 diabetes, particularly among individuals obesity, sedentary lifestyle, poor dietary habits, genetic factors, hypertension, and dyslipidemia, commonly linked with type 2 diabetes	A later meta-analysis discovered a link between non-alcoholic fatty liver disease and both the occurrence and frequency of chronic kidney disease in individuals Moreover, it was found that chronic kidney disease was more prevalent in individuals with non-alcoholic steatohepatitis compared to those with simple steatosis.	The mechanisms of NAFLD, notably systemic inflammation, oxidative stress, adipokines, endoplasmic reticulum stress, cardiac lipotoxicity, and microbiota dysbiosis, are involved in cardiovascular diseases. including, adipose tissue releases numerous factors potentially implicated in atherogenesis.	Substantial evidence indicates that NAFLD could elevate the risk of developing specific cancer types, including colorectal, pancreatic, esophageal, gastric, uterine, intrahepatic cholangiocarcinoma, breast, kidney/bladder, and melanom.
Reference			
(Anstee *et al.*, 2013)	**(Hydes *et al.*, 2020)**	**(Byrne and Targher, 2015; Mitsala *et al.*, 2022).**	**(Mitsala *et al.*, 2022)**

I.6. Pathophysiology of NAFLD

I.6.1. NON ALCOOLIC STEATOSIS NAFLD

The development of NAFLD involves an intricate interplay of factors: a surge in dietary fatty acids, disruptions in the gut-liver axis due to dysbiosis, breakdown of adipose tissues, hepatic fat synthesis, and reduced elimination and oxidation of fatty acids **(Jasirwan *et al.*, 2019).** This imbalance leads to lipid accumulation in hepatocytes, followed by inflammation and fibrosis triggered by pro-inflammatory cytokine influx (TNF-α, IL6, IL-8, IL1β) which activate several serine kinases (Figure 4), including IκB kinase (IKK) and JNK **(Gual *et al.*,**

2005) and heightened oxidative stress **(Aleksandrova *et al*., 2014; Cassard-Doulcier and Perlemuter, 2011).** Obesity and/or insulin resistance leads to an increase of lipolysis which induced an overflow of triglycerides derived free fatty acids (FFA) **(Sethi and Vidal-Puig, 2007).** These FFAs can activate inflammatory pathways and impair insulin signaling PI3K/Akt ***(McArdle et al*., 2013).** The accumulation of triglycerides in the liver leads to insulin resistance, which increases insulin's inhibitory (Figure 4) effect on the production of glucose (gluconeogenesis) and triglycerides (De novo lipogenesis) by the liver **(Saltiel and Kahn, 2001).** The disruption of mitochondrial biogenesis and oxidative function also plays a significant role in the development of insulin resistance and hepatic steatosis. **(Jornayvaz *et al*., 2010; Smith and Adams, 2011).** Histologically, steatosis in NAFLD is predominantly macrovesicular ***(Wree et al*. 2014),** although it can comprise a combination of large and small vacuoles **(Brown and Kleiner, 2016).** Microvesicular steatosis, identified by its characteristic foamy cytoplasmic appearance, can also manifest in individual hepatocytes or in small clusters **(Chalasani *et al*. 2008).**

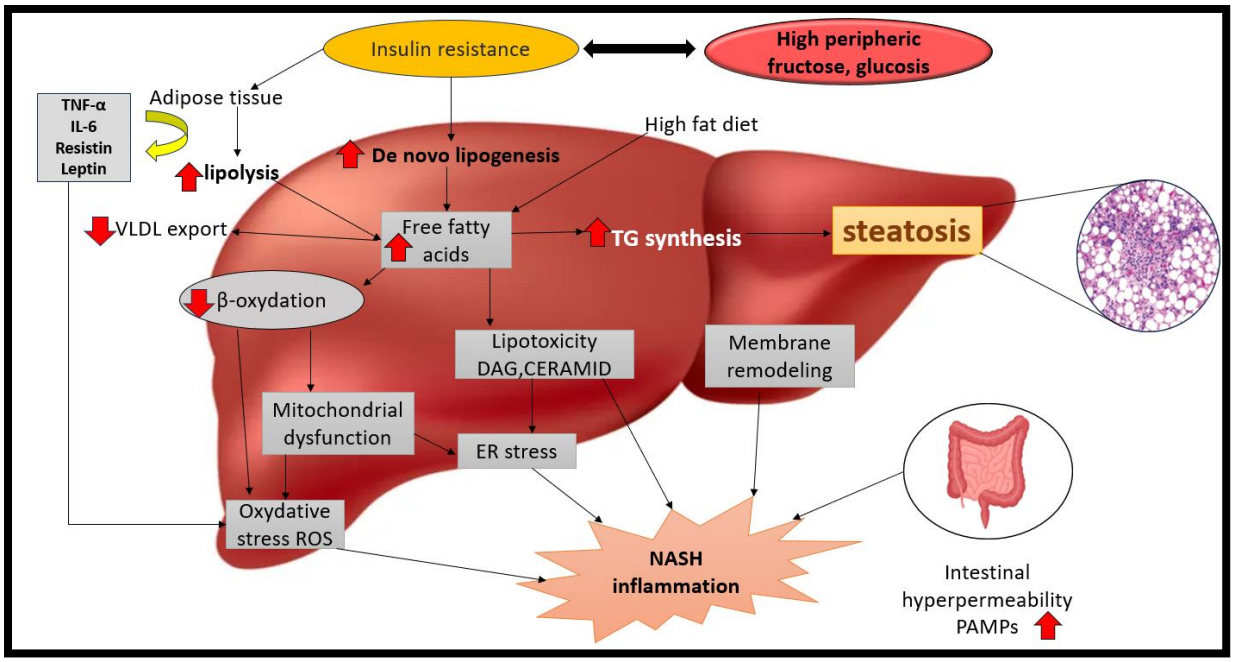


Figure 4. Pathophysiological mechanisms contributing to NAFLD progression **(Mi *et al.*, 2024).** ***TNF-α**: Tumor Necrosis Factor-alpha.* ***IL-6:** Interleukin-6.* ***TG:** Triglycerides.* ***VLDL:** Very Low-Density Lipoprotein.* ***DAG:** Diacylglycerol.* ***ROS:** Reactive Oxygen Species.* ***ER:** Endoplasmic Reticulum.* ***NASH:** Non-Alcoholic Steatohepatitis.* ***PAMPs:** Pathogen-Associated Molecular Patterns.*

I.6.2. FIBROSIS

Fibrogenesis is initiated by the activation of Kupffer cells and monocytes, which result in an activation of TGF-β signaling pathway and the production of cytokines such as IL-1β **(Koyama and Brenner, 2017)** and subsequently activating hepatic stellate cells (HSC) (Dooley and Ten Dijke, 2012). Therefore, apoptotic bodies from dead hepatocytes can be phagocytosed by stellate cells, further promoting their activation **(Jiang *et al.*, 2009).** Once activated, these stellate cells express new receptors, including the platelet-derived growth factor receptor (PDGF) and the transforming growth factor β receptor (TGF-β) **(Khanam *et al.*, 2021).** These stellate cells subsequently (Figure 5) differentiate into myofibroblasts with fibrogenic properties **(Zhang *et al.*, 2016).** This differentiation can worsen inflammation, primarily through the Toll Like Receptor 4 (TLR4) receptor on their surface **(Guo and Friedman, 2010).** This process culminates in the excessive deposition of collagen and other extracellular matrix proteins by the stellate cells, resulting in scar tissue formation **(Khurana *et al.*, 2021).** Fibrosis is frequently observed in NASH, usually manifesting as perisinusoidal/pericellular fibrosis in zone 3, and includes a mixed infiltrate of inflammatory cells such as lymphocytes, neutrophils, eosinophils, and Kupffer cells (KC) **(Méndez-Sánchez *et al.*, 2020).** Masson's trichrome stain accentuates the accumulation of collagen and other extracellular matrix proteins along the sinusoids surrounding the hepatocytes **(Czuppon *et al.* 1993).**

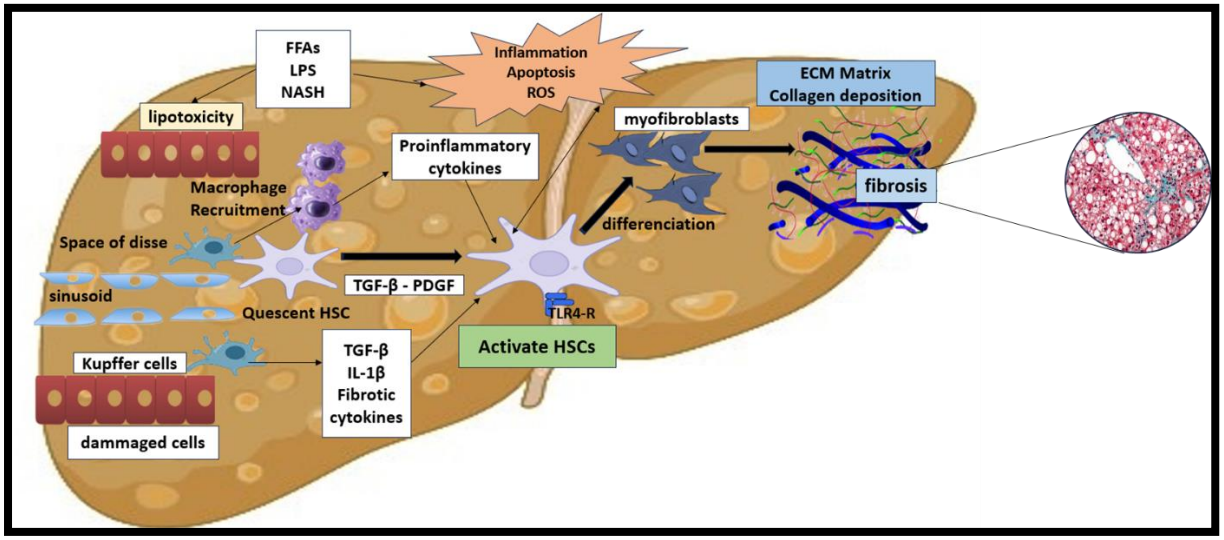


Figure 5. Fibrosis pathophysiology **(Khanam, Saleeb, and Kottilil 2021).** ***FFAs**: Free Fatty Acids* ***LPS**: Lipopolysaccharides.* ***NASH**: Non-Alcoholic Steatohepatitis.* ***ROS**: Reactive Oxygen Species.* ***TGF-β**: Transforming Growth Factor Beta.* ***PDGF**: Platelet-Derived Growth Factor.* ***IL-1β**: Interleukin 1 Beta.* ***HSCs**: Hepatic Stellate Cells.* ***ECM**: Extracellular Matrix.* ***TLR4-R**: Toll-Like Receptor 4.*

I.6.3. CIRRHOSIS

Cirrhosis marks a significant phase in the advancement of hepatic fibrosis triggered by various chronic liver diseases, such as non-alcoholic fatty liver disease **(Schuppan and Afdhal, 2008).** The complications associated with cirrhosis (Figure 6) can be potentially severe, encompassing portal hypertension (PHT) resulting in bleeding from esophageal or gastric varices, hepatic encephalopathy, spontaneous bacterial peritonitis, hepatorenal syndrome, and hepatocellular carcinoma (HCC) **(Sawadogo *et al.*, 2007).** In cirrhosis, the space of Disse becomes filled with fibrotic septa and the endothelial fenestrations are lost, a phenomenon known as sinusoidal capillarization **(Schaffner and Poper, 1963).** This phenomenon leads to significant microvascular changes within the liver and the development of intrahepatic shunts, primarily caused by angiogenesis and loss of parenchymal cells, as well as hepatic endothelial dysfunction **(Tsochatzis *et al.*, 2014).** In fact, due to the elevated portal pressure in cirrhosis, portosystemic anastomoses develop in the lower esophagus **(Williams and Iredale, 1998).** This leads to the formation of collateral vessels, providing an alternative route for blood returning to the systemic circulation without passing through the liver **(Maruyama and Yokosuka, 2012).** However, as the disease advances and splenic arterial vasodilation increases, blood pressure decreases, resulting in the activation of the renin-angiotensin system, elevated levels of circulating Antidiuretic Hormone (ADH), and retention of sodium and water by the kidneys **(Arroyo and Colmenero, 2003).** The appearance of ascites is often the initial sign of decompensation in many cirrhotic patients **(Williams and Iredale, 1998).**

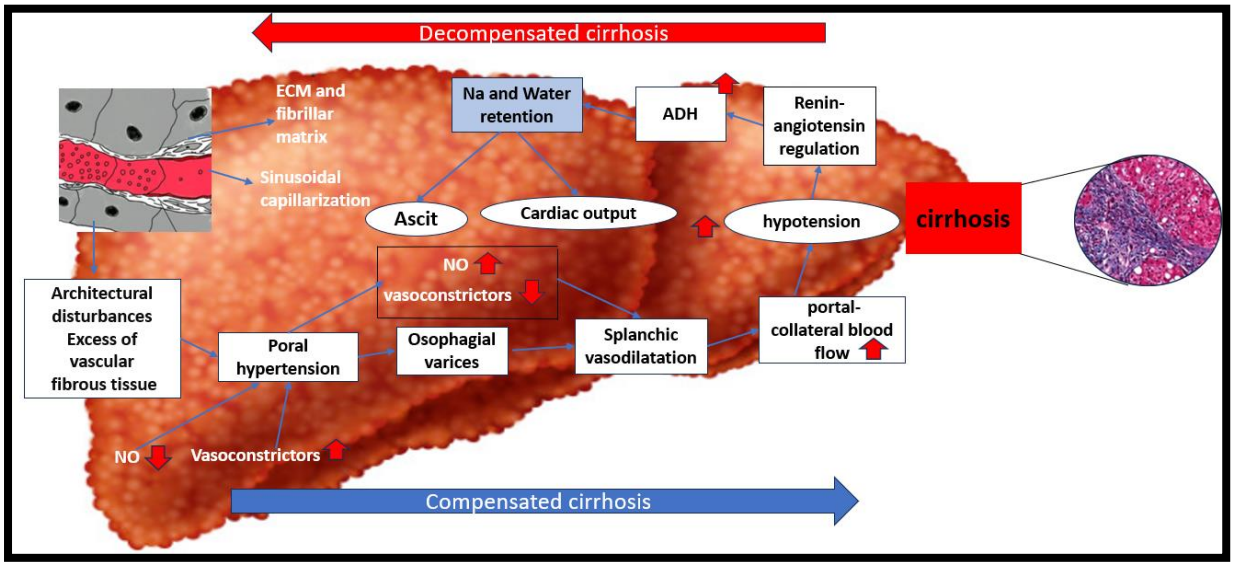


Figure 6. Pathophysiology of portal hypertension **(García-Pagán *et al.*, 2012).** ***ECM****: Extracellular Matrix.* ***NO****: Nitric Oxide.* ***ADH****: Antidiuretic Hormone.*

I.6.4. Hepatocellular carcinoma

Cirrhosis remains the primary risk factor for hepatocellular carcinoma development **(Tarao et al., 2019).**Within the context of cirrhosis, repetitive cycles of compensatory proliferation enhance carcinogenesis **(Nakagawa and Maeda, 2012).** Subsequent to sustained insulin resistance and heightened metabolic stress, intestinal dysbiosis ensues **(Roh and Seki, 2013).** Activation of the specific lipopolysaccharides (LPS) receptor, TLR-4, on Kupffer cells initiates an inflammatory cascade triggered by TNF-α **(Soares *et al.*, 2010).** Chronic inflammation precedes immune system dysregulation **(Anstee *et al.*, 2013).** The tumor microenvironment induces the differentiation of myeloid cells (macrophages) into immunoregulatory cells, including tumor-associated macrophages (TAMs), which exert a pro-tumoral function by stimulating proliferation, angiogenesis, invasion, and immunosuppression **(Tan *et al.*, 2021).** Notably they facilitate the generation of regulatory T lymphocytes **(Zhang *et al.*, 2019)**, and Activation of lipogenesis leading to the excessive production of free fatty acids (FFA) that act as both lipotoxic and pro-tumorigenic agents **(Wang and Malhi, 2018).** Specifically, FFAs induce the activation oncogenic transcription factor, promoting tumor growth **(Longo *et al.*, 2021).**

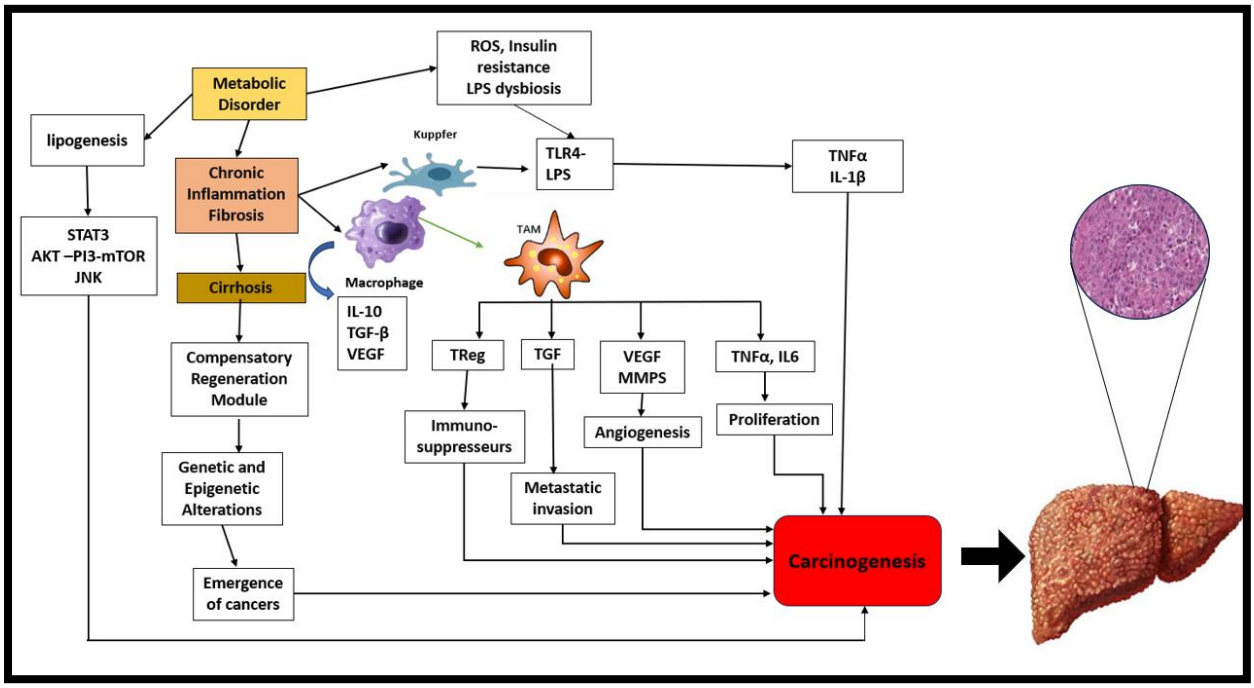


Figure 7. Summary of mechanisms and serum markers of obesity-associated hepatocellular carcinoma (HCC) **(Schwabe and Greten, 2020).** ***STAT3****: Signal Transducer and Activator of Transcription 3,* ***AKT:*** *Protein Kinase B.* ***PI3:*** *Phosphoinositide 3.* ***TOR****: Mechanistic Target of Rapamycin.* ***JNK****: c-Jun N-terminal Kinase.* ***ROS****: Reactive Oxygen Species.* ***LPS:*** *Lipopolysaccharides.****TLR4:*** *Toll-Like Receptor 4.* ***TNFα:*** *Tumor Necrosis Factor Alpha.* ***IL-1β :*** *Interleukin 1 Beta.* ***IL-10 :*** *Interleukin 10.* ***TGF-β:*** *Transforming Growth Factor Beta.* ***VEGF:*** *Vascular Endothelial Growth Factor.* ***TAM:*** *Tumor-*

Associated Macrophage. ***TReg:*** *Regulatory T Cells.* ***TGF:*** *Transforming Growth Factor.* ***MMPs:*** *Matrix Metalloproteinases.* ***IL6:*** *Interleukin 6.*

I.7. G protein-coupled receptors (GCPRs)

I.7.1. Definition

G protein-coupled receptors (GPCRs) are essential proteins located in the cell membrane that play a crucial role in sensing signaling molecules **(Venkatakrishnan *et al.*, 2013).** preserved structure comprising seven transmembrane domains (TMD). G proteins are composed of Gα, Gβ, and Gγ subunits **(Wingler and Lefkowitz., 2020).** They facilitate signal transmission between the interior and exterior of cells. Binding of an external signal to a specific GPCR initiates a series of intracellular reactions **(Rehman *et al.*, 2024).** These reactions begin with a receptor conformational change triggered by ligand binding to its transmembrane region. This change activates associated G proteins, to activate various intracellular signaling pathways **(Miller and Lappin, 2024).** The cascade of reactions has broad consequences, regulating cellular processes such as cell proliferation and gene transcription modulation. At a broader level, these processes regulate key physiological functions including sensory perception, immune response, cell communication, and neurotransmission **(Nair *et al.*, 2019).** Due to their role in physiological regulation and involvement in human pathologies, GPCRs are prime targets for drug development **(Eglen and Reisine, 2011).** GPCRs are divided into five groups: Glutamate, Rhodopsin, Adhesion, Frizzled, and Secretin, collectively known as the GRAFS system **(Fredriksson *et al.*, 2003).**

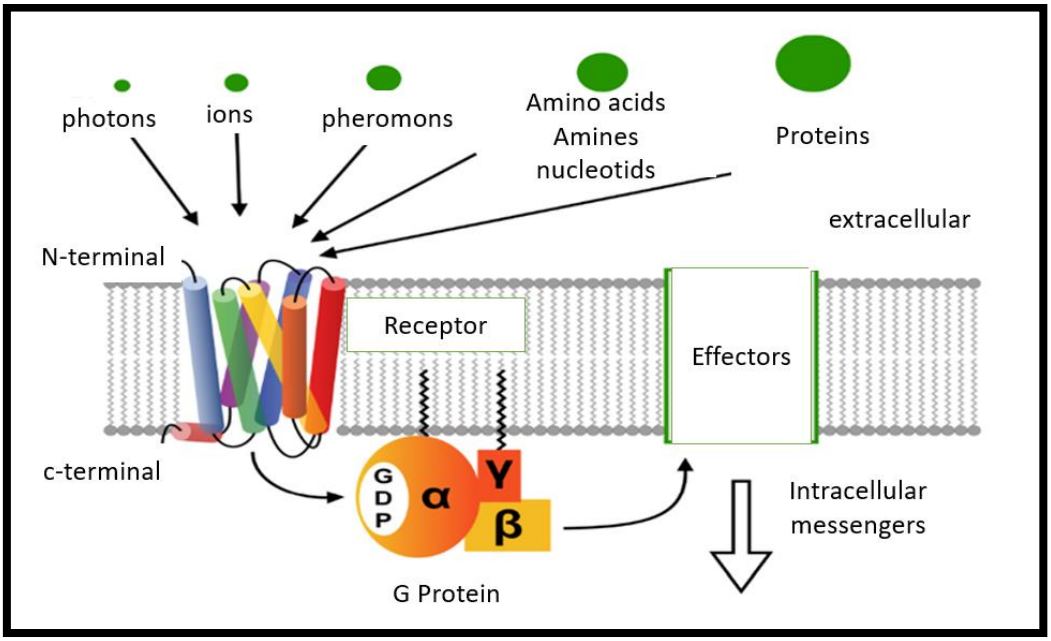


Figure 8. Signal transduction of GCPR **(Bockaert, 1999).**

I.7.2. Activation of GPCR

While inactive, the constituents of G proteins are bonded together, with Gα coupled to GDP. Upon receptor activation, GDP is liberated and substituted with GTP, inducing heterotrimeric dissociation into Gα-GTP and linked Gβγ (Figure 8). This division empowers each subunit to initiate diverse responses within the cell **(Wingler and Lefkowitz, 2020).** They activate additional effectors, thereby amplifying the signal. Upon conclusion, the hydrolysis of GTP into GDP is facilitated by the GTPase activity of Gα. Gα-GDP reverts to its quiescent state through reassociation. Various proteins interact, including β-arrestins and RGS proteins. GPCRs stand as significant targets for addressing heart diseases, inflammation, and central nervous system (CNS) disorder **(DeWire *et al*., 2007; Kobilka, 2013).**

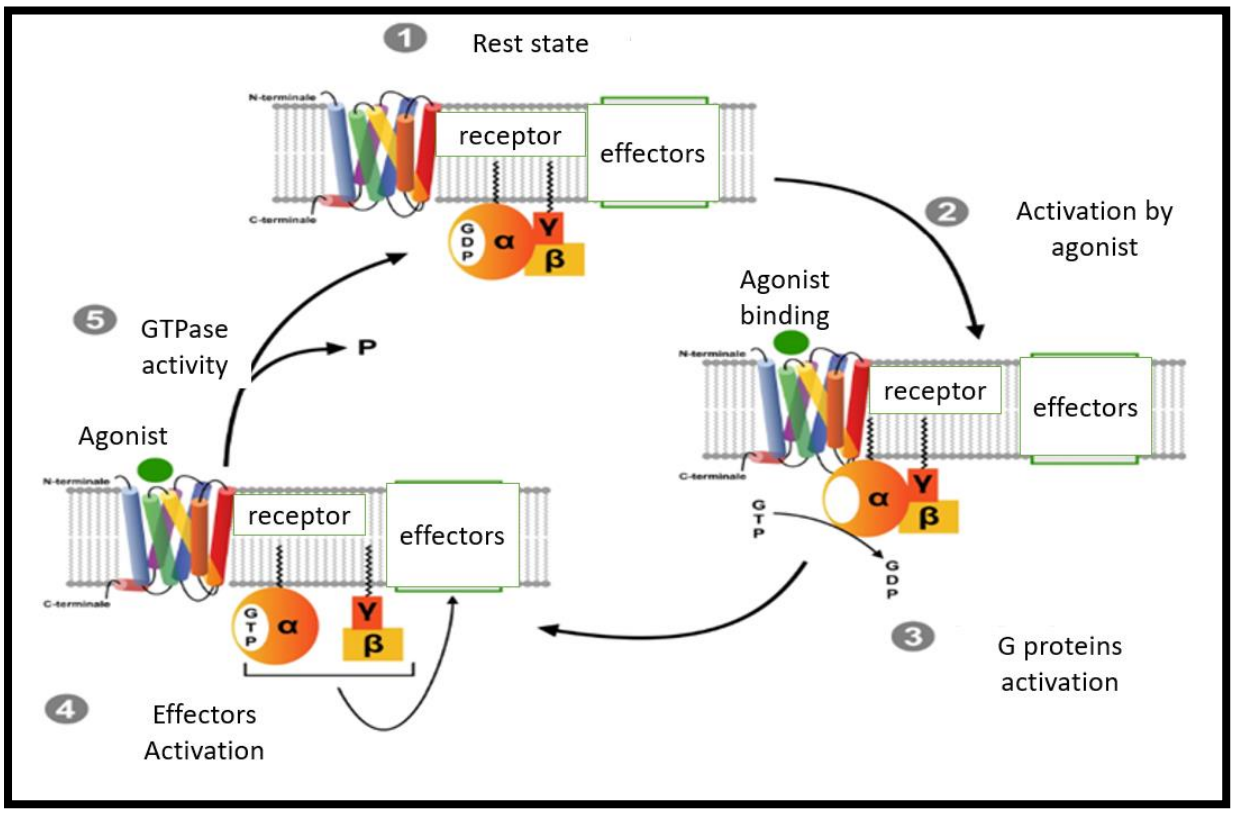


Figure 9. Activation cycle of GCPR **(Tuteja, 2009).**

I.7.3. The β-adrenergic receptors

β-adrenergic receptors are predominantly postsynaptic receptors, consist of three subtypes, β1, β2, and β3, encoded by distinct genes (Figure 9). They primarily interact with Gs

proteins, initiating adenylate cyclase activation, leading to elevated intracellular cyclic AMP levels and subsequent activation of protein kinase A (PKA) **(Amin *et al.*, 2011).** Their native ligands are catecholamines: adrenaline and noradrenaline, synthesized by the adrenal medulla glands and regulated by the sympathetic nervous system. Their physiological effects vary significantly due to diverse second messengers and multiple tissue distributions. They are distributed throughout the body, including the central nervous system. **(Brodde and Michel, 1999).**

I.7.4. Activation of β2-adrenergic receptors

The binding of an agonist triggers the binding of the β2 receptor-agonist complex to the Gs transduction protein that binds GTP (Figure 10). This G protein enables the coupling of the ligand-receptor complex to adenylate cyclase. The activated adenylate cyclase converts ATP into cAMP. This cAMP activates a family of enzymes, the protein kinases A (PKA), that regulates the activity of several cellular proteins, including the L-type Ca2+ channel and β2AR itself (Figure 10). This process results in the relaxation of smooth muscles and other cellular responses **(Devillier *et al.*, 1996).** The β2AR couples to both Gαs and Gαi proteins in cardiac myocytes **(Xiao *et al.*, 1999).**

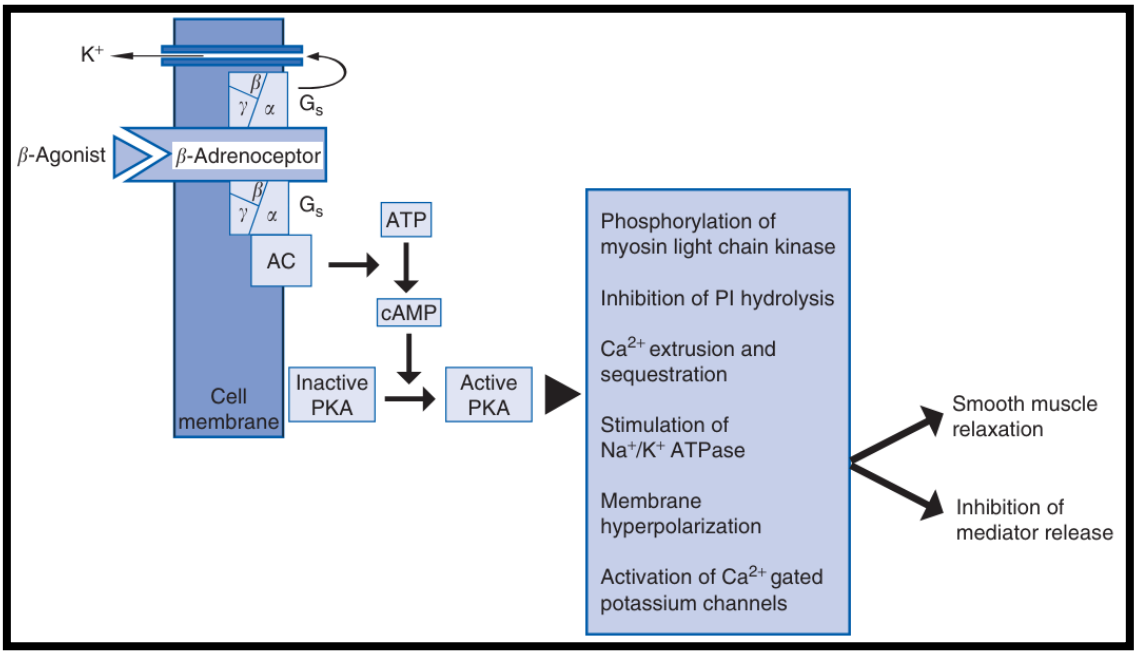


Figure 10. Schematic representation of the signaling pathway following stimulation of the b-receptor **(Tattersfield, 2006).** ***K⁺:*** *Potassium Ion.* ***Gs:*** *Stimulatory G Protein.* ***AC****: Adenylyl Cyclase.* ***ATP****: Adenosine Triphosphate.* ***cAMP****: Cyclic Adenosine Monophosphate.* ***PKA****: Protein Kinase A.* ***PI****: Phosphoinositide.* ***Na⁺/K⁺ ATPase****: Sodium-Potassium Adenosine Triphosphatase.* ***Ca²⁺****: Calcium Ion.*

I.7.5. Regulation of β2-adrenergic receptors

β-2 adrenergic receptors undergo regulatory processes. Phosphorylation of specific serine and threonine residues on these receptors leads to the dissociation of the receptor-Gs protein complex **(Ferguson *et al*., 1995).** These phosphorylated sites are targeted by serine-threonine kinases such as protein kinase A (PKA) (Figure 10), protein kinase (PKC), and G protein-coupled receptor kinases (GRK), which induces desensitization of receptors, resulting in a decrease in their affinity for ligands **(Pitcher *et al*., 1998).** This phosphorylation recruits β-arrestins. Acting as scaffolding proteins, β-arrestins promote the binding of other proteins, particularly phosphodiesterase 4 (PDE4). They activate extracellular signal-regulated kinase (ERK), block G protein activation, and facilitate receptor internalization via clathrin-coated pits **(Rosenbaum *et al*., 2009).** Once bound, PDE4 metabolizes cAMP (cyclic adenosine monophosphate), thereby limiting the propagation of the cellular signal (Figure 11) **(Lefkimmiatis and Zaccolo, 2014)**.

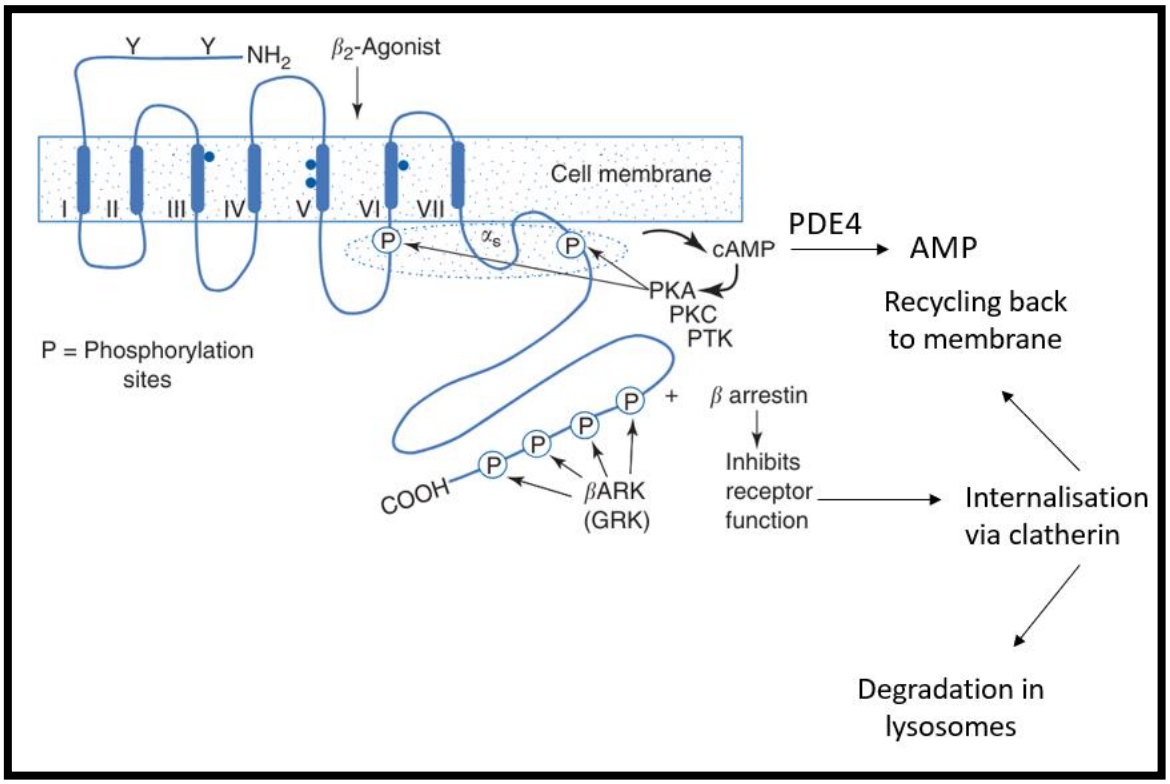


Figure 11. Schematic representation of the signaling pathway following stimulation of the b-receptor (**Tattersfield**, **2006**). *β₂-Agonist: Beta-2 adrenergic receptor agonist.* ***cAMP:*** *Cyclic adenosine monophosphate.* ***PDE4:*** *Phosphodiesterase 4.* ***AMP:*** *Adenosine monophosphate.* ***PKA****: Protein kinase A.****PKC:*** *Protein kinase C.* ***PTK:*** *Protein tyrosine kinase.* ***βARK (GRK):*** *Beta-adrenergic receptor kinase (G-protein-coupled receptor kinase).* ***P****: Phosphorylation sites.*

I.8. β2-Agonists

β-2 adrenergic agonists are a drug class used as a mainstay treatment for respiratory diseases such as bronchial asthma and chronic obstructive pulmonary disease (COPD) **(Hsu and Bajaj, 2024).** They replicate the functions of catecholamines such as epinephrine, norepinephrine, and dopamine in producing different autonomic responses within the body Specifically, the smooth muscle of the airway **(Paravati *et al*., 2024),** uterus **(Klukovits *et al*., 2004)**, intestine **(Díez-Sampedro *et al*., 2011)**, and systemic vasculature are areas where beta-2 agonists have the greatest effect **(Fur *et al*., 2012).**

I.8.1. Affinity of β2-agonists for β2-adrenergic receptors

β-adrenergic receptors have two affinity states for agonists, regulated by guanine nucleotides. When GDP is present, agonist binding leads to a long-lasting ternary complex formation with high agonist binding affinity **(De Lean *et al*., 1980).** In the absence of G protein or when GTP enables receptor-catalyzed G protein activation the receptor in a low-affinity state. Considerable evidence suggests that beta-2 adrenergic receptor agonists bind to either a hydrophobic pocket or an active region situated at a depth of at least 11 angstroms within the core of the beta-2 adrenergic receptor **(Xiao *et al*., 1999).** This particular site aligns with the anticipated location of several crucial amino acid residues, including aspartate 113, serine 204, serine 207, and phenylalanine 290. These residues are acknowledged as indispensable for ligand binding **(Isin *et al*., 2012).**

Table II. β2-agonists affinity for β2-adrenoreceptors **(Anderson, 2006; Lafontan *et al*., 1988; Lötvall, 2001).**

β-agonists	**Nature**	**Interaction with the β2 adrenergic receptor**	**Action**	**Onset of action**
Formoterol	Lipophilic	Directly to the active site	Fast	Long
Salbutamol	Hydrophilic	Directly to the active site	Fast	Court
Salmeterol	Lipophilic	Diffuses through the cell membrane to reach the active site	Slow	Long
Clenbuterol	Lipophilic	Directly to the active site	Slow	Long

I.8.2. Effects of β2-agonists on different tissues

Table III. Effects of β2-agonists on different tissues.

Targets	Effects	References
Myocardium	Treatment with **formoterol**, **salmeterol, clenbuterol**, **fenoterol** or **isoproterenol**: • Elevation in heart rate post administration. • Hypertrophy, marked by a rise in the protein content within the heart, coincides with significant structural and functional alterations. • Cellular necrosis subsequent to an increase in apoptosis, in reaction to cellular necrosis, there is an observed fibrosis, likely triggered by the stimulation of collagen synthesis.	**(Eisemann *et al*., 1988)** **(Bates and Pell, 1991; Deshaies *et al*., 1981)** **(Patiyal and Katoch, 2006)**
Airway smooth muscles	Treatment of **Chronic Obstructive Pulmonary Disease** with **salmeterol:** • Increase of forced expiratory volume (FEV) and inspiratory capacity (IC). • Relaxation by catalysis of the activation of protein kinase A (PKA) which in turn leads to the phosphorylation of key regulatory proteins responsible for muscle tone control.	**(Boyd *et al*., 1997)**
Adipose tissue	Treatment with **salbutamol**, **isoproterenol** or **clenbuterol**: • A notable decrease in the amount of adipose tissue. • Restriction of adipogenesis. • Rise in lipolysis.	**(Wenkeová *et al*., 1976)** **(Bricout *et al*., 2004)** **(Kearns *et al*., 2002)**
Adrenal gland	Treatment with **clenbuterol**: • Cellular hyperplasia in the adrenal cortex coincides with elevated levels of corticosterone and adrenaline secretion.	**(Illera *et al*., 1998)**
Skeletal muscle	Treatment with **formoterol**: • Hypertrophy by activating the Akt-mTOR pathway.	**(Joassard *et al*., 2013)**
Liver	Treatment with **clenbuterol**: • Decrease in liver mass (8 to 9 %). • Increase in liver glycogen concentration.	**(MacLennan and Edwards 1989)** **(Xydas *et al*., 2006)**

Chapter II: Materials and methods

II.1. Experimental protocol

The protocol involves inducing steatosis through a specially prepared high fat diet to evaluate the progression of the disease among four groups: negative control with water ad libitum (CTL), HFD without treatment, treated with Formoterol (FOR), and treated with Salbutamol (SAL). The rats were identified using a color-coding system on their tails with indelible markers (red, green, black, and blue) and were weighed daily.

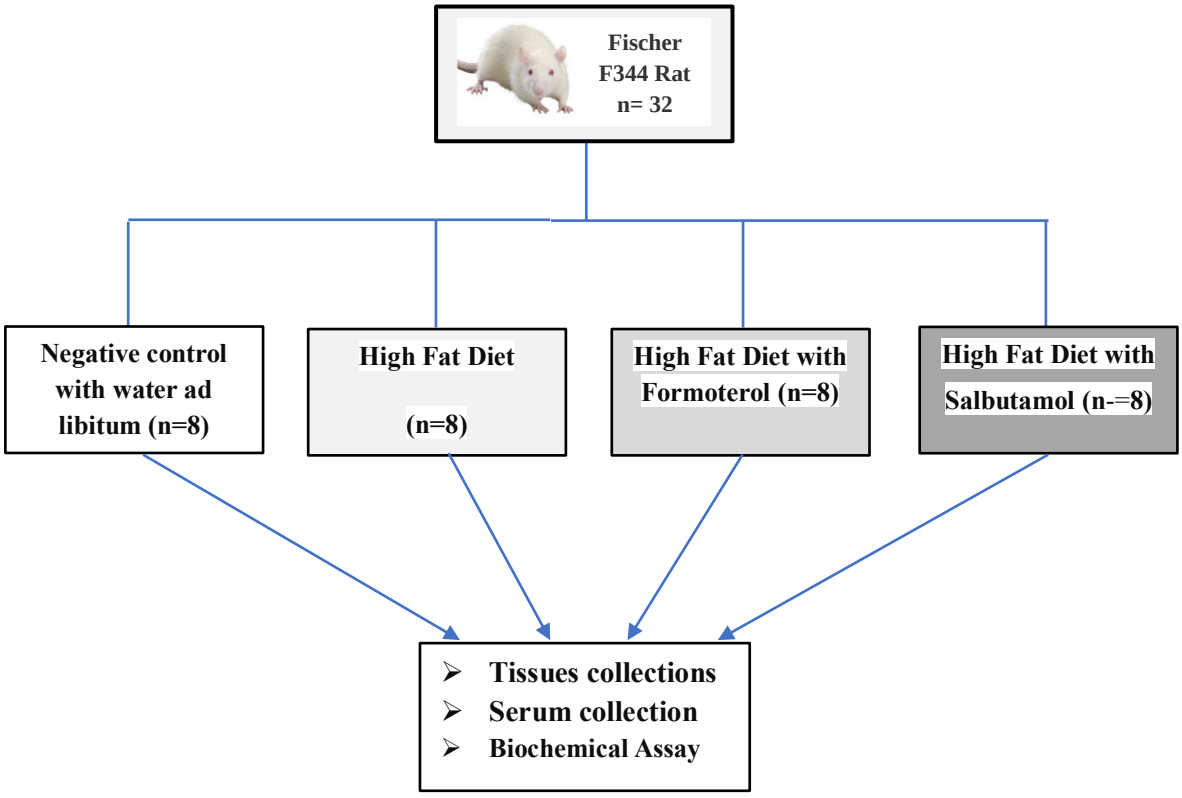


Figure 12. Experimental protocol. *Thirty-two Fischer F344 rats (n=32) were divided into four groups (n=8 per group). A negative control group receiving water ad libitum, a group receiving a high-fat diet (HFD) for 12 weeks, a group receiving HFD for 12 weeks followed by treatment with Formoterol (15 µg/kg) for 2 weeks, and a group receiving HFD for 12 weeks followed by treatment with Salbutamol (150 µg/kg) for 2 weeks. At the end of the treatment period, tissue and serum samples were collected for biochemical assays to evaluate the effects of the treatments.*

II.2. Housing conditions

The rats were housed in the university animal facility in an environment conducive to experimentation: ambient temperature, in plastic cages, labeled, lined with wood shavings, with stainless steel closures.

II.3. Treatment, administration and samples collections

The treatment was prepared in the university's physico-chemical laboratory as an injectable solution. For in vivo experiments, 8-week-old rats received daily subcutaneously injections with formoterol at a concentration of 15µg/kg or salbutamol at a concentration 150 µg/kg. For control, age matched rats were injected with a sterile saline solution. After 2 weeks, the animals were anesthetized with intraperitoneally injections with ketamine (50 mg/kg) and xylazine (5 mg/kg).

Organs (liver, heart, adipose tissue and lungs) were weighed and stored at -20°C. Blood samples were collected from the jugular vein in dry tubes, labeled, and centrifuged. The first centrifugation was at 4000 rpm for 10 minutes at 4°C to collect serum, which was then pipetted into Eppendorf tubes. The second centrifugation was at 2000 rpm for 10 minutes at 4°C to remove cellular debris, and the serum was stored at -20°C for subsequent biochemical tests. Vital organs (liver, heart, kidneys, lungs, spleen, pancreas, and adipose tissue) were dissected, weighed and stored at -20°C in 10% formalin.

II.4. Biochemical Assay

II.4.1. Aspartate aminotransferase (ASAT) Activity

Accurate measurement of enzyme activity is crucial in various biochemical and clinical analyses. Aspartate aminotransferase (ASAT) is a key enzyme whose activity can indicate liver function and damage.

Method developed by Karmen et al. and optimized by Henry et al (In accordance with IFCC recommendations) **(Lustig *et al.*, 1988).** The decrease in absorbance is proportional to ASAT activity in the specimen measured at 340 nm. Serum samples are prepared by centrifugation and stored at -20°C. The ASAT reagent, including buffers, substrates (L-aspartate and 2-oxoglutarate), and NADH, is prepared and equilibrated to room temperature. 100 µl of the

serum sample is mixed with 1000 µl of the reagent, incubated at 37°C, and the absorbance decrease at 340 nm is measured over a 5 minutes interval. The ASAT activity, expressed in U/L, is proportional to the rate of NADH oxidation.

The reaction scheme is as follows:

ASAT (Aspartate Aminotransferase) reaction :

MDH (Malate Dehydrogenase) reaction :

II.4.2. Alanine aminotransferase (ALAT) Activity

Accurate assessment of alanine aminotransferase (ALAT) activity is critical for evaluating liver health and detecting liver damage.

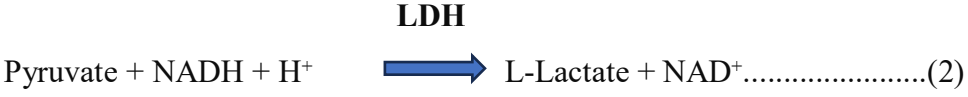
Method developed by Wrobleski and La Due, optimized by Henry and Bergmeyer (in accordance with IFCC recommendations) **(Okorodudu *et al*., 1989).** The decrease in absorbance is proportional to ALAT activity in the specimen measured at 340 nm.

Serum samples are prepared by centrifugation and stored at -20°C. The ALAT reagent, containing buffers, L-alanine, 2-oxoglutarate, and NADH, is prepared and equilibrated to room temperature. After mixing 100 µl of the serum sample with 1000 µl of the reagent, the reaction mixture is incubated at 37°C, and the absorbance decrease at 340 nm is measured over a 5 minutes interval to determine ALAT activity, expressed in U/L.

The reaction scheme is as follows:

ALAT (Alanine Aminotransferase) reaction :

LDH (Lactate Dehydrogenase) reaction :



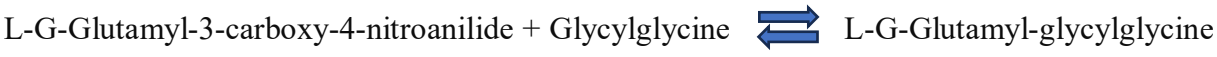
II.4.3. GAMMA GT Activity

Accurate measurement of gamma-glutamyltransferase (GGT) activity is vital for assessing liver and bile duct function, as well as for diagnosing various liver diseases.

Method based on the work of Szasz, Rosalki, and Tarlow. The reaction scheme is as **(Szasz *et al*., 2019).** The rate of formation of p-nitroaniline is directly proportional to GGT activity in the specimen measured at 405 nm.

Serum samples are prepared by prompt separation and storage at -20°C. The GGT reagent, comprising appropriate buffers and gamma-glutamyl-p-nitroanilide substrate, is prepared and equilibrated to room temperature. Upon mixing 50 µl of the serum with 1000 µl of the reagent, the reaction is incubated at 37°C, and absorbance at 405 nm is monitored over time to quantify GGT activity. Results are expressed in U/L, where one unit represents the enzyme activity that generates one micromole of product per minute under standard assay conditions.

The reaction scheme is as follows:



II.4.4. Triglycerides quantification

Accurate measurement of triglyceride levels is crucial in clinical diagnostics, providing insights into lipid metabolism and cardiovascular health.

Fossati and Prencipe method associated with the Trinder reaction **(Fossati *et al*., 1983; Trinder, 1969).** The absorbance of the colored complex (quinoneimine) is proportional to the amount of triglycerides in the specimen measured at 500 nm.

Serum samples are preferred and stored at -20°C if not immediately analyzed. The triglycerides reagent, containing buffers, glycerol kinase, glycerol-3-phosphate oxidase, and peroxidase, is prepared and brought to room temperature. After mixing 10 µl of the sample with

1000 µl of the reagent, the reaction mixture is incubated at 37°C, and the absorbance at 500 nm is measured using a spectrophotometer. Triglyceride concentrations are calculated using a standard curve and reported in mg/dL or mmol/L of serum.

The reaction scheme is as follows:

Triglycerides hydrolysis:

$$\text{Triglycerides} \xrightarrow{\textbf{Lipase}} \text{Glycerol + Free fatty acids} \quad \text{.....(1)}$$

GK (Glycerol Kinase) reaction:

$$\text{Glycerol + ATP} \xrightarrow{\textbf{GK}} \text{Glycerol 3-Phosphate + ADP} \quad \text{.....(2)}$$

GPO (Glycerol-3-Phosphate Oxidase) reaction:

$$\text{Glycerol 3-Phosphate + } O_2 \xrightarrow{\textbf{GPO}} \text{Dihydroxyacetone Phosphate + } H_2O_2 \quad \text{.....(3)}$$

POD (Peroxidase) reaction:

$$H_2O_2 + \text{4-Chlorophenol + PAP} \xrightarrow{\textbf{POD}} \text{Quinoneimine (pink) + } H_2O \quad \text{.....(4)}$$

II.4.5. Cholesterolimia quantification

Accurate measurement of serum cholesterol levels is essential in assessing cardiovascular risk and monitoring lipid metabolism.

Quantitative measurement of serum cholesterol was performed using the enzymatic method described by Allain et al **(Allain *et al.*, 1974)**. Serum samples were thawed to room temperature before analysis. The cholesterol reagent, containing buffers, cholesterol esterase, and cholesterol oxidase, was prepared and equilibrated to room temperature. 10 µl of serum was mixed with 1000 µl of the reagent in a cuvette, incubated at 37°C, and the absorbance was measured spectrophotometrically at 500 nm. Total cholesterol concentrations were determined using a standard curve and expressed in mg/dL or mmol/L of serum.

According to the following reaction scheme:

CE (Cholesterol Esterase) reaction :

CE

Cholesterol + O_2 ⟹ Cholesterol + Free Fatty Acids…….(1)

CO (Cholesterol Oxidase) reaction:

CO

Cholesterol + O_2 ⟹ Cholesten-4-one-3 + H_2O_2…….(2)

POD (Peroxidase) reaction:

POD

2 H_2O_2 + Phenol + PAP ⟹ Quinoneimine (pink) + 4 H_2 …….(3)

II.5. Histology

Liver tissues were fixed in 4% paraformaldehyde and embedded in paraffin. Sections of 4 µm were used for hematoxylin-eosin-safran (HES) to visualize the architecture of hepatic tissue and inflammatory cell infiltration.

Fixation

The livers of the rats were placed in 10% formalin suspension immediately after dissection.

Dehydration

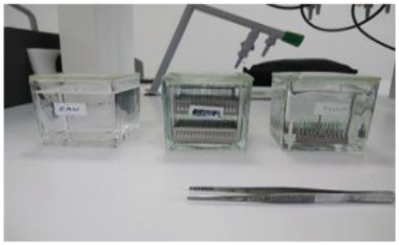
The samples are placed in histocassets and then in a semi-closed automatic tissue processor on the laboratory bench for a duration of 7 hours.

Paraffin embedding

The histocassets containing the samples are individually coated with paraffin using a paraffin embedding station.

Microtomy

After the paraffin-embedded samples cooled down, they were processed for microtomy. The very thin sections obtained from this microtomy are mounted on slides.



Rehydration

After the slides have been incubated for a minimum of 2 hours at 60°C, they are immersed in xylene for 10 minutes, then in ethanol for 10 minutes, and finally in water for 10 minutes.

Coloration

The slides were immersed in hematoxylin for 3-4 minutes and then in eosin for 1 minute.

Figure 13. Histomorphometry protocol.

II.5.1. Histological quantification

The surface area of lipid droplets within liver tissue samples was measured using ImageJ software. Five high-resolution images were captured to represent the entire liver for each sample. These images were imported into ImageJ, where its measurement tools were used to outline and quantify the surface area of each lipid droplet. The rule of three was then applied using the total surface area of the images to ensure accurate proportional representation.

II.6. Statistics

Various statistical tests including unpaired t-tests as well as one-way analysis of variance followed by Bonferroni test were used to determine whether specific group mean differences were significant. Each test performed is specified in the figure legends. The minimum a-level of significance was set at 0.05. Data are presented as means ± SEM throughout.

Chapter III: Results and discussion

III.1. Results

III.1.1. β2-agonist effect on adipose tissue

We aimed to assess the effects of administering formoterol and salbutamol on different tissues. Adipose tissue weight was significantly ($p<0,001$) different between HFD and others groups after two weeks of treatment. Noted that adipose tissue weight in the group HFD was significantly increased by 286% compared CTL group. In addition, adipose tissues weight was decreases in group FOR and SAL respectively 55% and 65% compared with HFD group.

Table IV. Weights of different tissues. *Values are means ± SE (n=6-8). *** P<0.001 relative to CTL: One-way ANOVA and Bonferroni as post hoc test. ns: not significant.*

Tissue	Group	Mean (g)	Std. Dev	Significance
Adipose tissue	CTL	5,95	1,49	
	HFD	17,03	3,59	***
	FOR	9,46	2,63	ns
	SAL	11,20	1,15	ns
Liver	CTL	13,28	2,03	
	HFD	15,69	3,16	ns
	FOR	14,06	1,76	ns
	SAL	12,85	1,41	ns
Lungs	CTL	2,84	0,78	
	HFD	2,65	0,68	ns
	FOR	2,90	0,70	ns
	SAL	3,45	0,52	ns
Heart	CTL	1,40	0,13	
	HFD	1,51	0,12	ns
	FOR	1,56	0,29	ns
	SAL	1,51	0,12	ns

III.1.2. β2-agonist effect on liver enzymes levels

ALAT, ASAT and ɣGT serum can be used to assess hepatic function and/or injury. ALAT is in the highest concentration in the liver. ASAT is also present in heart, muscle, kidney, brain, pancreas, lung. ɣGT is abundant in liver, kidney, pancreas and intestine.

We have expected by spectrophometry the level of alanine aminotransferase (ALAT) in different groups. As expected, the level of ALAT in HFD treated with formoterol or salbutamol groups shows a substantial decrease respectively by 36% ($p<0.05$) and 42% ($p<0.05$) compared to HFD without treatment group (Figure 14.A). Moreover, there is no significant difference of ALAT level between negative control and other groups (Figure 14.A). The FOR group has levels around 75%, while the SAL group shows levels slightly below the control at around 90%. Similarly, we have also observed that aspartate aminotransferase (ASAT) is decreased in HFD treated with formoterol or salbutamol groups compared with non-treated HFD group (Figure 13.B).

As ALAT level, we didn't expect any difference of ASAT level between negative control groups with HFD treated or HFD alone. Surprisingly, we assessed the level of ɣGT in different groups. As shown in (Figure 14.C), there is no significance difference of level ɣGT in different groups.

III.1.3. β2-agonist effect on Triglycerides level

Steatosis is characterized by the accumulation of triglycerides in the liver, which increases insulin's inhibitory effect on the production of glucose and triglycerides by the liver **(Saltiel and Kahn, 2001).** To determine whether high fat diet induces steatosis through triglyceride over synthesis, we measured the rate of triglyceride in serum of different groups. The HFD group shows an increase of triglyceride level by approximately 60% compared to control group. However, triglyceride level is significantly decreased in group HFD treated with salbutamol (Figure 14.D). Nevertheless, we don't find significant difference between HFD treated with formoterol and HFD group. Moreover, there is no significant difference on triglyceride level between negative control and other groups (Figure 14.D).

III.1.4. β2-agonist effect on total cholesterol level

Steatosis is considered the hepatic component of Metabolic syndrome (MetS) **(Flisiak-Jackiewicz *et al.*, 2021).** Metabolic syndrome is characterized by hypertriglyceridemia and decreased High-Density Lipoprotein (HDL) cholesterol **(Eckel *et al.* 2010)**. β2-agonist administration with formoterol or salbutamol does not alter total cholesterol level (Figure 14.E).

We did not find any difference of total cholesterol level between groups CTL, HFD, HFD-FOR and HFD-SAL.

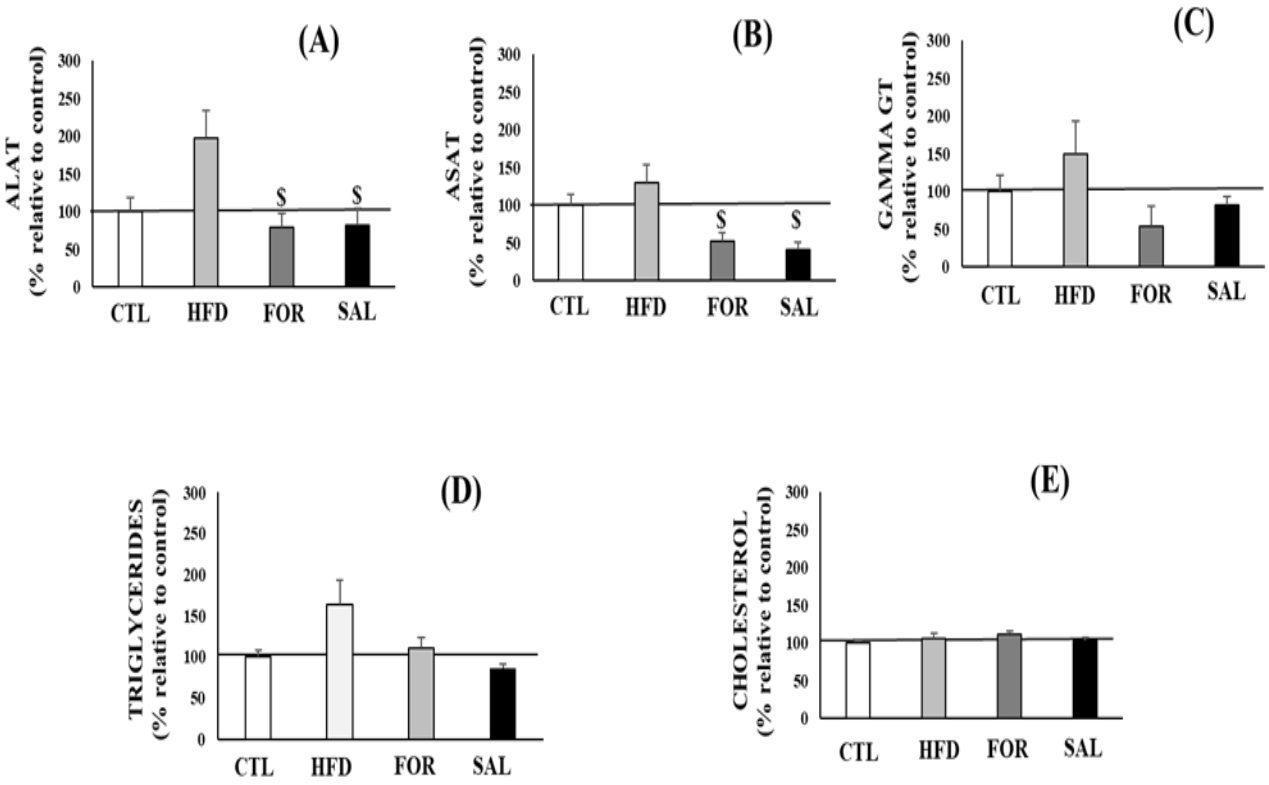


Figure 14. Plasma levels of different biochemical parameters. in different groups (CTL: Control, HFD: High Fat Diet, FOR: High Fat Diet Formoterol, SAL: High Fat Diet Salbutamol). *Values are means ± SE (n=6-8). $ P<0.05 relative to HFD: One-way ANOVA and Bonferroni as post hoc test.*

III.1.5. β2-agonist effect on steatosis

Non-alcoholic fatty liver disease (NAFLD) is defined as an excess of fat in the liver (≥ 5% of hepatocytes laden with lipid droplets upon histological analysis. The table presents the percentages of hepatic steatosis induced by a high-fat diet (HFD) for different groups (CTRL, HFD, FOR, SAL).

Table V. Steatosis percentage compared to CTL group. *Values are means ± SE (n=6-8). $ P<0.05 relative to HFD, * P<0,05 relative to CTL: One-way ANOVA and Bonferroni as post hoc test.*

	CTL	HFD	FOR	SAL
STEATOSIS %	0,33	5,23	2,36	1,04
STDEV	0,10	0,75	1,11	0,75
SIGNIFICANCE	$	*		$

After 12 weeks of high fat diet, we determined the accumulation of fat in the liver in different groups. As illustrated in (Figure 15), the steatosis is highly significant ($p<0.001$) increase in HFD group by about 5.23% compared to negative control (Table V). The steatosis in HFD-treated with formoterol or salbutamol is respectively significant 2.36% ($p<0.05$) and 1.04% compared to negative control (Table V). Moreover, there is no difference of percentage of steatosis between HFD-treated with salbutamol and the negative control. Finally, the effect of salbutamol administration is highly marked than formoterol treatment compared to the control. These data, suggest that salbutamol treatment is more efficient than formoterol treatment.

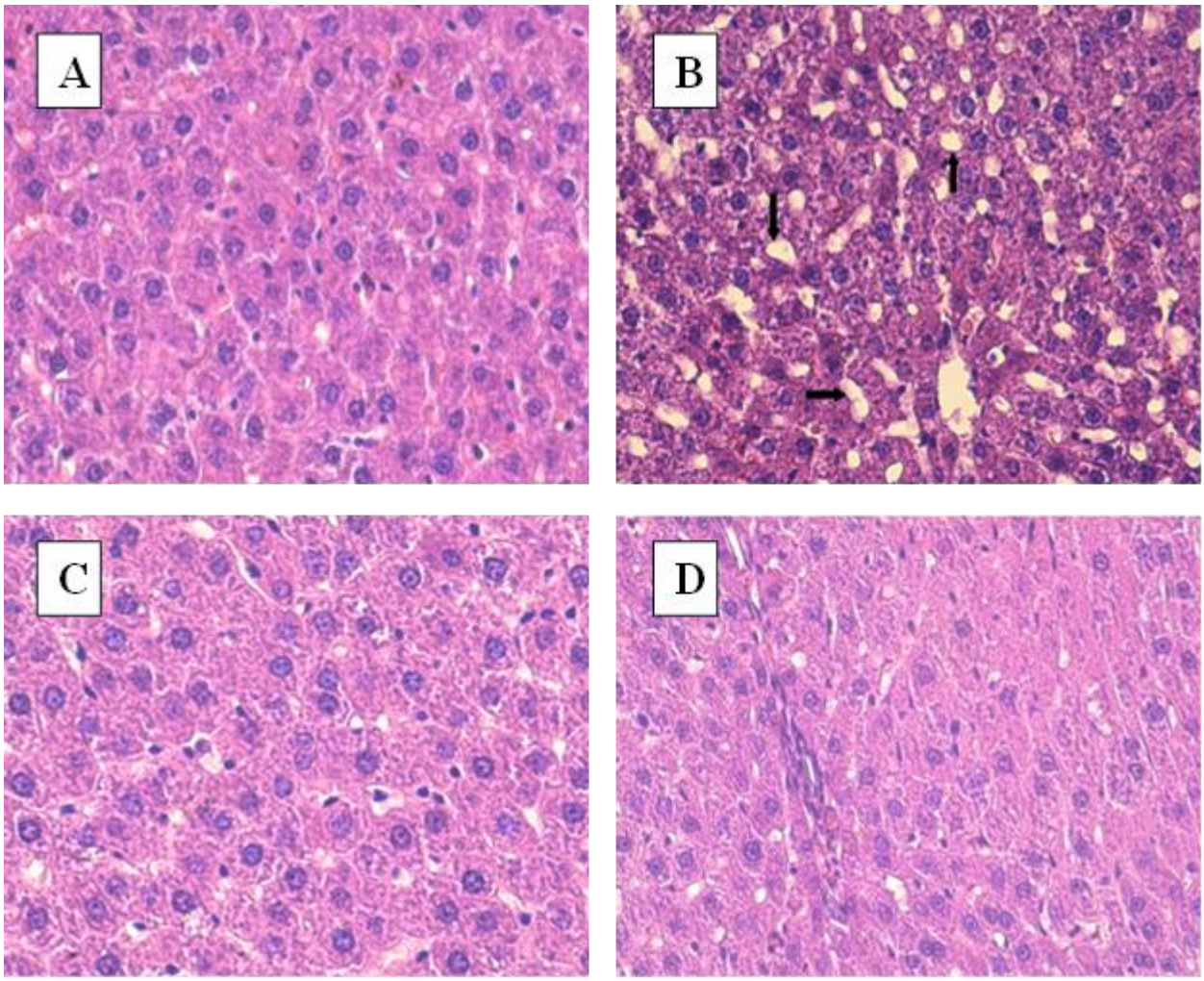


Figure 15. Hematoxylin-eosin staining of liver cross-sectional area. ***A :*** *Control group, healthy and uniform hepatocytes without any signs of damage (CTL),* ***B :*** *High Fat Diet (HFD) group, steatosis characterized by lipid droplets (indicated by arrows) and dilation of sinusoids which indicates toxicity,* ***C :*** *High Fat Diet Formoterol-treated group (FOR), uniform appearance of hepatocytes without obvious signs of swelling or degeneration,* ***D :****High Fat Diet Salbutamol-treated group (SAL), hepatocytes appear normal, with no obvious signs of cellular stress or degeneration. There are few lipid droplets.*

III.2. Discussion

The growing incidence of metabolic disorders linked to high-fat diets (HFD) necessitates the exploration of effective therapeutic interventions. The consumption of high-fat diets has been widely documented as a major factor contributing to the development of obesity, non-alcoholic fatty liver disease (NAFLD), and other metabolic syndromes **(Buettner et al., 2007).** β2-agonists such as formoterol (FOR) and salbutamol (SAL) are primarily known for their bronchodilatory effects in treating respiratory conditions like asthma and COPD **(Wood et al., 2011).** Moreover, their ability to stimulate lipolysis **(Haffner et al., 1993)** make them of interest in research and clinical settings focused on reducing metabolic disturbances caused by high-fat diets **(Wali et al., 2020).** Our study aims to investigate the effects of a high fat diet and treatments with FOR and SAL on various health parameters in rats, thereby assessing their potential utility in managing diet-induced metabolic disorders.

Aminotransferases are a group of enzymes that catalyze the transfer of amino groups, facilitating the interconversion between amino acids and keto acids **(Vroon and Israili, 1990)** and are released into the bloodstream following hepatocellular injury, thereby serving as direct indicators of hepatic damage **(Contreras-Zentella and Hernández-Muñoz, 2016).** Similarly, gamma-glutamyl transferase (γGT) is another important biomarker for liver health **(Koenig and Seneff, 2015),** reflecting cholestasis **(Xing et al., 2022)** and hepatic oxidative damage when elevated in the bloodstream **(Loguercio et al., 2001).** We revealed significant liver stress and damage in the HFD group, with GGT, ALAT, and ASAT levels rising. These elevations indicate considerable liver damage, consistent with Buettner et al, who reported similar hepatic enzymes increases in HFD-fed rodents **(Buettner et al., 2007).** Formoterol and salbutamol treatments showed partial protective effects, reducing ɣGT by 37% and 45%, ALT by 36% and 42%, and AST by 33% and 36%, respectively. This suggests that both treatments mitigate liver injury. It was also reported by Angelico et al that metformin can induce a certain decrease in these parameters in a study including randomized clinical trials assessing the effects of drugs improving insulin resistance for patients with NAFLD or NASH **(Angelico et al., 2005).** Metformin improved short-term fat accumulation in the liver induced by a high-fat diet, and this improvement is associated with the suppression of inflammation in adipose tissue **(Tajima et al., 2013).** Metformin may exert these effects by activating AMP-activated protein kinase (AMPK), which functions as a sensor of cellular energy status **(Kahn et al., 2005).** AMPK inhibits the mammalian target of rapamycin (mTOR), a downstream

effector of growth factor signaling that is often activated in malignant cells **(Jalving et al., 2010).**

In the context of a high-fat diet (HFD), elevated triglycerides levels indicate hypertriglyceridemia, a risk factor for atherosclerosis **(Luna-Castillo et al., 2022)** and pancreatitis **(Karanchi et al., 2024).** Similarly, increased cholesterol levels can contribute to the development of atherosclerotic plaques, leading to cardiovascular disease **(Simonen et al., 2023).** We demonstrated that triglycerides level was significantly elevated in the HFD group, but compared to formoterol-treated and salbutamol-treated groups it was reduced by 75% and 58%, respectively. Corroborating findings by Peng et al, who reported in a cross-sectional study of the associations of serum lipid indexes with NAFLD in adult males a similar increase in triglycerides level **(Peng et al., 2017).** The efficacy in managing HFD-induced hypertriglyceridemia by formoterol and salbutamol molecules is similar to the resmetirom (MGL-3196), thyroid hormone receptor-beta (THR-beta) agonist molecule studied by Wang et al, who reported it in an *in vitro* and *in vivo* study to further elucidate the role and the underlying mechanism of resmetirom molecule which is a liver-direct **(Wang et al., 2023).** Interestingly, there wasn't any significant difference of total cholesterol levels between the groups. Ours results complement several studies, which have previously reported that a high fat diet may not necessarily lead to elevated total cholesterol levels **(Pflugradt et al., 1981).** Salbutamol appears to be more effective than formoterol in reducing hepatic steatosis, suggesting it could be a more potent treatment for high-fat diet-induced hepatic steatosis. Intravenous salbutamol caused significant increases in plasma insulin **(Neville et al., 1977)** the insulin sensitization contribute to underproduction of FFAs by stopping de novo lipogenesis and regulating Sterol regulatory element-binding transcription factor 1 (SREBF1) **(Crewe et al., 2019)** and carbohydrate response element binding protein (ChREBP1) **(Iizuka and Horikawa, 2008).** As confirmed by the study carried out by Wong and Sul SREBP-1c can be induced by mammalian target of rapamycin complex 1 (mTORC1), mTORC1, bifurcating lipogenesis from AKT-activated gluconeogenesis **(Laplante and Sabatini, 2010).** Dysregulation of FFAs and TAG metabolism often contributes to metabolic diseases such as obesity, diabetes, and cardiovascular diseases **(Bravo-Ruiz et al., 2021).** Transcription factors and signaling molecules involved in transcriptional activation of FFAs and TAG synthesis represent attractive targets for the prevention and treatment of metabolic diseases **(Wong and Sul, 2010)** salbutamol is a short acting beta 2 agonist with immediate effect that can be a factor of more efficacy than formoterol treatment. β2 agonists possess ergogenic properties,

especially when administered at high doses, due to their stimulating effects on glycolysis and lipolysis, as well as their anabolic effects under certain conditions **(Fur et al., 2012).**

The main histological characteristic of NAFLD, as its name implies, is the accumulation of fat in the form of triglycerides within hepatocytes **(Basaranoglu and Neuschwander-Tetri, 2006).** The presence of > 5% steatotic hepatocytes in a liver tissue section is now accepted as the minimum criterion for the histological diagnosis of NAFLD **(Alswat et al., 2019).** We reported that global steatosis showed a significant increase in the HFD. Formoterol and salbutamol reduced steatosis by 55% and 80%, respectively. salbutamol provide a higher efficiency in repairing liver damage. Although formoterol did not completely normalize the steatosis levels, it decreased the lipid accumulation, indicating a promising therapeutic potential for improving high-fat diet-induced hepatic steatosis. these results correlate with those of Gao et al who investigated the effects of treatment with the adenosine monophosphate (AMP) analog, 5-aminoimidazole-4-carboxamide ribonucleotide (AICAR) which decreased steatosis in a mouse model fed a high-fat diet (HFD) **(Gao et al., 2018).**

CONCLUSION

The results indicate that a high-fat diet is the triggering factor of severe hepatic steatosis in rats, the liver biopsy reflected a recovery from steatosis after both treatments with formoterol and salbutamol reducing the lipid droplets accumulation with different degrees of efficacy. which can be explained by the difference in the duration of treatment and the mode of action of the two drugs. This effect was reflected in same time by hepatic and lipidic assessments.

Salbutamol, in particular, has higher therapeutic potential with a more pronounced reduction in steatosis. These findings suggest that these beta-agonists could be further explored for their effectiveness in treating hepatic steatosis, although additional studies are necessary to confirm these effects and understand the underlying mechanisms. The mechanisms through which salbutamol or formoterol enhances liver steatosis are currently unknown but could possibly involve 2- adrenoceptor coupling to Gαi and/or activation of Epac by cAMP. It is clear from these data that multiple pathways mediate the effects of salbutamol or formoterol in liver. A better knowledge of the actions of 2-agonists in liver could lead to the development of pharmacological strategies aimed at treating NAFLD.

Perspectives

Salbutamol and formoterol treatments being recognized by the FDA for its approved pharmacological properties and the health risk due to their use represents a strong point in order to adopt this treatment. One of the highlights of our study is the identification and exploitation of clinically relevant drugs for treating NAFLD. Such a repurposing strategy exploits the off-target effects of clinically approved drugs, thereby providing opportunities to accelerate the development of new drugs for NAFLD while reducing health. In summary, our results constitute proof-of-principle showing that administration of 2-agonists salbutamol or formoterol may prove beneficial as a novel therapeutic approach for NAFLD.

Bibliography

Abosamak, N.R., Shahin, M.H., 2024. Beta2 Receptor Agonists and Antagonists, in: StatPearls. StatPearls Publishing, Treasure Island (FL).

Adams, L.A., Anstee, Q.M., Tilg, H., Targher, G., 2017. Non-alcoholic fatty liver disease and its relationship with cardiovascular disease and other extrahepatic diseases. Gut 66, 1138–1153. https://doi.org/10.1136/gutjnl-2017-313884

Aleksandrova, K., Boeing, H., Nöthlings, U., Jenab, M., Fedirko, V., Kaaks, R., Lukanova, A., Trichopoulou, A., Trichopoulos, D., Boffetta, P. *et al.*, 2014. Inflammatory and metabolic biomarkers and risk of liver and biliary tract cancer. Hepatology 60, 858–871. https://doi.org/10.1002/hep.27016

Allain, C.C., Poon, L.S., Chan, C.S., Richmond, W., Fu, P.C., 1974. Enzymatic determination of total serum cholesterol. Clin. Chem. 20, 470–475.

Almobarak, A.O., Barakat, S., Khalifa, M.H., Elhoweris, M.H., Elhassan, T.M., Ahmed, M.H., 2014. Non alcoholic fatty liver disease (NAFLD) in a Sudanese population: What is the prevalence and risk factors? Arab J. Gastroenterol. 15, 12–15. https://doi.org/10.1016/j.ajg.2014.01.008

Alswat, K.A., Fallatah, H.I., Al-Judaibi, B., Elsiesy, H.A., Al-Hamoudi, W.K., Qutub, A.N., Alturaify, N., Al-Osaimi, A., 2019. Position statement on the diagnosis and management of non-alcoholic fatty liver disease. Saudi Med. J. 40, 531–540. https://doi.org/10.15537/smj.2019.6.23980

Amin, P., Singh, M., Singh, K., 2011. β -Adrenergic Receptor-Stimulated Cardiac Myocyte Apoptosis: Role of β 1 Integrins. J. Signal Transduct. 2011, 1–9. https://doi.org/10.1155/2011/179057

Anderson, G.P., 2006. Current Issues With β_2-Adrenoceptor Agonists: Pharmacology and Molecular and Cellular Mechanisms. Clin. Rev. Allergy Immunol. 31, 119–130. https://doi.org/10.1385/CRIAI:31:2:119

Angelico, F., Del Ben, M., Conti, R., Francioso, S., Feole, K., Fiorello, S., Cavallo, M.G., Zalunardo, B., Lirussi, F., Alessandri, C., Violi, F., 2005. Insulin Resistance, the Metabolic Syndrome, and Nonalcoholic Fatty Liver Disease. J. Clin. Endocrinol. Metab. 90, 1578–1582. https://doi.org/10.1210/jc.2004-1024

Anstee, Q.M., Castera, L., Loomba, R., 2022. Impact of non-invasive biomarkers on hepatology practice: Past, present and future. J. Hepatol. 76, 1362–1378. https://doi.org/10.1016/j.jhep.2022.03.026

Anstee, Q.M., Targher, G., Day, C.P., 2013. Progression of NAFLD to diabetes mellitus, cardiovascular disease or cirrhosis. Nat. Rev. Gastroenterol. Hepatol. 10, 330–344. https://doi.org/10.1038/nrgastro.2013.41

Aragno, M., Tomasinelli, C.E., Vercellinatto, I., Catalano, M.G., Collino, M., Fantozzi, R., Danni, O., Boccuzzi, G., 2009. SREBP-1c in nonalcoholic fatty liver disease induced by Western-type high-fat diet plus fructose in rats. Free Radic. Biol. Med. 47, 1067–1074. https://doi.org/10.1016/j.freeradbiomed.2009.07.016

Arroyo, V., Colmenero, J., 2003. Ascites and hepatorenal syndrome in cirrhosis: pathophysiological basis of therapy and current management. J. Hepatol. 38, 69–89. https://doi.org/10.1016/S0168-8278(03)00007-2

Barata, L., Feitosa, M.F., Bielak, L.F., Halligan, B., Baldridge, A.S., Guo, X., Yerges-Armstrong, L.M., Smith, A.V., Yao, J., Palmer, N.D *et al*., 2019. Insulin Resistance Exacerbates Genetic Predisposition to Nonalcoholic Fatty Liver Disease in Individuals Without Diabetes. Hepatol. Commun. 3, 894–907. https://doi.org/10.1002/hep4.1353

Barrera, F., George, J., 2014. The Role of Diet and Nutritional Intervention for the Management of Patients with NAFLD. Clin. Liver Dis. 18, 91–112. https://doi.org/10.1016/j.cld.2013.09.009

Basaranoglu, M., Neuschwander-Tetri, B.A., 2006. Nonalcoholic Fatty Liver Disease: Clinical Features and Pathogenesis. Gastroenterol. Hepatol. 2, 282–291.

Bashiardes, S., Shapiro, H., Rozin, S., Shibolet, O., Elinav, E., 2016. Non-alcoholic fatty liver and the gut microbiota. Mol. Metab. 5, 782–794. https://doi.org/10.1016/j.molmet.2016.06.003

Bates, P.C., Pell, J.M., 1991. Action and interaction of growth hormone and the β-agonist, clenbuterol, on growth, body composition and protein turnover in dwarf mice. Br. J. Nutr. 65, 115–129. https://doi.org/10.1079/BJN19910074

Bazick, J., Donithan, M., Neuschwander-Tetri, B.A., Kleiner, D., Brunt, E.M., Wilson, L., Doo, E., Lavine, J., Tonascia, J., Loomba, R., 2015. Clinical Model for NASH and Advanced Fibrosis in Adult Patients With Diabetes and NAFLD: Guidelines for Referral in NAFLD. Diabetes Care 38, 1347–1355. https://doi.org/10.2337/dc14-1239

Bedogni, G., Miglioli, L., Masutti, F., Castiglione, A., Crocè, L.S., Tiribelli, C., Bellentani, S., 2007. Incidence and natural course of fatty liver in the general population: The Dionysos study. Hepatology 46, 1387–1391. https://doi.org/10.1002/hep.21827

Benedict, M., Zhang, X., 2017. Non-alcoholic fatty liver disease: An expanded review. World J. Hepatol. 9, 715. https://doi.org/10.4254/wjh.v9.i16.715

Bernsmeier, C., Heim, M., 2011. Stéatose hépatique non alcoolique et stéatohépatite non alcoolique. Forum Méd. Suisse – Swiss Med. Forum 11. https://doi.org/10.4414/fms.2011.07418

Birkenfeld, A.L., Shulman, G.I., 2014. Nonalcoholic fatty liver disease, hepatic insulin resistance, and type 2 Diabetes. Hepatology 59, 713–723. https://doi.org/10.1002/hep.26672

Bockaert, J., 1999. Molecular tinkering of G protein-coupled receptors: an evolutionary success. EMBO J. 18, 1723–1729. https://doi.org/10.1093/emboj/18.7.1723

Boyd, G., Morice, A.H., Pounsford, J.C., Siebert, M., Peslis, N., Crawford, C., 1997. An evaluation of salmeterol in the treatment of chronic obstructive pulmonary disease (COPD). Eur. Respir. J. 10, 815–821.

Bravo-Ruiz, I., Medina, M.Á., Martínez-Poveda, B., 2021. From Food to Genes: Transcriptional Regulation of Metabolism by Lipids and Carbohydrates. Nutrients 13, 1513. https://doi.org/10.3390/nu13051513

Bricout, V. -A., Serrurier, B.D., Bigard, A.X., 2004. Clenbuterol treatment affects myosin heavy chain isoforms and MyoD content similarly in intact and regenerated soleus muscles. Acta Physiol. Scand. 180, 271–280. https://doi.org/10.1046/j.0001-6772.2003.01246.x

Brodde, O.E., Michel, M.C., 1999. Adrenergic and muscarinic receptors in the human heart. Pharmacol. Rev. 51, 651–690.

Brown, G.T., Kleiner, D.E., 2016. Histopathology of nonalcoholic fatty liver disease and nonalcoholic steatohepatitis. Metabolism 65, 1080–1086. https://doi.org/10.1016/j.metabol.2015.11.008

Buettner, R., Schölmerich, J., Bollheimer, L.C., 2007. High-fat Diets: Modeling the Metabolic Disorders of Human Obesity in Rodents. Obesity 15, 798–808. https://doi.org/10.1038/oby.2007.608

Bugianesi, E., Gastaldelli, A., Vanni, E., Gambino, R., Cassader, M., Baldi, S., Ponti, V., Pagano, G., Ferrannini, E., Rizzetto, M., 2005. Insulin resistance in non-diabetic patients with non-alcoholic fatty liver disease: sites and mechanisms. Diabetologia 48, 634–642. https://doi.org/10.1007/s00125-005-1682-x

Byrne, C.D., Targher, G., 2015. NAFLD: A multisystem disease. J. Hepatol. 62, S47–S64. https://doi.org/10.1016/j.jhep.2014.12.012

Cani, P.D., Amar, J., Iglesias, M.A., Poggi, M., Knauf, C., Bastelica, D., Neyrinck, A.M., Fava, F., Tuohy, K.M., Chabo, C *et al.*, 2007. Metabolic Endotoxemia Initiates Obesity and Insulin Resistance. Diabetes 56, 1761–1772. https://doi.org/10.2337/db06-1491

Cardoso, A.C., De Figueiredo-Mendes, C., A. Villela-Nogueira, C., 2021. Current management of NAFLD/NASH. Liver Int. 41, 89–94. https://doi.org/10.1111/liv.14869

Cassard-Doulcier, A.-M., Perlemuter, G., 2011. Inflammation hépatique liée à l'obésité (NASH). Ol. Corps Gras Lipides 18, 21–26. https://doi.org/10.1051/ocl.2011.0363

Cero, C., Lea, H.J., Zhu, K.Y., Shamsi, F., Tseng, Y.-H., Cypess, A.M., 2021. β3-Adrenergic receptors regulate human brown/beige adipocyte lipolysis and thermogenesis. JCI Insight 6, e139160. https://doi.org/10.1172/jci.insight.139160

Chalasani, N., Wilson, L., Kleiner, D.E., Cummings, O.W., Brunt, E.M., Ünalp, A., 2008. Relationship of steatosis grade and zonal location to histological features of steatohepatitis in adult patients with non-alcoholic fatty liver disease. J. Hepatol. 48, 829–834. https://doi.org/10.1016/j.jhep.2008.01.016

Chapman, R.W., Lynch, K.D., 2020. Obeticholic acid—a new therapy in PBC and NASH. Br. Med. Bull. 133, 95–104. https://doi.org/10.1093/bmb/ldaa006

Chaudhry, A., Noor, J., Batool, S., Fatima, G., Noor, R., 2023. Advancements in Diagnostic and Therapeutic Interventions of Non-alcoholic Fatty Liver Disease: A Literature Review. Cureus. https://doi.org/10.7759/cureus.44924

Chen, M.-M., Cai, J.-J., Yu, Y., She, Z.-G., Li, H., 2019. Current and Emerging Approaches for Nonalcoholic Steatohepatitis Treatment. Gene Expr. 19, 175–185. https://doi.org/10.3727/105221619X15536120524171

Choudhury, J., Sanyal, A.J., 2004. Clinical Aspects of Fatty Liver Disease. Semin. Liver Dis. 24, 349–362. https://doi.org/10.1055/s-2004-860864

Clemente-Suárez, V.J., Redondo-Flórez, L., Beltrán-Velasco, A.I., Martín-Rodríguez, A., Martínez-Guardado, I., Navarro-Jiménez, E., Laborde-Cárdenas, C.C., Tornero-Aguilera, J.F., 2023. The Role of Adipokines in Health and Disease. Biomedicines 11, 1290. https://doi.org/10.3390/biomedicines11051290

Cobbina, E., Akhlaghi, F., 2017. Non-alcoholic fatty liver disease (NAFLD) – pathogenesis, classification, and effect on drug metabolizing enzymes and transporters. Drug Metab. Rev. 49, 197–211. https://doi.org/10.1080/03602532.2017.1293683

Cohen, J.C., Horton, J.D., Hobbs, H.H., 2011. Human Fatty Liver Disease: Old Questions and New Insights. Science 332, 1519–1523. https://doi.org/10.1126/science.1204265

Contreras-Zentella, M.L., Hernández-Muñoz, R., 2016. Is Liver Enzyme Release Really Associated with Cell Necrosis Induced by Oxidant Stress? Oxid. Med. Cell. Longev. 2016, 1–12. https://doi.org/10.1155/2016/3529149

Crewe, C., Zhu, Y., Paschoal, V.A., Joffin, N., Ghaben, A.L., Gordillo, R., Oh, D.Y., Liang, G., Horton, J.D., Scherer, P.E., 2019. SREBP-regulated adipocyte lipogenesis is dependent on substrate availability and redox modulation of mTORC1. JCI Insight 4, e129397. https://doi.org/10.1172/jci.insight.129397

Czuppon, A.B., Marczynski, B., Scheer, E., Hartmann, R., Baur, X., 1993. Increased incidence of anti-dsDNA autoantibody concentration in sera of workers occupationally exposed to diisocyanates. Toxicol. Lett. 66, 29–34. https://doi.org/10.1016/0378-4274(93)90076-a

De Lean, A., Stadel, J.M., Lefkowitz, R.J., 1980. A ternary complex model explains the agonist-specific binding properties of the adenylate cyclase-coupled beta-adrenergic receptor. J. Biol. Chem. 255, 7108–7117.

Deshaies, Y., Willemot, J., Leblanc, J., 1981. Protein synthesis, amino acid uptake, and pools during isoproterenol-induced hypertrophy of the rat heart and tibialis muscle. Can. J. Physiol. Pharmacol. 59, 113–121. https://doi.org/10.1139/y81-020

Devillier, Ph., Molimard, M., Bessard, G., Advenier, Ch., 1996. Aspects actuels des récepteurs β2-adrénergiques et des β2-stimulants dans l'asthme. Rev. Fr. Allergol. Immunol. Clin. 36, 269–281. https://doi.org/10.1016/S0335-7457(96)80095-5

DeWire, S.M., Ahn, S., Lefkowitz, R.J., Shenoy, S.K., 2007. β-Arrestins and Cell Signaling. Annu. Rev. Physiol. 69, 483–510. https://doi.org/10.1146/annurev.physiol.69.022405.154749

Díez-Sampedro, A., Pérez, M., Cobo, M.T., Martínez, J.A., Barber, A., 2011. Effect of Different β -Adrenergic Agonists on the Intestinal Absorption of Galactose and Phenylalanine. J. Pharm. Pharmacol. 50, 907–911. https://doi.org/10.1111/j.2042-7158.1998.tb04007.x

Dimitrov, S., Hulteng, E., Hong, S., 2017. Inflammation and exercise: Inhibition of monocytic intracellular TNF production by acute exercise via β2-adrenergic activation. Brain. Behav. Immun. 61, 60–68. https://doi.org/10.1016/j.bbi.2016.12.017

Dongiovanni, P., Romeo, S., Valenti, L., 2015. Genetic Factors in the Pathogenesis of Nonalcoholic Fatty Liver and Steatohepatitis. BioMed Res. Int. 2015, 1–10. https://doi.org/10.1155/2015/460190

Dooley, S., Ten Dijke, P., 2012. TGF-β in progression of liver disease. Cell Tissue Res. 347, 245–256. https://doi.org/10.1007/s00441-011-1246-y

Dufour, J.-F., Anstee, Q.M., Bugianesi, E., Harrison, S., Loomba, R., Paradis, V., Tilg, H., Wong, V.W.-S., Zelber-sagi, S., 2022. Current therapies and new developments in NASH. Gut 71, 2123–2134. https://doi.org/10.1136/gutjnl-2021-326874

Eckel, R.H., Alberti, K., Grundy, S.M., Zimmet, P.Z., 2010. The metabolic syndrome. The Lancet 375, 181–183. https://doi.org/10.1016/S0140-6736(09)61794-3

Eglen, R.M., Reisine, T., 2011. GPCRs Revisited: New Insights Lead to Novel Drugs. Pharmaceuticals 4, 244–272. https://doi.org/10.3390/ph4020244

Eisemann, J.H., Huntington, G.B., Ferrell, C.L., 1988. Effects of Dietary Clenbuterol on Metabolism of the Hindquarters in Steers. J. Anim. Sci. 66, 342. https://doi.org/10.2527/jas1988.662342x

Estes, C., Razavi, H., Loomba, R., Younossi, Z., Sanyal, A.J., 2018. Modeling the epidemic of nonalcoholic fatty liver disease demonstrates an exponential increase in burden of disease. Hepatology 67, 123–133. https://doi.org/10.1002/hep.29466

Ferguson, S.S.G., Ménard, L., Barak, L.S., Koch, W.J., Colapietro, A.-M., Caron, M.G., 1995. Role of Phosphorylation in Agonist-promoted β2-Adrenergic Receptor Sequestration. J. Biol. Chem. 270, 24782–24789. https://doi.org/10.1074/jbc.270.42.24782

Flisiak-Jackiewicz, M., Bobrus-Chociej, A., Wasilewska, N., Lebensztejn, D.M., 2021. From Nonalcoholic Fatty Liver Disease (NAFLD) to Metabolic Dysfunction-Associated Fatty Liver Disease (MAFLD)—New Terminology in Pediatric Patients as a Step in Good Scientific Direction? J. Clin. Med. 10, 924. https://doi.org/10.3390/jcm10050924

Fossati, P., Prencipe, L., Berti, G., 1983. Enzymic creatinine assay: a new colorimetric method based on hydrogen peroxide measurement. Clin. Chem. 29, 1494–1496. https://doi.org/10.1093/clinchem/29.8.1494

Francque, S.M., Marchesini, G., Kautz, A., Walmsley, M., Dorner, R., Lazarus, J.V., Zelber-Sagi, S., Hallsworth, K., Busetto, L., Frühbeck, G *et al.*, 2021. Non-alcoholic fatty liver disease: A patient guideline. JHEP Rep. 3, 100322. https://doi.org/10.1016/j.jhepr.2021.100322

Fredriksson, R., Lagerström, M.C., Lundin, L.-G., Schiöth, H.B., 2003. The G-Protein-Coupled Receptors in the Human Genome Form Five Main Families. Phylogenetic Analysis, Paralogon Groups, and Fingerprints. Mol. Pharmacol. 63, 1256–1272. https://doi.org/10.1124/mol.63.6.1256

Friedman, S.L., Neuschwander-Tetri, B.A., Rinella, M., Sanyal, A.J., 2018. Mechanisms of NAFLD development and therapeutic strategies. Nat. Med. 24, 908–922. https://doi.org/10.1038/s41591-018-0104-9

Frith, J., Day, C.P., Henderson, E., Burt, A.D., Newton, J.L., 2009. Non-Alcoholic Fatty Liver Disease in Older People. Gerontology 55, 607–613. https://doi.org/10.1159/000235677

Frohnert, B.I., Jacobs, D.R., Steinberger, J., Moran, A., Steffen, L.M., Sinaiko, A.R., 2013. Relation Between Serum Free Fatty Acids and Adiposity, Insulin Resistance, and Cardiovascular Risk Factors From Adolescence to Adulthood. Diabetes 62, 3163–3169. https://doi.org/10.2337/db12-1122

Fur, M.A.L., Sanchez, A.M.J., Candau, R., 2012. Effets ergogéniques des ?2 agonistes : mode d'action et enjeux pour la lutte antidopage. Mov. Sport Sci. n° 77, 29. https://doi.org/10.3917/sm.077.0029

Gao, J., Xiong, R., Xiong, D., Zhao, W., Zhang, S., Yin, T., Zhang, X., Jiang, G., Yin, Z., 2018. The Adenosine Monophosphate (AMP) Analog, 5-Aminoimidazole-4-Carboxamide Ribonucleotide (AICAR) Inhibits Hepatosteatosis and Liver Tumorigenesis in a High-Fat Diet Murine Model Treated with Diethylnitrosamine (DEN). Med. Sci. Monit. 24, 8533–8543. https://doi.org/10.12659/MSM.910544

García-Pagán, J.-C., Gracia-Sancho, J., Bosch, J., 2012. Functional aspects on the pathophysiology of portal hypertension in cirrhosis. J. Hepatol. 57, 458–461. https://doi.org/10.1016/j.jhep.2012.03.007

Grgurevic, I., Bozin, T., Mikus, M., Kukla, M., O'Beirne, J., 2021. Hepatocellular Carcinoma in Non-Alcoholic Fatty Liver Disease: From Epidemiology to Diagnostic Approach. Cancers 13, 5844. https://doi.org/10.3390/cancers13225844

Grzych, G., Bernard, L., Lestrelin, R., Tailleux, A., Staels, B., 2023. État des lieux sur la physiopathologie, le diagnostic et les traitements de la stéato-hépatite non alcoolique (NASH). Ann. Pharm. Fr. 81, 183–201. https://doi.org/10.1016/j.pharma.2022.09.005

Gual, P., Le Marchand-Brustel, Y., Tanti, J.-F., 2005. Positive and negative regulation of insulin signaling through IRS-1 phosphorylation. Biochimie 87, 99–109. https://doi.org/10.1016/j.biochi.2004.10.019

Guo, J., Friedman, S.L., 2010. Toll-like receptor 4 signaling in liver injury and hepatic fibrogenesis. Fibrogenesis Tissue Repair 3, 21. https://doi.org/10.1186/1755-1536-3-21

Haffner, C., Kendall, M., Maxwell, S., Hughes, B., 1993. The lipolytic effect of beta 1- and beta 2-adrenoceptor activation in healthy human volunteers. Br. J. Clin. Pharmacol. 35, 35–39. https://doi.org/10.1111/j.1365-2125.1993.tb05667.x

Haldar, D., Kern, B., Hodson, J., Armstrong, M.J., Adam, R., Berlakovich, G., Fritz, J., Feurstein, B., Popp, W., Karam, V *et al.*, 2019. Outcomes of liver transplantation for non-alcoholic steatohepatitis: A European Liver Transplant Registry study. J. Hepatol. 71, 313–322. https://doi.org/10.1016/j.jhep.2019.04.011

Hamaguchi, M., Kojima, T., Takeda, N., Nakagawa, T., Taniguchi, H., Fujii, K., Omatsu, T., Nakajima, T., Sarui, H., Shimazaki, M *et al*., 2005. The Metabolic Syndrome as a Predictor of Nonalcoholic Fatty Liver Disease. Ann. Intern. Med. 143, 722. https://doi.org/10.7326/0003-4819-143-10-200511150-00009

Han, D., Chen, J., Liu, S., Zhang, Z., Zhao, Z., Jin, W., Xin, Y., 2021. Serum Resistin Levels in Adult Patients with Nonalcoholic Fatty Liver Disease: A Systematic Review and Meta-analysis. J. Clin. Transl. Hepatol. 000, 000–000. https://doi.org/10.14218/JCTH.2021.00018

Hsu, E., Bajaj, T., 2024. Beta2-Agonists, in: StatPearls. StatPearls Publishing, Treasure Island (FL).

Hydes, T.J., Summers, N., Brown, E., Alam, U., Thomaides-Brears, H., Wilding, J.P.H., Cuthbertson, D.J., 2020. Mechanisms, screening modalities and treatment options for individuals with non-alcoholic fatty liver disease and type 2 diabetes. Diabet. Med. 37, 1793–1806. https://doi.org/10.1111/dme.14356

Iizuka, K., Horikawa, Y., 2008. ChREBP: A Glucose-activated Transcription Factor Involved in the Development of Metabolic Syndrome. Endocr. J. 55, 617–624. https://doi.org/10.1507/endocrj.K07E-110

Illera, J.C., Silván, G., Blass, A., Martínez, M.M., Illera, M., 1998. The effect of clenbuterol on adrenal function in rats†. The Analyst 123, 2521–2524. https://doi.org/10.1039/a805360j

Isin, B., Estiu, G., Wiest, O., Oltvai, Z.N., 2012. Identifying Ligand Binding Conformations of the β2-Adrenergic Receptor by Using Its Agonists as Computational Probes. PLoS ONE 7, e50186. https://doi.org/10.1371/journal.pone.0050186

Jalving, M., Gietema, J.A., Lefrandt, J.D., Jong, S.D., Reyners, A.K.L., Gans, R.O.B., Vries, E.G.E.D., 2010. Metformin: Taking away the candy for cancer? Eur. J. Cancer 46, 2369–2380. https://doi.org/10.1016/j.ejca.2010.06.012

Jang, W., Song, J.S., 2023. Non-Invasive Imaging Methods to Evaluate Non-Alcoholic Fatty Liver Disease with Fat Quantification: A Review. Diagnostics 13, 1852. https://doi.org/10.3390/diagnostics13111852

Jasirwan, C.O.M., Lesmana, C.R.A., Hasan, I., Sulaiman, A.S., Gani, R.A., 2019. The role of gut microbiota in non-alcoholic fatty liver disease: pathways of mechanisms. Biosci. Microbiota Food Health 38, 81–88. https://doi.org/10.12938/bmfh.18-032

Jiang, J.X., Mikami, K., Venugopal, S., Li, Y., Török, N.J., 2009. Apoptotic body engulfment by hepatic stellate cells promotes their survival by the JAK/STAT and Akt/NF-κB-

dependent pathways. J. Hepatol. 51, 139–148. https://doi.org/10.1016/j.jhep.2009.03.024

Joassard, O.R., Durieux, A.-C., Freyssenet, D.G., 2013. β2-Adrenergic agonists and the treatment of skeletal muscle wasting disorders. Int. J. Biochem. Cell Biol. 45, 2309–2321. https://doi.org/10.1016/j.biocel.2013.06.025

Jornayvaz, F.R., Samuel, V.T., Shulman, G.I., 2010. The Role of Muscle Insulin Resistance in the Pathogenesis of Atherogenic Dyslipidemia and Nonalcoholic Fatty Liver Disease Associated with the Metabolic Syndrome. Annu. Rev. Nutr. 30, 273–290. https://doi.org/10.1146/annurev.nutr.012809.104726

Juanola, O., Martínez-López, S., Francés, R., Gómez-Hurtado, I., 2021. Non-Alcoholic Fatty Liver Disease: Metabolic, Genetic, Epigenetic and Environmental Risk Factors. Int. J. Environ. Res. Public. Health 18, 5227. https://doi.org/10.3390/ijerph18105227

Kahn, B.B., Alquier, T., Carling, D., Hardie, D.G., 2005. AMP-activated protein kinase: Ancient energy gauge provides clues to modern understanding of metabolism. Cell Metab. 1, 15–25. https://doi.org/10.1016/j.cmet.2004.12.003

Kahn, B.B., Flier, J.S., 2000. Obesity and insulin resistance. J. Clin. Invest. 106, 473–481. https://doi.org/10.1172/JCI10842

Kalinovich, A., Dehvari, N., Åslund, A., Van Beek, S., Halleskog, C., Olsen, J., Forsberg, E., Zacharewicz, E., Schaart, G., Rinde, M *et al.*, 2020. Treatment with a β-2-adrenoceptor agonist stimulates glucose uptake in skeletal muscle and improves glucose homeostasis, insulin resistance and hepatic steatosis in mice with diet-induced obesity. Diabetologia 63, 1603–1615. https://doi.org/10.1007/s00125-020-05171-y

Karanchi, H., Muppidi, V., Wyne, K., 2024. Hypertriglyceridemia, in: StatPearls. StatPearls Publishing, Treasure Island (FL).

Karim, G., Department of Medicine, Mount Sinai Israel, New York, NY, USA, Bansal, M.B., Division of Liver Diseases, Department of Medicine, Icahn School of Medicine at Mount Sinai, New York, NY, USA, 2023. Resmetirom: An Orally Administered, Small-molecule, Liver-directed, β-selective THR Agonist for the Treatment of Non-alcoholic Fatty Liver Disease and Non-alcoholic Steatohepatitis. Eur. Endocrinol. 19, 60. https://doi.org/10.17925/EE.2023.19.1.60

Kearns, S.R., Connolly, E.M., McNally, S., McNamara, D.A., Deasy, J., 2002. Randomized clinical trial of diathermy *versus* scalpel incision in elective midline laparotomy. Br. J. Surg. 88, 41–44. https://doi.org/10.1046/j.1365-2168.2001.01625.x

Khanam, A., Saleeb, P.G., Kottilil, S., 2021. Pathophysiology and Treatment Options for Hepatic Fibrosis: Can It Be Completely Cured? Cells 10, 1097. https://doi.org/10.3390/cells10051097

Khurana, A., Sayed, N., Allawadhi, P., Weiskirchen, R., 2021. It's all about the spaces between cells: role of extracellular matrix in liver fibrosis. Ann. Transl. Med. 9, 728–728. https://doi.org/10.21037/atm-20-2948

Kindermann, W., Meyer, T., 2006. Inhaled β_2 agonists and performance in competitive athletes. Br. J. Sports Med. 40, i43–i47. https://doi.org/10.1136/bjsm.2006.027748

Kleiner, D.E., Makhlouf, H.R., 2016. Histology of Nonalcoholic Fatty Liver Disease and Nonalcoholic Steatohepatitis in Adults and Children. Clin. Liver Dis. 20, 293–312. https://doi.org/10.1016/j.cld.2015.10.011

Klukovits, A., Ducza, E., Földesi, I., Falkay, G., 2004. β_2 -agonist treatment enhances uterine oxytocin receptor mRNA expression in pregnant rats. Mol. Reprod. Dev. 69, 60–65. https://doi.org/10.1002/mrd.20158

Kobilka, B., 2013. The Structural Basis of G-Protein-Coupled Receptor Signaling (Nobel Lecture). Angew. Chem. Int. Ed. 52, 6380–6388. https://doi.org/10.1002/anie.201302116

Koenig, G., Seneff, S., 2015. Gamma-Glutamyltransferase: A Predictive Biomarker of Cellular Antioxidant Inadequacy and Disease Risk. Dis. Markers 2015, 1–18. https://doi.org/10.1155/2015/818570

Kojta, I., Chacińska, M., Błachnio-Zabielska, A., 2020. Obesity, Bioactive Lipids, and Adipose Tissue Inflammation in Insulin Resistance. Nutrients 12, 1305. https://doi.org/10.3390/nu12051305

Koyama, Y., Brenner, D.A., 2017. Liver inflammation and fibrosis. J. Clin. Invest. 127, 55–64. https://doi.org/10.1172/JCI88881

Kwok, R., Choi, K.C., Wong, G.L.-H., Zhang, Y., Chan, H.L.-Y., Luk, A.O.-Y., Shu, S.S.-T., Chan, A.W.-H., Yeung, M.-W., Chan, J.C.-N *et al*., 2016. Screening diabetic patients for non-alcoholic fatty liver disease with controlled attenuation parameter and liver stiffness measurements: a prospective cohort study. Gut 65, 1359–1368. https://doi.org/10.1136/gutjnl-2015-309265

Lafontan, M., Berlan, M., Prud'Hon, M., 1988. Les agonistes bêta-adrénergiques. Mécanismes d'action : lipomobilisation et anabolisme. Reprod. Nutr. Dév. 28, 61–84. https://doi.org/10.1051/rnd:19880104

Laplante, M., Sabatini, D.M., 2010. mTORC1 activates SREBP-1c and uncouples lipogenesis from gluconeogenesis. Proc. Natl. Acad. Sci. 107, 3281–3282. https://doi.org/10.1073/pnas.1000323107

Lau, L.H.S., Wong, S.H., 2018. Microbiota, Obesity and NAFLD, in: Yu, J. (Ed.), Obesity, Fatty Liver and Liver Cancer, Advances in Experimental Medicine and Biology. Springer Singapore, Singapore, pp. 111–125. https://doi.org/10.1007/978-981-10-8684-7_9

Laureys, G., Clinckers, R., Gerlo, S., Spooren, A., Wilczak, N., Kooijman, R., Smolders, I., Michotte, Y., De Keyser, J., 2010. Astrocytic β2-adrenergic receptors: From physiology to pathology. Prog. Neurobiol. 91, 189–199. https://doi.org/10.1016/j.pneurobio.2010.01.011

Lefkimmiatis, K., Zaccolo, M., 2014. cAMP signaling in subcellular compartments. Pharmacol. Ther. 143, 295–304. https://doi.org/10.1016/j.pharmthera.2014.03.008

Lessard, S.J., Rivas, D.A., Chen, Z.-P., Van Denderen, B.J., Watt, M.J., Koch, L.G., Britton, S.L., Kemp, B.E., Hawley, J.A., 2009. Impaired Skeletal Muscle β-Adrenergic Activation and Lipolysis Are Associated with Whole-Body Insulin Resistance in Rats Bred for Low Intrinsic Exercise Capacity. Endocrinology 150, 4883–4891. https://doi.org/10.1210/en.2009-0158

Loguercio, C., De Girolamo, V., Federico, A., Feng, S.L., Crafa, E., Cataldi, V., Gialanella, G., Moro, R., Del Vecchio Blanco, C., 2001. Relationship of Blood Trace Elements to Liver Damage, Nutritional Status, and Oxidative Stress in Chronic Nonalcoholic Liver Disease. Biol. Trace Elem. Res. 81, 245–254. https://doi.org/10.1385/BTER:81:3:245

Lonardo, A., Ballestri, S., Mantovani, A., Targher, G., Bril, F., 2024. Endpoints in NASH Clinical Trials: Are We Blind in One Eye? Metabolites 14, 40. https://doi.org/10.3390/metabo14010040

Longo, S.K., Guo, M.G., Ji, A.L., Khavari, P.A., 2021. Integrating single-cell and spatial transcriptomics to elucidate intercellular tissue dynamics. Nat. Rev. Genet. 22, 627–644. https://doi.org/10.1038/s41576-021-00370-8

Loomba, R., Morgan, E., Watts, L., Xia, S., Hannan, L.A., Geary, R.S., Baker, B.F., Bhanot, S., 2020. Novel antisense inhibition of diacylglycerol O-acyltransferase 2 for treatment of non-alcoholic fatty liver disease: a multicentre, double-blind, randomised, placebo-controlled phase 2 trial. Lancet Gastroenterol. Hepatol. 5, 829–838. https://doi.org/10.1016/S2468-1253(20)30186-2

Loomis, A.K., Kabadi, S., Preiss, D., Hyde, C., Bonato, V., St. Louis, M., Desai, J., Gill, J.M.R., Welsh, P., Waterworth, D *et al.*, 2016. Body Mass Index and Risk of Nonalcoholic Fatty

Liver Disease: Two Electronic Health Record Prospective Studies. J. Clin. Endocrinol. Metab. 101, 945–952. https://doi.org/10.1210/jc.2015-3444

Lötvall, J., 2001. Pharmacological similarities and differences between β2-agonists. Respir. Med. 95, S7–S11. https://doi.org/10.1053/rmed.2001.1139

Luna-Castillo, K.P., Olivares-Ochoa, X.C., Hernández-Ruiz, R.G., Llamas-Covarrubias, I.M., Rodríguez-Reyes, S.C., Betancourt-Núñez, A., Vizmanos, B., Martínez-López, E., Muñoz-Valle, J.F., Márquez-Sandoval, F *et al*., 2022. The Effect of Dietary Interventions on Hypertriglyceridemia: From Public Health to Molecular Nutrition Evidence. Nutrients 14, 1104. https://doi.org/10.3390/nu14051104

Lustig, V., Papanastasiou-Diamandis, A., Goldberg, D.M., 1988. Evaluation of commercially formulated aspartate aminotransferase and alanine aminotransferase activity determinations by the scandinavian committee on enzymes and IFCC methods as modified for use with automated enzyme analysers. Clin. Biochem. 21, 283–290. https://doi.org/10.1016/S0009-9120(88)80082-1

Marchisello, S., Pino, A.D., Scicali, R., Urbano, F., Piro, S., Purrello, F., Rabuazzo, A.M., 2019. Pathophysiological, Molecular and Therapeutic Issues of Nonalcoholic Fatty Liver Disease: An Overview. Int. J. Mol. Sci. 20, 1948. https://doi.org/10.3390/ijms20081948

Markham, A., Keam, S.J., 2016. Obeticholic Acid: First Global Approval. Drugs 76, 1221–1226. https://doi.org/10.1007/s40265-016-0616-x

Maruyama, H., Yokosuka, O., 2012. Pathophysiology of Portal Hypertension and Esophageal Varices. Int. J. Hepatol. 2012, 1–7. https://doi.org/10.1155/2012/895787

McArdle, M.A., Finucane, O.M., Connaughton, R.M., McMorrow, A.M., Roche, H.M., 2013. Mechanisms of Obesity-Induced Inflammation and Insulin Resistance: Insights into the Emerging Role of Nutritional Strategies. Front. Endocrinol. 4. https://doi.org/10.3389/fendo.2013.00052

McDonald, J.F., Ayala, F.J., 1978. Gene regulation in adaptive evolution. Can. J. Genet. Cytol. J. Can. Genet. Cytol. 20, 159–175. https://doi.org/10.1139/g78-018

Méndez-Sánchez, N., Valencia-Rodríguez, A., Coronel-Castillo, C., Vera-Barajas, A., Contreras-Carmona, J., Ponciano-Rodríguez, G., Zamora-Valdés, D., 2020. The cellular pathways of liver fibrosis in non-alcoholic steatohepatitis. Ann. Transl. Med. 8, 400–400. https://doi.org/10.21037/atm.2020.02.184

Mi, A., Hu, Q., Liu, Y., Zhao, Y., Shen, F., Lan, J., Lv, K., Wang, B., Gao, R., Yu, X., 2024. Hepatoprotective efficacy and interventional mechanism of the panaxadiol saponin

component in high-fat diet-induced NAFLD mice. Food Funct. 15, 794–808. https://doi.org/10.1039/D3FO03572G

Miller, E.J., Lappin, S.L., 2024. Physiology, Cellular Receptor, in: StatPearls. StatPearls Publishing, Treasure Island (FL).

Mitsala, A., Tsalikidis, C., Romanidis, K., Pitiakoudis, M., 2022. Non-Alcoholic Fatty Liver Disease and Extrahepatic Cancers: A Wolf in Sheep's Clothing? Curr. Oncol. 29, 4478–4510. https://doi.org/10.3390/curroncol29070356

Moslehi, A., Hamidi-zad, Z., 2018. Role of SREBPs in Liver Diseases: A Mini-review. J. Clin. Transl. Hepatol. 6, 1–7. https://doi.org/10.14218/JCTH.2017.00061

Mundi, M.S., Velapati, S., Patel, J., Kellogg, T.A., Abu Dayyeh, B.K., Hurt, R.T., 2020. Evolution of NAFLD and Its Management. Nutr. Clin. Pract. 35, 72–84. https://doi.org/10.1002/ncp.10449

Nagashimada, M., Ota, T., 2019. Role of vitamin E in nonalcoholic fatty liver disease. IUBMB Life 71, 516–522. https://doi.org/10.1002/iub.1991

Nair, A., Chauhan, P., Saha, B., Kubatzky, K.F., 2019. Conceptual Evolution of Cell Signaling. Int. J. Mol. Sci. 20, 3292. https://doi.org/10.3390/ijms20133292

Nakagawa, H., Maeda, S., 2012. Molecular Mechanisms of Liver Injury and Hepatocarcinogenesis: Focusing on the Role of Stress-Activated MAPK. Pathol. Res. Int. 2012, 1–14. https://doi.org/10.1155/2012/172894

National Guideline Centre (UK), 2016. Non-Alcoholic Fatty Liver Disease: Assessment and Management, National Institute for Health and Care Excellence: Guidelines. National Institute for Health and Care Excellence (NICE), London.

Neville, A., Palmer, J.B., Gaddie, J., May, C.S., Palmer, K.N., Murchison, L.E., 1977. Metabolic effects of salbutamol: comparison of aerosol and intravenous administration. BMJ 1, 413–414. https://doi.org/10.1136/bmj.1.6058.413

Okorodudu, A.O., Pelletier, P.R., Valcour, A.A., Bowers, G.N., McComb, R.B., 1989. Evaluation of the IFCC reference method for alanine aminotransferase: spurious blank ALT activity due to contamination of D-alanine with L-alanine, and recommendations for a correction. Clin. Chem. 35, 153–156.

Pacana, T., Sanyal, A.J., 2012. Vitamin E and nonalcoholic fatty liver disease: Curr. Opin. Clin. Nutr. Metab. Care 15, 641–648. https://doi.org/10.1097/MCO.0b013e328357f747

Papatheodoridi, M., Cholongitas, E., 2019. Diagnosis of Non-alcoholic Fatty Liver Disease (NAFLD): Current Concepts. Curr. Pharm. Des. 24, 4574–4586. https://doi.org/10.2174/1381612825666190117102111

Paravati, S., Rosani, A., Warrington, S.J., 2024. Physiology, Catecholamines, in: StatPearls. StatPearls Publishing, Treasure Island (FL).

Park, K.S., Lee, Y.S., Park, H.W., Seo, S.H., Jang, B.G., Hwang, J.Y., Cho, K.B., Hwang, J.S., Ahn, S.H., Kang, Y.N., Kim, G.C., 2004. Factors Associated or Related to with Pathological Severity of Nonalcoholic Fatty Liver Disease. Korean J. Intern. Med. 19, 19–26. https://doi.org/10.3904/kjim.2004.19.1.19

Paschos, P., Paletas, K., 2009. Non alcoholic fatty liver disease and metabolic syndrome. Hippokratia 13, 9–19.

Patiyal, S., Katoch, S., 2006. Tissue specific and variable collagen proliferation in Swiss albino mice treated with clenbuterol. Physiol. Res. 97–104. https://doi.org/10.33549/physiolres.930677

Pearce, S.G., Thosani, N.C., Pan, J.-J., 2013. Noninvasive biomarkers for the diagnosis of steatohepatitis and advanced fibrosis in NAFLD. Biomark. Res. 1, 7. https://doi.org/10.1186/2050-7771-1-7

Peng, K., Mo, Z., Tian, G., 2017. Serum Lipid Abnormalities and Nonalcoholic Fatty Liver Disease in Adult Males. Am. J. Med. Sci. 353, 236–241. https://doi.org/10.1016/j.amjms.2017.01.002

Perumpail, B.J., Khan, M.A., Yoo, E.R., Cholankeril, G., Kim, D., Ahmed, A., 2017. Clinical epidemiology and disease burden of nonalcoholic fatty liver disease. World J. Gastroenterol. 23, 8263–8276. https://doi.org/10.3748/wjg.v23.i47.8263

Peterla, T.A., Scanes, C.G., 1990. Effect of β-adrenergic agonists on lipolysis and lipogenesis by porcine adipose tissue in vitro. J. Anim. Sci. 68, 1024–1029. https://doi.org/10.2527/1990.6841024x

Pflugradt, K., Voss, Ch., Hartmann, N., Ratzmann, L. -K., 1981. Zum Einfluß einer Fettdiät auf den Gehalt an Gesamtcholesterol, freiem Cholesterol und Estercholesterol in Serum und Leber von Ratten. Food Nahr. 25, 937–941. https://doi.org/10.1002/food.19810251007

Piribauer, M., Jiang, L., Kostov, T., Parr, M., Steidel, S., Bizjak, D.A., Steinacker, J.M., Diel, P., 2023. Combinatory in vitro effects of the β2-agonists salbutamol and formoterol in skeletal muscle cells. Toxicol. Lett. 378, 10–18. https://doi.org/10.1016/j.toxlet.2023.02.007

Pitcher, J.A., Freedman, N.J., Lefkowitz, R.J., 1998. G PROTEIN–COUPLED RECEPTOR KINASES. Annu. Rev. Biochem. 67, 653–692. https://doi.org/10.1146/annurev.biochem.67.1.653

Pougeois, R., Satre, M., Vignais, P.V., 1979. Reactivity of mitochondrial F1-ATPase to dicyclohexylcarbodiimide. Inactivation and binding studies. Biochemistry 18, 1408–1413. https://doi.org/10.1021/bi00575a003

Pouwels, S., Sakran, N., Graham, Y., Leal, A., Pintar, T., Yang, W., Kassir, R., Singhal, R., Mahawar, K., Ramnarain, D., 2022. Non-alcoholic fatty liver disease (NAFLD): a review of pathophysiology, clinical management and effects of weight loss. BMC Endocr. Disord. 22, 63. https://doi.org/10.1186/s12902-022-00980-1

Ratziu, V., Harrison, S.A., Francque, S., Bedossa, P., Lehert, P., Serfaty, L., Romero-Gomez, M., Boursier, J., Abdelmalek, M., Caldwell, S *et al*., 2016. Elafibranor, an Agonist of the Peroxisome Proliferator−Activated Receptor−α and −δ, Induces Resolution of Nonalcoholic Steatohepatitis Without Fibrosis Worsening. Gastroenterology 150, 1147-1159.e5. https://doi.org/10.1053/j.gastro.2016.01.038

Rehman, S., Rahimi, N., Dimri, M., 2024. Biochemistry, G Protein Coupled Receptors, in: StatPearls. StatPearls Publishing, Treasure Island (FL).

Ribback, S., Peters, K., Yasser, M., Prey, J., Wilhelmi, P., Su, Q., Dombrowski, F., Bannasch, P., 2024. Hepatocellular Ballooning is Due to Highly Pronounced Glycogenosis Potentially Associated with Steatosis and Metabolic Reprogramming. J. Clin. Transl. Hepatol. 12, 52–61. https://doi.org/10.14218/JCTH.2023.00242

Rinaldi, L., Pafundi, P.C., Galiero, R., Caturano, A., Morone, M.V., Silvestri, C., Giordano, M., Salvatore, T., Sasso, F.C., 2021. Mechanisms of Non-Alcoholic Fatty Liver Disease in the Metabolic Syndrome. A Narrative Review. Antioxidants 10, 270. https://doi.org/10.3390/antiox10020270

Roh, Y.S., Seki, E., 2013. Toll-like receptors in alcoholic liver disease, non-alcoholic steatohepatitis and carcinogenesis. J. Gastroenterol. Hepatol. 28, 38–42. https://doi.org/10.1111/jgh.12019

Rosato, V., Masarone, M., Dallio, M., Federico, A., Aglitti, A., Persico, M., 2019. NAFLD and Extra-Hepatic Comorbidities: Current Evidence on a Multi-Organ Metabolic Syndrome. Int. J. Environ. Res. Public. Health 16, 3415. https://doi.org/10.3390/ijerph16183415

Rosenbaum, D.M., Rasmussen, S.G.F., Kobilka, B.K., 2009. The structure and function of G-protein-coupled receptors. Nature 459, 356–363. https://doi.org/10.1038/nature08144

Saltiel, A.R., Kahn, C.R., 2001. Insulin signalling and the regulation of glucose and lipid metabolism. Nature 414, 799–806. https://doi.org/10.1038/414799a

Sanyal, A.J., Campbell–Sargent, C., Mirshahi, F., Rizzo, W.B., Contos, M.J., Sterling, R.K., Luketic, V.A., Shiffman, M.L., Clore, J.N., 2001. Nonalcoholic steatohepatitis:

Association of insulin resistance and mitochondrial abnormalities. Gastroenterology 120, 1183–1192. https://doi.org/10.1053/gast.2001.23256

Sawadogo, A., Dib, N., Cales, P., 2007. Physiopathologie de la cirrhose et de ses complications. Réanimation 16, 557–562. https://doi.org/10.1016/j.reaurg.2007.09.001

Schaffner, F., Poper, H., 1963. Capillarization of hepatic sinusoids in man. Gastroenterology 44, 239–242.

Schuppan, D., Afdhal, N.H., 2008. Liver cirrhosis. The Lancet 371, 838–851. https://doi.org/10.1016/S0140-6736(08)60383-9

Schwabe, R.F., Greten, T.F., 2020. Gut microbiome in HCC – Mechanisms, diagnosis and therapy. J. Hepatol. 72, 230–238. https://doi.org/10.1016/j.jhep.2019.08.016

Sethi, J.K., Vidal-Puig, A.J., 2007. Thematic review series: Adipocyte Biology. Adipose tissue function and plasticity orchestrate nutritional adaptation. J. Lipid Res. 48, 1253–1262. https://doi.org/10.1194/jlr.R700005-JLR200

Sheridan, D.A., Aithal, G., Alazawi, W., Allison, M., Anstee, Q., Cobbold, J., Khan, S., Fowell, A., McPherson, S., Newsome, P.N., Oben, J., Tomlinson, J., Tsochatzis, E., 2017. Care standards for non-alcoholic fatty liver disease in the United Kingdom 2016: a cross-sectional survey. Frontline Gastroenterol. 8, 252–259. https://doi.org/10.1136/flgastro-2017-100806

Shojima, N., Sakoda, H., Ogihara, T., Fujishiro, M., Katagiri, H., Anai, M., Onishi, Y., Ono, H., Inukai, K., Abe, M *et al.*, 2002. Humoral Regulation of Resistin Expression in 3T3-L1 and Mouse Adipose Cells. Diabetes 51, 1737–1744. https://doi.org/10.2337/diabetes.51.6.1737

Silva Rosa, S.C., Nayak, N., Caymo, A.M., Gordon, J.W., 2020. Mechanisms of muscle insulin resistance and the cross-talk with liver and adipose tissue. Physiol. Rep. 8. https://doi.org/10.14814/phy2.14607

Simonen, P., Öörni, K., Sinisalo, J., Strandberg, T.E., Wester, I., Gylling, H., 2023. High cholesterol absorption: A risk factor of atherosclerotic cardiovascular diseases? Atherosclerosis 376, 53–62. https://doi.org/10.1016/j.atherosclerosis.2023.06.003

Smagris, E., Gilyard, S., BasuRay, S., Cohen, J.C., Hobbs, H.H., 2016. Inactivation of Tm6sf2, a Gene Defective in Fatty Liver Disease, Impairs Lipidation but Not Secretion of Very Low Density Lipoproteins. J. Biol. Chem. 291, 10659–10676. https://doi.org/10.1074/jbc.M116.719955

Smith, B.W., Adams, L.A., 2011. Nonalcoholic fatty liver disease and diabetes mellitus: pathogenesis and treatment. Nat. Rev. Endocrinol. 7, 456–465. https://doi.org/10.1038/nrendo.2011.72

Soares, J.-B., Pimentel-Nunes, P., Roncon-Albuquerque, R., Leite-Moreira, A., 2010. The role of lipopolysaccharide/toll-like receptor 4 signaling in chronic liver diseases. Hepatol. Int. 4, 659–672. https://doi.org/10.1007/s12072-010-9219-x

Sodum, N., Kumar, G., Bojja, S.L., Kumar, N., Rao, C.M., 2021. Epigenetics in NAFLD/NASH: Targets and therapy. Pharmacol. Res. 167, 105484. https://doi.org/10.1016/j.phrs.2021.105484

Strawbridge, R.J., Laumen, H., Hamsten, A., Breier, M., Grallert, H., Hauner, H., Arner, P., Dahlman, I., 2016. Effects of Genetic Loci Associated with Central Obesity on Adipocyte Lipolysis. PLOS ONE 11, e0153990. https://doi.org/10.1371/journal.pone.0153990

Szasz, A.M., Minnaar, C.A., Szentmártoni, G., Szigeti, G.P., Dank, M., 2019. Review of the Clinical Evidences of Modulated Electro-Hyperthermia (mEHT) Method: An Update for the Practicing Oncologist. Front. Oncol. 9, 1012. https://doi.org/10.3389/fonc.2019.01012

Szendrei, B., González-Lamuño, D., Amigo, T., Wang, G., Pitsiladis, Y., Benito, P.J., Gomez-Candela, C., Calderón, F.J., Cupeiro, R., PRONAF Study Group, 2016. Influence of ADRB2 Gln27Glu and ADRB3 Trp64Arg polymorphisms on body weight and body composition changes after a controlled weight-loss intervention. Appl. Physiol. Nutr. Metab. 41, 307–314. https://doi.org/10.1139/apnm-2015-0425

Tajima, K., Nakamura, A., Shirakawa, J., Togashi, Y., Orime, K., Sato, K., Inoue, H., Kaji, M., Sakamoto, E., Ito, Y *et al*., 2013. Metformin prevents liver tumorigenesis induced by high-fat diet in C57Bl/6 mice. Am. J. Physiol.-Endocrinol. Metab. 305, E987–E998. https://doi.org/10.1152/ajpendo.00133.2013

Tan, Y., Wang, M., Zhang, Y., Ge, S., Zhong, F., Xia, G., Sun, C., 2021. Tumor-Associated Macrophages: A Potential Target for Cancer Therapy. Front. Oncol. 11, 693517. https://doi.org/10.3389/fonc.2021.693517

Tarao, K., Nozaki, A., Ikeda, T., Sato, A., Komatsu, H., Komatsu, T., Taguri, M., Tanaka, K., 2019. Real impact of liver cirrhosis on the development of hepatocellular carcinoma in various liver diseases-meta-analytic assessment. Cancer Med. 8, 1054–1065. https://doi.org/10.1002/cam4.1998

Tattersfield, A.E., 2006. ADRENERGIC RECEPTORS, in: Encyclopedia of Respiratory Medicine. Elsevier, pp. 53–57. https://doi.org/10.1016/B0-12-370879-6/00018-1

Tesfay, M., Goldkamp, W.J., Neuschwander-Tetri, B.A., 2018. NASH: The Emerging Most Common Form of Chronic Liver Disease. Mo. Med. 115, 225–229.

Trinder, P., 1969. A Simple Turbidimetric Method for the Determination of Serum Cholesterol. Ann. Clin. Biochem. Int. J. Lab. Med. 6, 165–166. https://doi.org/10.1177/000456326900600505

Tsochatzis, E.A., Bosch, J., Burroughs, A.K., 2014. Liver cirrhosis. The Lancet 383, 1749–1761. https://doi.org/10.1016/S0140-6736(14)60121-5

Tuteja, N., 2009. Signaling through G protein coupled receptors. Plant Signal. Behav. 4, 942–947. https://doi.org/10.4161/psb.4.10.9530

Utzschneider, K.M., Kahn, S.E., 2006. The Role of Insulin Resistance in Nonalcoholic Fatty Liver Disease. J. Clin. Endocrinol. Metab. 91, 4753–4761. https://doi.org/10.1210/jc.2006-0587

Venkatakrishnan, A.J., Deupi, X., Lebon, G., Tate, C.G., Schertler, G.F., Babu, M.M., 2013. Molecular signatures of G-protein-coupled receptors. Nature 494, 185–194. https://doi.org/10.1038/nature11896

Vilar-Gomez, E., Calzadilla-Bertot, L., Wai-Sun Wong, V., Castellanos, M., Aller-de La Fuente, R., Metwally, M., Eslam, M., Gonzalez-Fabian, L., Alvarez-Quiñones Sanz, M., Conde-Martin, A.F *et al*., 2018. Fibrosis Severity as a Determinant of Cause-Specific Mortality in Patients With Advanced Nonalcoholic Fatty Liver Disease: A Multi-National Cohort Study.Gastroenterology 155, 443-457.e17. https://doi.org/10.1053/j.gastro.2018.04.034

Vroon, D.H., Israili, Z., 1990. Alkaline Phosphatase and Gamma Glutamyltransferase, in: Walker, H.K., Hall, W.D., Hurst, J.W. (Eds.), Clinical Methods: The History, Physical, and Laboratory Examinations. Butterworths, Boston.

Wagenknecht, L.E., Scherzinger, A.L., Stamm, E.R., Hanley, A.J.G., Norris, J.M., Chen, Y.I., Bryer-Ash, M., Haffner, S.M., Rotter, J.I., 2009. Correlates and Heritability of Nonalcoholic Fatty Liver Disease in a Minority Cohort. Obesity 17, 1240–1246. https://doi.org/10.1038/oby.2009.4

Wali, J.A., Jarzebska, N., Raubenheimer, D., Simpson, S.J., Rodionov, R.N., O'Sullivan, J.F., 2020. Cardio-Metabolic Effects of High-Fat Diets and Their Underlying Mechanisms—A Narrative Review. Nutrients 12, 1505. https://doi.org/10.3390/nu12051505

Wang, D., He, H., Wei, C., 2023. Cellular and potential molecular mechanisms underlying transovarial transmission of the obligate symbiont *SULCIA* in cicadas. Environ. Microbiol. 25, 836–852. https://doi.org/10.1111/1462-2920.16310

Wang, X., Rao, H., Liu, F., Wei, L., Li, H., Wu, C., 2021. Recent Advances in Adipose Tissue Dysfunction and Its Role in the Pathogenesis of Non-Alcoholic Fatty Liver Disease. Cells 10, 3300. https://doi.org/10.3390/cells10123300

Wang, X.J., Malhi, H., 2018. Nonalcoholic Fatty Liver Disease. Ann. Intern. Med. 169, ITC65. https://doi.org/10.7326/AITC201811060

Wei, Y., Rector, R.S., Thyfault, J.P., Ibdah, J.A., 2008. Nonalcoholic fatty liver disease and mitochondrial dysfunction. World J. Gastroenterol. 14, 193. https://doi.org/10.3748/wjg.14.193

Weiss, R., Caprio, S., 2005. The metabolic consequences of childhood obesity. Best Pract. Res. Clin. Endocrinol. Metab. 19, 405–419. https://doi.org/10.1016/j.beem.2005.04.009

Wellen, K.E., Hotamisligil, G.S., 2005. Inflammation, stress, and diabetes. J. Clin. Invest. 115, 1111–1119. https://doi.org/10.1172/JCI25102

Wenkeová, J., Kuhn, E., Wenke, M., 1976. Some adrenomimetic drugs affecting lipolysis in human adipose tissue in vitro. Eur. J. Pharmacol. 35, 1–6. https://doi.org/10.1016/0014-2999(76)90294-6

Westphal, S.A., 2008. Obesity, Abdominal Obesity, and Insulin Resistance. Clin. Cornerstone 9, 23–31. https://doi.org/10.1016/S1098-3597(08)60025-3

Williams, E.J., Iredale, J.P., 1998a. Liver cirrhosis. Postgrad. Med. J. 74, 193–202. https://doi.org/10.1136/pgmj.74.870.193

Williams, E.J., Iredale, J.P., 1998b. Liver cirrhosis. Postgrad. Med. J. 74, 193–202. https://doi.org/10.1136/pgmj.74.870.193

Wingler, L.M., Lefkowitz, R.J., 2020. Conformational Basis of G Protein-Coupled Receptor Signaling Versatility. Trends Cell Biol. 30, 736–747. https://doi.org/10.1016/j.tcb.2020.06.002

Wong, R.H., Sul, H.S., 2010. Insulin signaling in fatty acid and fat synthesis: a transcriptional perspective. Curr. Opin. Pharmacol. 10, 684–691. https://doi.org/10.1016/j.coph.2010.08.004

Wood, L.G., Garg, M.L., Gibson, P.G., 2011. A high-fat challenge increases airway inflammation and impairs bronchodilator recovery in asthma. J. Allergy Clin. Immunol. 127, 1133–1140. https://doi.org/10.1016/j.jaci.2011.01.036

Wree, A., Schlattjan, M., Bechmann, L.P., Claudel, T., Sowa, J.-P., Stojakovic, T., Scharnagl, H., Köfeler, H., Baba, H.A., Gerken, G *et al*., 2014. Adipocyte cell size, free fatty acids and apolipoproteins are associated with non-alcoholic liver injury progression in severely obese patients. Metabolism 63, 1542–1552. https://doi.org/10.1016/j.metabol.2014.09.001

Xiao, R.-P., Avdonin, P., Zhou, Y.-Y., Cheng, H., Akhter, S.A., Eschenhagen, T., Lefkowitz, R.J., Koch, W.J., Lakatta, E.G., 1999. Coupling of β_2-Adrenoceptor to G_i Proteins and Its Physiological Relevance in Murine Cardiac Myocytes. Circ. Res. 84, 43–52. https://doi.org/10.1161/01.RES.84.1.43

Xing, M., Gao, M., Li, J., Han, P., Mei, L., Zhao, L., 2022. Characteristics of peripheral blood Gamma-glutamyl transferase in different liver diseases. Medicine (Baltimore) 101, e28443. https://doi.org/10.1097/MD.0000000000028443

Xydas, S., Kherani, A.R., Chang, J.S., Klotz, S., Hay, I., Mutrie, C.J., Moss, G.W., Gu, A., Schulman, A.R., Gao, D *et al*., 2006. β_2-Adrenergic Stimulation Attenuates Left Ventricular Remodeling, Decreases Apoptosis, and Improves Calcium Homeostasis in a Rodent Model of Ischemic Cardiomyopathy. J. Pharmacol. Exp. Ther. 317, 553–561. https://doi.org/10.1124/jpet.105.099432

Yamauchi, T., Tobe, K., Tamemoto, H., Ueki, K., Kaburagi, Y., Yamamoto-Honda, R., Takahashi, Y., Yoshizawa, F., Aizawa, S., Akanuma, Y *et al*., 1996. Insulin Signalling and Insulin Actions in the Muscles and Livers of Insulin-Resistant, Insulin Receptor Substrate 1-Deficient Mice. Mol. Cell. Biol. 16, 3074–3084. https://doi.org/10.1128/MCB.16.6.3074

Younossi, Z., Anstee, Q.M., Marietti, M., Hardy, T., Henry, L., Eslam, M., George, J., Bugianesi, E., 2018. Global burden of NAFLD and NASH: trends, predictions, risk factors and prevention. Nat. Rev. Gastroenterol. Hepatol. 15, 11–20. https://doi.org/10.1038/nrgastro.2017.109

Younossi, Z., Tacke, F., Arrese, M., Chander Sharma, B., Mostafa, I., Bugianesi, E., Wai-Sun Wong, V., Yilmaz, Y., George, J., Fan, J *et al*., 2019. Global Perspectives on Nonalcoholic Fatty Liver Disease and Nonalcoholic Steatohepatitis. Hepatology 69, 2672–2682. https://doi.org/10.1002/hep.30251

Younossi, Z.M., Koenig, A.B., Abdelatif, D., Fazel, Y., Henry, L., Wymer, M., 2016. Global epidemiology of nonalcoholic fatty liver disease—Meta-analytic assessment of prevalence, incidence, and outcomes. Hepatology 64, 73–84. https://doi.org/10.1002/hep.28431

Zhang, C.-Y., Yuan, W.-G., He, P., Lei, J.-H., Wang, C.-X., 2016. Liver fibrosis and hepatic stellate cells: Etiology, pathological hallmarks and therapeutic targets. World J. Gastroenterol. 22, 10512. https://doi.org/10.3748/wjg.v22.i48.10512

Zhang, Q., He, Y., Luo, N., Patel, S.J., Han, Y., Gao, R., Modak, M., Carotta, S., Haslinger, C., Kind, D *et al.*, 2019. Landscape and Dynamics of Single Immune Cells in Hepatocellular Carcinoma. Cell 179, 829-845.e20. https://doi.org/10.1016/j.cell.2019.10.003

Zheng, Y., Wang, S., Wu, J., Wang, Y., 2023. Mitochondrial metabolic dysfunction and non-alcoholic fatty liver disease: new insights from pathogenic mechanisms to clinically targeted therapy. J. Transl. Med. 21, 510. https://doi.org/10.1186/s12967-023-04367-1

Summary

NAFLD is related to obesity and metabolic syndrome, affecting 20-30% of individuals and sometimes progressing to NASH. We aimed to evaluate formoterol and salbutamol in treating NAFLD using thirty-two male Fischer F344 rats on a high-fat, high-sucrose diet for 12 weeks. The rats were divided into four groups: control (CTL), untreated HFD, formoterol-treated (15 µg/kg), and salbutamol-treated (150 µg/kg). Results showed significant reductions in hepatic steatosis and liver enzymes in treated rats, with salbutamol showing superior efficacy. This study innovates by using β-adrenergic agonists for hepatic steatosis treatment. The main limitation is the use of an animal model, necessitating further human studies.

Résumé

La NAFLD est liée à l'obésité et au syndrome métabolique, touchant 20 à 30 % des individus et pouvant évoluer en NASH. Nous avons évalué l'efficacité du formotérol et du salbutamol pour traiter la NAFLD en utilisant trente-deux rats mâles Fischer F344 nourris avec un régime riche en graisses et en saccharose pendant 12 semaines. Les rats ont été répartis en quatre groupes : témoin (CTL), HFD non traité, traité avec formotérol (15 µg/kg) et traité avec salbutamol (150 µg/kg). Les résultats ont montré des réductions significatives de la stéatose hépatique et des enzymes hépatiques chez les rats traités, avec une efficacité supérieure du salbutamol. Cette étude innove en utilisant des agonistes β-adrénergiques pour traiter la stéatose hépatique. La principale limitation est l'utilisation d'un modèle animal, nécessitant des études supplémentaires chez l'homme.

ملخص

مرض الكبد الدهني غير الكحولي هو حالة مرتبطة بالسمنة ومتلازمة الأيض، تؤثر على 20-30% من الأفراد وقد تتطور أحيانًا إلى التهاب الكبد الدهني غير الكحولي. هدفنا هو تقييم فعالية الفورموتيرول والسالبوتامول في علاج المرض باستخدام اثنين وثلاثين فأرًا ذكرًا من نوع فيشر 344 تم تغذيتهم بنظام غذائي عالي الدهون والسكروز لمدة 12 أسبوعًا. تم تقسيم الفئران إلى أربع مجموعات: مجموعة تحكم، مجموعة غير معالجة، مجموعة عولجت بالفورموتيرول 15 ميكروغرام/كغ، ومجموعة عولجت بالسالبوتامول 150 ميكروغرام/كغ. أظهرت النتائج انخفاضًا كبيرًا في التنكس الدهني الكبدي وإنزيمات الكبد في الفئران المعالجة، مع فعالية أكبر للسالبوتامول. تميزت هذه الدراسة باستخدام ناهضات مستقبلات بيتا الأدرينالية لعلاج التنكس الدهني الكبدي. القيد الرئيسي هو استخدام نموذج حيواني، مما يتطلب دراسات إضافية على البشر