Pathogenesis and Therapeutic Considerations for Nonalcoholic Fatty Liver Disease

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A Senior Thesis submitted in partial fulfillment
of the requirements for graduation
in the Honors Program
Liberty University
Spring 2024

Acceptance of Senior Honors Thesis

This Senior Honors Thesis is accepted in partial
fulfillment of the requirements for graduation from the
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April 23, 2024
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Abstract

A product of the Westernized, high-fat diet, non-alcoholic fatty liver disease (NAFLD) has emerged as the leading cause of chronic liver disease, affecting one-fourth of the world's population. NAFLD is a progressive disease arising from a multisystem response to excess lipids in the blood, adipose tissue, and liver. Despite the prevalence of NAFLD and its well-studied bidirectional association with obesity and type 2 diabetes mellitus and obesity, there is a shocking scarcity of available treatments aside from diet and lifestyle changes. Thus, further research on NAFLD and potential therapies is urgently needed. This paper will illustrate the major pathways associated with NAFLD and address the relevance of adipose tissue and insulin resistance to identify targets for potential medications and treatments.

Introduction

The liver is the largest visceral organ in the body and performs over 200 metabolic functions, including bile and cholesterol production, blood filtration, detoxification, vitamin mineral storage, and the storage and distribution of carbohydrates and lipids. The liver is heavily involved in digestion and is the first internal organ, following intestinal absorption, to process and distribute post-prandial nutrients. The process of digestion begins with the mechanical and chemical digestion of carbohydrates, lipids, and proteins throughout the gastrointestinal tract until eventual absorption in the lumen of the small intestine by specialized epithelial cells called enterocytes is accomplished (Adeva-Adany et al., 2016). Monosaccharides and amino acids are absorbed into the enterocytes and are transported into the blood (Adeva-Adany et al., 2016). Sugars and amino acids reach the liver via the hepatic portal vein, while fatty acids first travel through the lymph until dumped into the left subclavian vein and eventually reach the liver through the hepatic artery. The liver employs various specialized cells to perform its biochemical and metabolic functions. Hepatocytes serve as the liver's primary parenchymal cell and are specialized epithelial cells that perform most of the liver's essential biochemical and metabolic processes (Alves-Bezerra and Cohen, 2017; Rui, 2014). Other relevant cell types include cholangiocytes, stellate cells, liver sinusoidal endothelial cells (LSECs), and Kupffer cells which are highly involved in the liver's inflammation response (Alves-Bezerra and Cohen, 2017). This paper will begin by exploring insulin signaling (with a special focus on the p300-mediated pathway) and how it interacts with normal liver physiology and its role in metabolism. From there, the effects of obesity and insulin resistance and how they affect the innate immune system and increase inflammation will be discussed. Finally, the pathophysiology of non-alcoholic fatty liver disease (NAFLD) will

be integrated with the discussion on obesity and insulin resistance. Potential treatments will be described.

Overview of Insulin Signaling Pathway

Insulin signaling is essential to understanding the liver's role in glucose and lipid metabolism due to its systemic role in regulating blood sugar level and overall adiposity. Insulin signaling is a mechanism driven by a progression of kinase phosphorylation and is regulated through select phosphatases and competition with acetylation, methylation, ubiquitylation, and other types of post-translational modifications. Two main pathways are known: the PI3K/Akt metabolic pathway (Figure 1) and the mitosis-related Raf/Ras/MEK/MAPK pathway (De Meyts 2016). P300 is only known to impact the PI3K/Akt metabolic pathway, primarily in a insulin-dependent fashion. The PI3K/Akt pathway begins with the reception of insulin at the insulin receptor (IR) which triggers autophosphorylation in the cytoplasmic domain of the IR. The phosphorylated IR then recruits insulin receptor substrate 1 (IRS1). IRS2 is another structurally and functionally similar insulin signaling adaptor with minor distinctions across different tissue types (Eckstein, Weigert, Lehmann, 2017) (Figure 1). Following tyrosine-phosphorylation by the IR, IRS1 acts as a docking site for PI3K, a phosphatidylinositol heterodimer of p85 and p110. PI3K then phosphorylates the plasma membrane lipid PI-(4,5)-biphosphate (PIP_2) into PI-(3,4,5)-biphosphate (PIP_3). PIP_3 binds to 3-PI-dependent protein kinase-1 (PDK1) and Akt (a.k.a. protein kinase B) and catalyzes the phosphorylation of Akt at Thr-308 by PDK1. Akt is also phosphorylated by mechanistic target of rapamycin complex 2 (mTORC2) at Ser-473. Phosphorylation at both sites is essential for proper function of Akt. Akt has several roles such as the inhibition of AS160 and GSK3β via phosphorylation. AS160 is a GTPase-activating protein that deactivates Rab family proteins, a set of proteins responsible for vesicle movement and fusion

(Mîinea et al. 2005). Inhibition of AS160 maintains the active GTP-bound forms of the Rab proteins and allows for translocation of GLUT4, a glucose transporter predominately localized to skeletal muscle and adipose tissue, to the plasma membrane and increased glucose uptake. GSK3β is a serine-threonine kinase that inhibits glycogen synthase (GS) through phosphorylation. Inhibition of GSK3β releases GS which converts absorbed glucose into glycogen for storage and reduces serum glucose levels (LaBarge, Migdal, Schenk 2015).

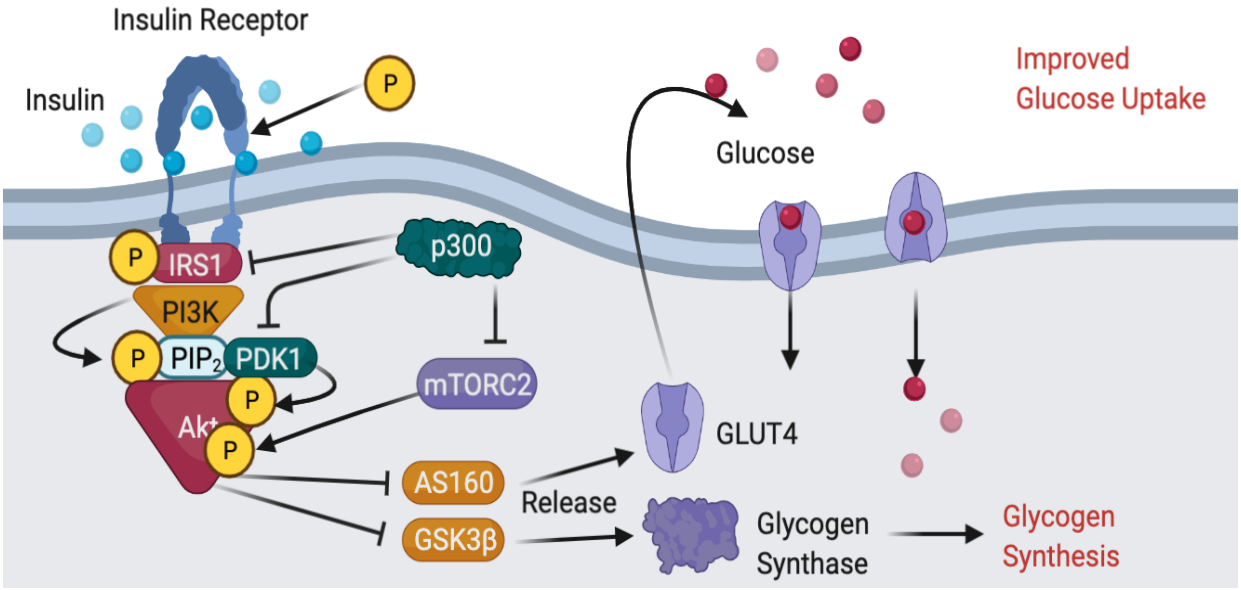

Figure 1. Insulin Signaling Pathway

Insulin triggers a signal cascade wherein the IR phosphorylates IRS1* which recruits PI3K to phosphorylate PIP_2 to PIP_3 and provide a docking site for Akt and PDK1. Phosphorylation by PDK1 thus activates Akt, which then phosphorylates and inhibits AS160 and GSK3β. Inhibition of AS160 and GSK3β lowers serum glucose concentration by improving glucose uptake and stimulating glycogen synthesis. This signal cascade can be fully silenced by the presence of cytoplasmic p300 which acetylates IRS1 and IRS2. Acetylation of IRS1 and IRS2 prevents translocation of GLUT4 and release of glycogen synthase, thus keeping serum glucose levels high when cytoplasmic p300 is elevated. P300 can also acetylate PDK1 and mTORC2 to inhibit their kinase activity.

*Note that recruitment of IRS1 or IRS2 is dependent on tissue-type and the timing of food consumption. IRS1 is dominant in skeletal muscle alone, while IRS1 and IRS2 are both present in hepatocytes, yet are recruited depending on how recently food has been consumed (Eckstein, Weigert, Lehmann 2017).

P300's Structure and Role in Insulin Signaling

P300 Structure

P300 is a histone acetyltransferase (HAT) that serves a critical role in cell proliferation and differentiation and therefore serves as an interesting protein target for medications. P300 is characterized by several key protein interaction domains: nuclear receptor interaction domain (NRID), two cysteine/histidine domains (TAZ1 and TAZ2), the interferon binding domain (IBiD), and the specialized KIX domain (Figure 2) (Piskacek, 2016). The most important domain is the histone acetyltransferase domain which binds to p53 transcription factors to upregulate expression of p53. P300 and its closely related coactivator CREB binding protein, most commonly bind to Ser-133 of cAMP-binding protein (CREB) to enhance transcription of the target gene (He, 2021). Additionally, p300 bears a PHD finger motif and RING domain suspected to serve ambiguous regulatory functions over the HAT domain (Ringel and Wolberger, 2013; Rack, 2014).

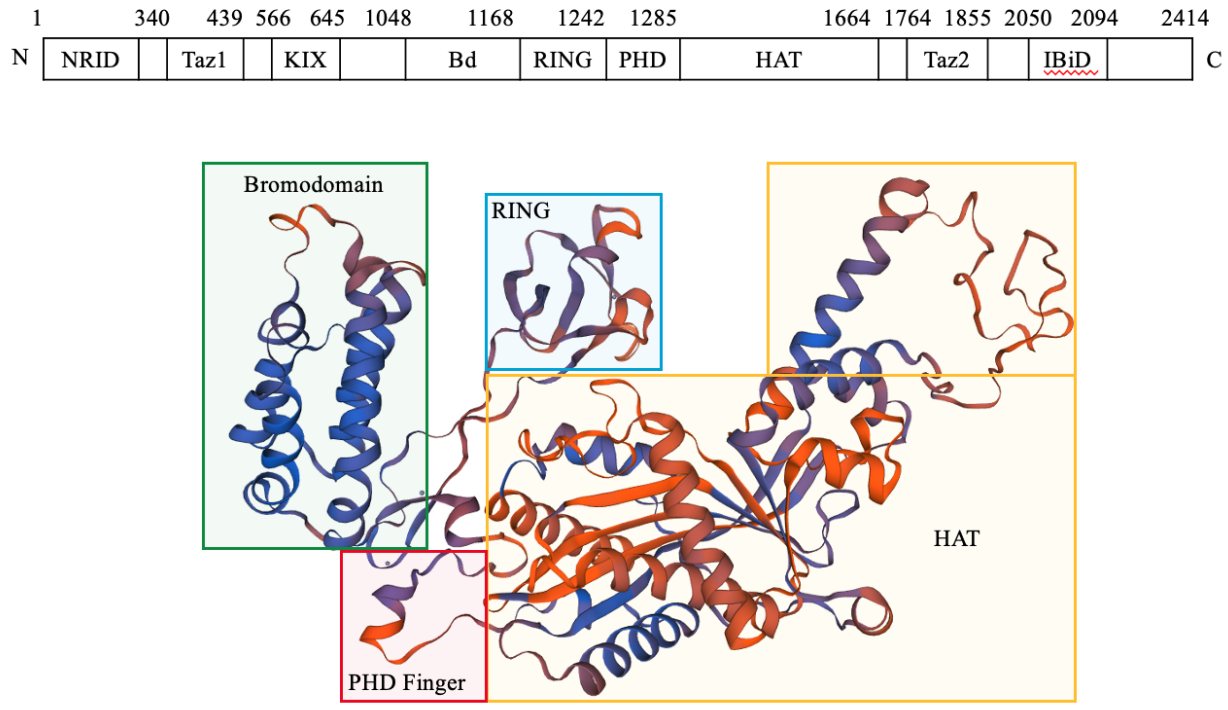

Figure 2. Primary and Tertiary Structure of p300

The primary structure diagram numbers by amino acid residues. The key functional domains of the tertiary structure of p300 are labelled according to data from Ringel and Wolberger (2013) and the NIH gene library. SWISS-MODEL software was used to model the human p300.

Lipopolysaccharide-Induced p300 Effects on Glucogenesis and Insulin Resistance

Under normal conditions, glucogenesis is triggered in response to hypoglycemia to supply cells with glucose for normal function and health. The liver responds to several regulatory mechanisms (i.e. glucagon, epinephrine, and glucocorticoid secretions) through the cAMP-PKA signaling pathway (He, 2021). The activation of PKA by these hormones leads to the phosphorylation of CREB at Ser-133 which subsequently triggers the recruitment of co-activators, p300, CBP, and CRTC2. This complex greatly enhances the expression of *Pck1* and *G6pc*, two rate-limiting glucogenic genes, thus leading to hepatic glucose production. Additionally, p300 can acetylate CRTC2 at Lys-628 which protects the CRTC2 from degradation and further activates glucogenic gene expression. Finally, p300 and CREB will bind to the *Foxo1* gene to express FOXO1, another transcriptional enhancer of glucogenic gene expression (He, 2021) (Figure 3).

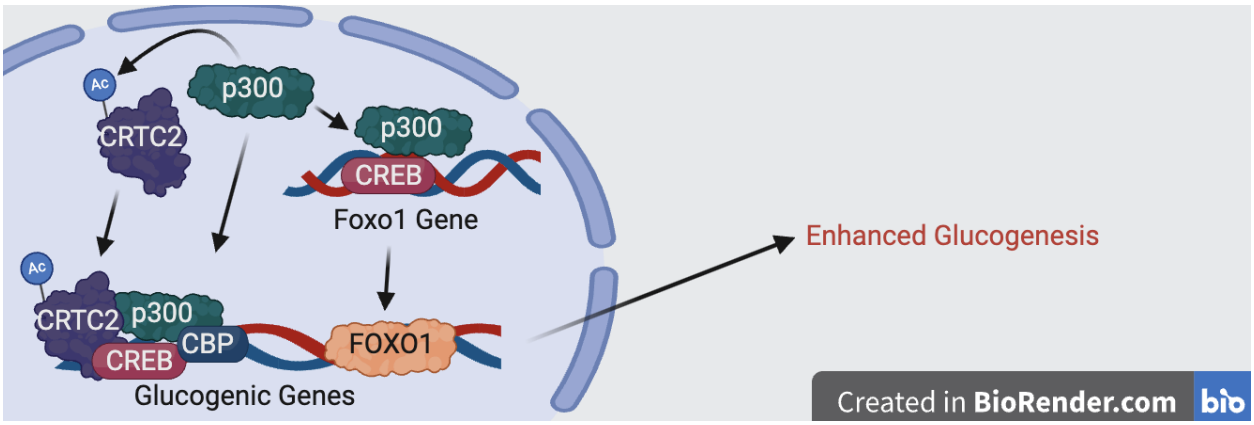

Figure 3. Glucogenesis Pathway in Hepatocytes

Nuclear p300 in hepatocytes further maintains high blood sugar by promoting transcription of glucogenic proteins via two mechanisms. p300 alone will bind to CREB and promote FOXO1 expression. FOXO1 then promotes transcription of the rate-limiting glucogenic genes Pck1 and

G6pc among other glucogenic genes. p300 will also acetylate CRTC2 and form a complex with the acetylated CRTC2 and CBP to further promote the glucogenic gene expression.

Lipolysaccharides (LPS) are common cell membrane components in many symbiotic gut bacteria populations in the human digestive tract. In individuals with elevated enteric and serum LPS, overnutrition and elevated levels of LPS causes distress to the endoplasmic reticulum and trigger the unfolded protein response and ultimately increase lipid deposition and inflammation in the liver. Particularly, the IRE1-XBP1 pathway leads to increased production of cytoplasmic and nuclear p300 (He, 2021). Additional studies in Hepa1-6 cells have revealed that p300 is induced by LPS through an unknown protection mechanism involving ubiquitination and degradation (He, 2021). Nuclear p300 will further increase glucogenesis via the cAMP-PKA pathway and expression of *Foxo1*. Notably, activation of *Foxo1* is sufficient in inducing hyperglycemia, insulin resistance, and organ failure, which are common sequelae associated with type 2 diabetes (Guo, 2014; Yang, 2018). Studies have shown that deletion of *Foxo1* is effective in preventing insulin resistance and associated heart failure in mice (Yang, 2018). In addition to enhancing gluconeogenesis in the liver, cytoplasmic p300 will acetylate IRS1 and IRS2, competitively inhibiting phosphorylation by the insulin receptor and effectively systemically inhibiting GLUT4 translocation and glucose uptake (He 2021). Combined with enhanced glucogenesis, the inhibition of glucose uptake will exacerbate the degree of hyperglycemia in individuals with elevated enteric and serum LPS. These effects of LPS-induced p300 are corroborated by improved insulin sensitivity in HFD mouse samples treated with shP300 and monitored in a hyperinsulinemic-euglycemic clamp experiment and as well as in samples isolated from HFD mice treated with deacetylase SIRT1 (He, 2021). Interestingly, p300 compounds the negative effects of LPS-induced p300 through its hyperacetylation and impairment of farnesoid X-activated receptor (FXR) which

results in ectopic liver and muscle lipid deposition, ultimately causing hepatic inflammation (Fukinishi et al., 2014; Guo, 2014).

Additional Information on the p300 Mechanism

In an recent study led by Vitor Martins, male mice were treated with CreLoxP to induce muscle-specific partial knockout of p300 or CBP and were placed on a calorie-restricted diet (CRD) or high fat diet (HFD) to determine if p300/CBP affects skeletal muscle sensitivity (Martins et al., 2019). Complete knockout of p300 and CBP was not possible due to embryonic lethality. Ultimately, the study revealed that even with up to 90% knockout of p300 and CBP, that p300 and CBP knockout alone did not affect insulin sensitivity. Therefore, it is unlikely that the acetyltransferase activity of p300 alone negatively regulates the insulin signaling pathway. However, even with significant p300 knockout, mice fed HFD diets still displayed remarkable insulin resistance (Martins et al., 2019). Thus, while the influence of p300 on insulin resistance and hyperglycemia are well-known, further research on other acetyltransferases involved in negatively regulating insulin signaling is necessary to better understand other underlying causes of insulin resistance and other potential health hazards of Western-style, high-fat diets. Further understanding of this mechanism can help identify potential targets for medications that can prevent or mitigate liver dysfunction at a systemic level rather than a tissue-specific level.

Normal Liver Physiology

Carbohydrate Metabolism

Following a meal, the body satisfies immediate energy needs before entering the postprandial state (6-12 hours). In the postprandial state, anabolic reactions are prioritized. As the first major metabolic organ to receive sugars, the liver directly consumes 33-60% of glucose from a meal (Alves-Bezerra and Cohen, 2017). This glucose is absorbed for intracellular modification

by the plasma membrane glucose transporter, GLUT2, a bidirectional channel that allows both absorption and secretion of glucose (as needed) (Alves-Bezerra and Cohen, 2017; Rui, 2014). Once in the hepatocyte, the enzyme glucokinase phosphorylates glucose into glucose-6-phosphate (G6P). This chemical modification effectively decreases intracellular glucose concentration and maintains a concentration gradient for glucose uptake while also preventing G6P from exiting the hepatocytes. Glucokinase is an isoenzyme of hexokinase, which exists as three other isozymes in most other cells. While all these isozymes perform the same function (produce G6P for various uses), they are regulated differently. Hexokinase 1-3 experiences feedback inhibition from G6P, while glucokinase is solely inhibited by GKRP (glucokinase regulatory protein). The lack of feedback inhibition allows glucokinase to process glucose efficiently into G6P and maintain a strong concentration gradient for glucose. This is achieved in part due to the relatively high Km (low affinity) of glucokinase compared to the other isoforms of hexokinase. The Km of glucokinase approximates the healthy blood glucose concentration at 5mM, meaning that glucokinase will slow and eventually stop phosphorylating glucose when blood sugar has dropped to normal levels (Alves-Bezerra and Cohen, 2017). This prevents hepatocytes from lowering blood sugar to dangerously low levels.

From here, G6P can take four paths: cellular respiration for energy production, glycogen synthesis, hexosamine production, or the pentose phosphate pathway (Adeva-Adany et al., 2016). Regardless of whether the body is fed or fasted, the liver will continuously send G6P through glycolysis to maintain energy levels for its other metabolic processes. The second pathway, glycogenesis, produces glycogen for energy storage. 50% of glucose from the postprandial state ends up in glycogen (Alves-Bezerra and Cohen, 2017). The third and fourth pathways begin with the isomerization of G6P into fructose-6-phosphate (F6P) via the enzyme glucose phosphate

isomerase (Rui, 2014). F6P can be combined with glutamate to initiate the hexosamine biosynthetic pathway to form UDP-*N*-acetylglucosamine, an essential precursor for O-Glc-n-acylation reactions. The final path, the pentose phosphate pathway, uses G6P to produce reducing equivalents (NADPH) and nucleic acid precursors (ribulose-5-phosphate) (Alves-Bezerra and Cohen, 2017; Trefts et al., 2017). The reducing equivalents act as electron scavengers and protect the body from reactive oxygen species (ROS)

In the fasted state (12-24 hours after a meal), glycogenolysis and gluconeogenesis are activated in the liver in response to the body's energy demands and the depleted of blood glucose levels. Therefore, the liver prioritizes metabolic pathways that can produce ATP to satisfy energy needs. Glycogenolysis and glucogenesis drive hepatic glucose production. Glycogenolysis is the liberation of glucose through the catabolism of glycogen. Gluconeogenesis is the *de novo* synthesis of glucose from non-carbohydrate sources such as amino acids and glycerol (Alves-Bezerra and Cohen, 2017; Trefts et al., 2017). In healthy individuals, glycogenolysis accounts for 85% of initial hepatic glucose output. However, as glycogen is depleted, glucogenesis increases to 77-94% (Alves-Bezerra and Cohen, 2017).

Lipid Metabolism

Unlike carbohydrates, the liver receives dietary lipids after they have had some time to circulate throughout the body. In the fed state, dietary lipids circulate the blood in the form of lipoproteins called chylomicrons. These chylomicrons are digested by lipoprotein lipase (LPL) in the capillaries of muscles and adipose tissue for absorption. Thus, when the remnants of the chylomicrons reach the liver, minimal amounts of glycerol, fatty acids, and cholesterol are available for metabolism. Excess carbohydrates are often converted to fatty acids via de novo lipogenesis. These fatty acids activate acyl CoA synthetase upon entry into the cell and esterified

to glycerol to produce triglycerides. These triglycerides are then either stored as lipid droplets in the liver or packaged onto very low-density lipoproteins (VLDLs) for transport to adipose or muscle tissue (Adeva-Adany et al., 2016). In the fasted state, fatty acid oxidation often supplements energy production when glycogen stores begin to wane (Adeva-Adany et al., 2016; Alves-Bezerra and Cohen, 2017). Fatty acids are converted into acyl-CoA and transported into mitochondria through the carnitine shuttle system to be processed into acetyl-CoA via beta-oxidation to be used in the TCA cycle (Adeva-Adany et al., 2016; Rui, 2014). Fatty acid oxidation also produces NADH and FADH2, which are also products of the TCA cycle that are used in the electron transport chain to drive ATP production.

Hormonal Regulation of Liver Metabolism

Naturally, as a metabolic organ, the liver operates to maintain homeostasis in the body. Thus, the liver responds to hormones that indicate different stressors. Most notably, the liver interacts with the pancreas and thyroid. The pancreas is responsible for insulin secretion, a hormone secreted in the postprandial state to stimulate anabolic pathways. Notably, insulin leads to a signaling cascade that promotes glycogenesis (and suppression of glycogenolysis and glucogenesis), and lipogenesis. The promotion of glycogenesis and lipogenesis occurs through the phosphorylation of enzymes in the PI3K/Akt pathway (Titchenell et al., 2017). Thyroid hormone (TH) refers to both T4 (thyroxine) and T3 (triiodothyronine), hormones commonly known for regulating growth and development and adult metabolism (Mullur et al., 2014). Through interactions with the TH receptor (TR) and liver X receptor (LXR), T4 and T3 modulate hepatic insulin sensitivity and are significant in both the anabolic and catabolic processes in carbohydrate and lipid metabolism.

NAFLD Pathophysiology

Insulin Resistance and Steatosis

Hepatic steatosis, the first stage of NAFLD, results from lipogenesis and lipid import (from adipose tissue and the diet) exceeding lipid oxidation and export from hepatocytes in the long term (Ipsen et al., 2018). Substantial increases in dietary carbohydrates (which undergo lipogenesis) and lipids lead to an overload of the liver's immediate ability to process and export lipids. When this excessive consumption of carbohydrates and lipids persists for an extended period, hyperinsulinemia occurs as the body continues to elevate insulin in response to the significant dietary macromolecule absorption (Ipsen et al., 2018). Hyperinsulinemia often develops into insulin resistance which then progresses to type 2 diabetes. Even if overeating and overconsumption of fats and carbohydrates did not result in obesity, hyperinsulinemia, and insulin resistance further contribute to an imbalanced accumulation of lipids throughout the blood and peripheral tissues, resulting in a condition known as dyslipidemia. This condition is primarily the result of the prolonged inhibition of lipolysis by excessive insulin levels. The reduction of lipolysis has several effects that lead to obesity. Reduced lipolysis prevents fat loss by preventing lipid breakdown in adipose tissue. It also encourages overeating by stimulating hunger in hyperinsulinemic patients who struggle to acquire energy from fat due to the inhibition of lipid oxidation by insulin. Finally, weight gain can occur as limited access to lipid energy stores results in feelings of tiredness that often encourage sedentary lifestyles. The resulting increases in adiposity (i.e., obesity) and hepatic lipid accumulation (i.e., steatosis) due to insulin resistance and type 2 diabetes explain the common concomitance of these three metabolic issues.

Adipose Tissue Under Duress

While most well-known for its role in energy storage, adipose tissue serves various biological roles throughout the body depending on type and anatomical location (Altintas et al., 2011). With diet-induced obesity, an increase in cell size (hypertrophy) and cell number (hyperplasia) is primarily seen in white adipose tissue (WAT) which includes subcutaneous and visceral fat. Tissue remodeling is also observed in these two compartments as a result. Adipose tissue bears significant endocrine functions to help mediate metabolism through the release of cytokines and adipokines such as leptin and adiponectin. Leptin, "the satiety hormone," helps signal fullness to the brain and is responsible for mediating some pro-inflammatory functions. Adiponectin, on the other hand functions through the downregulation of TNF, MCP-1, and IL6. Adipose tissue also regulates metabolism through AMPK, which helps balance anabolic and catabolic pathways according to the body's needs. Adipose tissue participates in cross-talk with the immune response through these endocrine functions.

The accumulation of lipids in adipose and hepatic tissue can cause insulin resistance due to the role of adipose tissueas an endocrine mediator with influence over hepatic insulin sensitivity. When lipid storage is overwhelmed by sustained caloric surplus and weight gain, ectopic lipid deposition and toxic lipid accumulation (lipotoxicity) occur. This process is driven by rising levels of leptin, decreasing adiponectin levels, and apoptosis in visceral adipose tissue (Hildebrandt et al., 2022). In obese individuals, rapid adipose tissue turnover is observed in response to lipotoxicity and inflammation and further stimulates macrophage recruitment. Oxidative stress from the lipotoxicity and apoptosis results in increased expression of soluble cell adhesion molecules (ICAM-1, PECAM-1, VCAM-1, and von Willebrand factor) and chemotactic factors (ex. CCL2, CCL5) adipose tissue endothelial capillaries , which leads to increased recruitment and infiltration

of macrophages to the tissue (Brestoff et al., 2021; Obstfeld, 2010). The leptin receptor in endothelial cells also helps to increase the capillary permeability and activate macrophage infiltration (Curat et al., 2004). It is worth noting that the endothelial factors released by the adipose capillaries enter circulation, increasing macrophage infiltration (primarily within the liver) and causing chronic, systemic inflammation (Liu et al., 2015).

In the visceral adipose tissue, macrophages will present as solitary macrophages or in crown-like structures (CLS). The regions of apoptosis in the visceral adipose tissue lead to the clustering of macrophages in CLSs as they attempt to manage the tissue damage (Altintas et al., 2012). As macrophages proliferate and migrate throughout the visceral tissue, mitochondrial transfer from the adipocytes to the macrophages will occur. Mitochondrial transfer occurs to help increase the strength and survivability of the macrophages and occurs through a heparan sulfate pathway. In obese individuals, mitochondrial transfer is observed to be increased compared to lean individuals as the body attempts to reinforce the immune response to the oxidative stress of lipotoxicity, apoptosis, and the resulting necrosis of the adipose tissue (Brestoff et al., 2021). In addition to effects on adipose tissue, adiponectin promotes lipid oxidation and inhibits lipid accumulation directly on the liver (Galvan-Martinez, 2023; Cobbina and Akhlagi, 2017). Thus, adiponectin is critical in sensitizing the liver to insulin and regulating lipid and carbohydrate homeostasis by regulating the organs responsible for lipid distribution. Studies have revealed hypoadiponectinemia often occurs due to NAFLD and insulin resistance (Cobbina and Akhlagi, 2017). In summary, hepatic steatosis results from, and is exacerbated by the diet-induced impairment of lipid metabolism via hepatic inflammation resulting from increased macrophage infiltration, hyperinsulinemia and hypoadiponectinemia.

Effect on Macrophage Populations

Overall, while 40-50% of WAT is already made up of macrophages, the macrophage population in WAT is increased by the increased permeability of adipose capillaries and the release of chemotactic factors by adipose-derived endothelial cells (Hildebrandt et al., 2022). The recruited macrophages are the classic pro-inflammatory M1 macrophages responding and attempting to unsuccessfully remove the source of oxidative stress in the visceral adipose tissue. M1 macrophages seek to recruit more myeloid cells and maintain inflammation through the secretion of TNF-α, IL-1β, IL-12, and IL-23. This secretion will be maintained if the stimulus is kept. In the long term, this will lead to tissue damage, most visibly in the liver. Inflammation will be further localized to the liver due to CCL2-specific recruitment of M1 macrophages to hepatic tissue and the production of acute phase proteins (Jamialahmadi et al., 2023). In contrast, M2 macrophages secrete IL-10 and TGF-β to quell inflammation and initiate tissue repair. M2 macrophages can also initiate the development of WAT into the more metabolically active beige adipose tissue (Li et al., 2023). In response to immune distress, pathogens and pro-inflammatory cytokines will trigger M1-polarization (Brestoff et al., 2021; Liu et al., 2015). To further explain why M1 macrophages persist in obese individuals, Liu et al., 2015 discovered ATG-5-dependent autophagy was defective that in mice with diet-induced obesity. ATG-5-dependent autophagy is an essential immune response that sequesters M1 macrophages in autophagosomes and allows M2 macrophages to initiate anti-inflammatory pathways (Liu et al., 2015).

Adipose Tissue Interaction with Toll-like Receptors (TLRs)

The innate immune system acts as the second line of defense to the natural barriers of the skin and mucosal membranes. The innate immune response consists of several pattern recognition receptors (PRRs) that recognize nonspecific pathogen-associated molecular patterns (PAMPs) or

damage-associated molecular patterns (DAMPs). These PRRs are located on the membranes of endothelial cells, dendritic cells, and macrophages. Specifically, a subset of PRRs, known as toll-like receptors (TLRs), mediate monocyte and macrophage release of pro-inflammatory cytokines (IL1β, IL6, and TNFα) by upregulating NF-kB, interferon regulatory factor (IRF) and AP-1 transcription factors. Diet-induced obesity leads to increased systemic inflammation and recruitment of naïve monocytes into adipose tissue. These changes have demonstrated obesity-induced alterations to TLRs. TLR4 is particularly interesting as it best demonstrates responsiveness to free fatty acids and can also be found on adipocytes (McFarlin et al., 2012). TLR4 is also shown to have synergistic interactions with TLR2 when interacting with environmental inflammatory signals and may have unexplained roles in promoting obesity-induced inflammation (McKernan et al., 2020). This chronic inflammation will generate immune system vulnerabilities and sensitivities. One such example is the increased sensitivity of TLR4 to LPS (Brestoff et al., 2021). Through these means, obesity will continually present excess lipid accumulation and apoptotic visceral adipose cells as DAMPs and, therefore, cause chronic inflammation which in turn generates some immune system vulnerabilities.

Other Effects of Obesity-induced Inflammation

The persistent polarization towards M1 macrophages has long-term impacts on adipose tissue function as it inhibits adipogenesis while causing insulin resistance and fibrosis (Li et al., 2023). Additionally, through IFN-γ secretion, M1 polarization impairs the aforementioned heparan sulfate pathway and thus increases phagocytosis and macropinocytosis in an attempt to clear apoptotic and necrotic adipose tissue. This impairment changes the WAT energy expenditure and contributes to increased adiposity and obesity (Brestoff et al. 2021). Additionally, obesity and hyperlipidemia often leave ectopic deposits on the liver and promote liver inflammation (hepatitis).

This inflammation is tied to specialized hepatic macrophages known as Kupffer cells that are overrecruited and overactivated through CCL2 secretion and impairment of Atg5-mediated autophagy (Liu et al., 2015; Obstfeld et al., 2010). While this section focuses on the interactions between obesity and the innate immune response, studies have also found that the adaptative immune response also plays a role in adipose tissue inflammation, insulin resistance, and glucose dysregulation in response to obesity. Due to their involvement in metabolism, the metabolic dysregulation of obesity consequently affects B and T cell class switching and their relative population sizes (McLaughlin et al., 2017). Clinically, some studies have shown the reversal of insulin resistance in obese mice when treated with anti-CD3 T cell or anti-CD20 B cell antibodies. If these treatments were further investigated, the use of these antibodies could attenuate oxidate stress on the liver by addressing the issue of insulin resistance.

Adipose tissue has a far more significant influence in the body than most people expect. Primarily through its endocrine role in the release of adipokines and cytokines, adipose tissue exerts both local and systemic influence on the immune response as it seeks to maintain metabolic homeostasis. With diet-induced obesity, sustained hyperlipidemia generates oxidative stress and apoptosis that triggers a systemic inflammation response primarily through M1 macrophage polarization and its respective pro-inflammatory secretions.

Through metabolic dysregulation, obesity also triggers immune dysregulation. Currently, only exercise and dieting are the prescribed treatments for obesity and its symptoms. While several macrophage pathways have been identified as potential targets for treatments for obesity-related inflammation, more research is needed to identify specific areas and trial possible treatments for this metabolic and immune disorder (Li et al., 2023; Liu et al., 2015). Preventative therapies and treatments that can effectively address inflammation at the systemic level are of great interest as

non-alcoholic steatohepatitis and hepatic fibrosis are often viewed as “points of no return” when observing fatty liver disease.

NASH and Fibrosis

NASH is the second stage of NAFLD, an immune response caused by elevated gut-derived endotoxins as well as elevated adipose secretion of TNF-α, IL-1β, and IL-6 (Cobbina and Akhlagi, 2017, Marjot et al., 2019). Elevation of adipose proinflammatory cytokines is caused by adipose tissue hypertrophy from overnutrition and hyperinsulinemia. Kupffer cells line the liver sinusoids and can be activated into two types: the proinflammatory M1 Kupffer cell and the anti-inflammatory M2 Kupffer cell. A substantial increase in the ratio of M1:M2 Kupffer cells is a strong indicator of NASH. Injury to hepatocytes leads to the release of biomolecules termed damage-associated molecular patterns (DAMPs). These DAMPs interact with the pattern recognition receptor on Kupffer cells to trigger M1 activation (Kitade et al., 2017; Marjot et al., 2019). M1 Kupffer cells further intensify NASH by driving additional production of TNF-α, IL-1β, and IL-6 and secreting TGFβ and PDGF to stimulate hepatic stellate cells (HSC). Kupffer cells also produce reactive oxygen species (ROS) that add to the oxidative strain on hepatocytes and cause DAMP release (Cobbina and Akhlagi, 2017, Marjot et al., 2019). This effect is amplified by Kupffer cell recruitment of neutrophils and NK cells, which further contribute to inflammation. Endotoxins from the digestion of bacterial LPS further activate Kupffer cells and HSCs. HSCs are responsible for hepatic fibrosing by promoting smooth muscle actin, desmin and type I collagen polymerization (Marjot et al., 2019).

Cirrhosis and Hepatocellular Carcinoma

Prolonged inflammation and fibrosing of the liver eventually lead to cirrhosis, the extensive scarring of the liver that leaves patients susceptible to severe complications. While hepatocellular

carcinoma (HCC) can develop without NASH and cirrhosis, statistical studies have shown higher rates of NASH-related HCC development, with 10-15% of those with NASH developing decompensated liver disease and HCC (Marjot et al., 2019). Considering the increasing prevalence of NAFLD and the 10-fold increase in NAFLD-related HCC incidence over the last decade, there should be a more significant concern for the emergence of HCC as a fatal disease (Byrne and Targher, 2019; Marjot et al., 2019). Despite research evidence strongly linking HCC to obesity, type 2 diabetes, NAFLD, and cirrhosis, the underlying means of action are not well understood. Regarding obesity and type 2 diabetes, it is proposed that there are unidentified diabetes and obesity factors, shared pathologies, or cellular pathways that are yet to be discovered. It is better understood that aspects of insulin resistance, oxidative stress, hepatic inflammation, and gut-derived endotoxins (i.e., LPS) symptomatic of NASH and type 2 diabetes are responsible for inducing HCC (Byrne and Targher, 2015). Specifically, the metabolic stress from these disorders induces stress-response pathways that utilize microRNA, PTEN (phosphatase and tensin homolog), one-carbon metabolism and NF-κB that seem to lead to HCC (Byrne and Targher, 2015). Furthermore, biological sex and genetics have unexplained effects evidenced by the 2.-2.5:1 ratio of HCC incidence and mortality between men and women and the clinical observation of the development of HCC at different stages of NASH in patients (Byrne and Targher, 2015; Marjot et al., 2019). Despite solid associations between HCC and the complications of NAFLD, NASH, and liver cirrhosis, the exact, shared cellular and physiological pathways demand further research.

Role of Thyroid Hormone

TH is an essential regulator of metabolism and generally promotes hepatic lipolysis and removal, lowering blood triglycerides (TAG) and LDL cholesterol (LDL-C) concentrations.

Interestingly, TH has been shown to have variable effects on lipogenesis that are still poorly understood (Sinha et al., 2019). Nonetheless, hypothyroidism is known to elevate blood concentrations of TAGs and LDL-C and causes increased adiposity that can directly contribute to NAFLD. Notably, TH metabolites, T2 and T1AM, and their analogs have demonstrated greater therapeutic effects on lipid metabolism than T4 and T3 (Sinha et al., 2019). Development of T2 and T1AM analogs that target the liver-specific TH receptor β bear great promise as a potential treatment for NAFLD and its complications.

Review of Recent Research into Potential Medications and Treatments

Intermittent Sympathetic Activation Attenuates NAFLD Symptoms

The stress response is essential to maintain physiological homeostasis due to internal and environmental stressors. Due to chronic exposure to the stress hormone corticosterone, with its role in aggravating liver disorder symptoms and triggering immunosuppression, chronic stress has been shown to promote hepatocyte injury and steatosis (Lee et al., 2019). Interestingly, some studies noted that adaptable stress showed promise in helping to reactivate the metabolism in metabolically compromised individuals. This study employed a murine model to observe the effects of intermittent restraint stress (induction of stress through physical restraints). A sample of 28 male mice was primed with a high-fat diet (HFD) to induce steatosis. Intermittent stress was shown to decrease body weight in both the non-HFD and HFD mice but only reduced serum cholesterol and TAGs in the HFD mice (Lee et al., 2019). Intermittent restraint stress was also shown not to increase blood corticosterone levels while substantially raising blood epinephrine (an effect further augmented in the HFD mice). The increase in blood epinephrine levels is proposed to drive increased lipolysis and reduced adiposity in HFD mice(Lee et al., 2019). This increase in epinephrine is not inhibited by the internal stress generated by prolonged corticosterone

elevations(Lee et al., 2019). Additional measurements of other inflammation biomarkers (such as TNF-α), hepatic adrenergic receptors, adipose tissue browning, and serum insulin further corroborate the conclusion that intermittent stress attenuates NAFLD's severe signs and symptoms (Lee et al., 2019). While the study results are robust, several factors must be considered. The use of only male mice only makes the study possibly generalizable to males, as NAFLD progression is significantly affected by the genetic and physiological distinctions between men and women (Lonardo et al., 2019). For example, estrogen plays a role in the upregulation of alpha-2-adrenergic receptors, which are antilipolytic in nature (Lonardo et al., 2019). Furthermore, considerations must be made regarding the differences in the experience of stress between mice and humans. Especially when discussing mental illness, the broader array of sources of psychological distress in humans compared to mice demands further research into potential differences in the stress responses of the two species. There are significant questions about what forms of stress may replicate the attenuation of NAFLD symptoms in humans.

Inulin Ameliorates Hepatic Pathology while Exacerbating Hypertriglyceridemia

Dietary fibers are carbohydrates that have been shown to reduce the risk of obesity through effects on energy intake, the intestinal microbiome, and intestinal barriers. Overall, limited studies have demonstrated the positive effects of dietary fiber on healthy and type-2-diabetic individuals in relation to glucose metabolism and cardiovascular pathologies (Kometsu et al., 2021). The inbred DalhS.Z-*Lepr*$^{+}$/*Lepr*$^{+}$ rat model was employed due to the induced leptin missense mutation that induced an array of metabolic disorders (associated with NAFLD). Inulin was selected as the only dietary fiber supplemented into a purified diet at 5% and 20% due to its effective absorption into the colon (Kometsu et al., 2021; Afinjuomo et al., 2021). Analysis of blood lipid concentrations revealed that fasting concentrations of total and non-HDL cholesterol were lower

in metabolically impaired rats on the high-fiber (high-inulin) diet compared to the rats on the control diet. Reduction of total cholesterol levels limits TAG export from tissues and exacerbates hypertriglyceridemia. Unfortunately, despite the high-fiber group's inhibition of adipose and liver inflammation, effects on insulin resistance were minimal. Overall, the researchers concluded that high dietary fiber (represented by inulin) ameliorates NAFLD development by attenuating lipogenesis and increasing VLDL secretion in metabolically compromised rats but is limited by the exacerbation of hypertriglyceridemia (Kometsu et al., 2021; Afinjuomo et al., 2021). While general recommendations of increased dietary fiber intake and its well-understood effects in combating obesity and type 2 diabetes can theoretically ameliorate NAFLD by reducing adiposity, this study does not provide sufficient evidence that general increases in dietary fiber can effectively and significantly reduce NAFLD symptoms. The use of only male rats presents the aforementioned issues, while the genetic induction of metabolic disorders may have additional effects that confound the resulting interactions with dietary fibers. Additionally, using a purified diet with a simple inulin supplement is not representative of a typical diet or dietary fiber consumption. Thus, further research must be conducted to observe how inulin and other dietary fibers may interact with other dietary components and in doing so, affect absorption and tissue penetration. Inulin's strong tissue penetration and potential as a drug delivery system make it easier to observe its effects in an experimental context, however it is not an appropriate representative of all other dietary fibers (Afinjuomo et al., 2021).

Dietary Ketone Ester Mitigates NAFLD Symptoms

Ketosis results from a high fat and protein and low carbohydrate diet. This type of diet is characterized by the lack of carbohydrate sources and the consumption of lipids in glucogenesis, which raises levels of ketone bodies in the blood and tissues. Nutritional ketosis is a proposed

therapy for obesity and NAFLD, in which ketone ester supplements are administered to effectively induce ketosis. Particularly, the administration of the dietary ketone R,S-1,3-butanediol diacetoacetate (BD-$AcAc_2$) was observed for its ability to induce ketosis and attenuate NASH markers and hepatic fibrosis (Moore et al., 2021). A male mouse model was used and primed with HFD to induce steatosis. After steatosis was induced, the mice were kept on the HFD or moved to a calorie-restricted diet or the (partial) dietary ketone ester replacement group. At the end of the eight-week study, steatosis, inflammation markers, and fibrosis were significantly attenuated in the ketone ester and calorie-restricted groups. The calorie-restricted group demonstrated less attenuation than the ketone-ester group. Interestingly, while overall proinflammatory marker concentrations decreased in the ketone ester group, the M1 proinflammatory protein CD11b and the M2 anti-inflammatory protein CD163 were significantly higher in the ketone ester group (Moore et al., 2021). The relative increase of CD163 compared to the calorie-restricted and control group was substantially greater than the CD11b and thus anti-inflammatory through the reduction of the M1:M2 Kupffer cell ratio. In total, the data strongly indicated that dietary ketosis did benefit mice with NAFLD. However, the longitudinal distinctions between the improvements of mice on the calorie-restricted diet and dietary ketone ester diet must be investigated to preclude potential issues that arise from dietary ketone ester replacement. Additionally, the long-term effectiveness of ketone ester dietary supplements compared to calorie restriction was not explored in this study and is essential in determining ketone ester supplements as valid treatments. Another issue that must be further researched is the increase in the M1 protein, CD11b, as a potential concern for patients taking dietary ketone esters. Finally, the most crucial concern that must be addressed is the purified diet of the mice. The diet of the mice across all experimental groups is not representative of the human diet and therefore raises questions as to how the nutritional profile of

a typical human diet may interact with ketone supplements and affect other systems. Consequently, nutritional ketosis cannot currently be recommended above diet and lifestyle changes until these studies are complete.

Conclusion

The liver is a critical metabolic organ responsible for several significant roles in carbohydrate and lipid metabolism. Hepatocytes act as the primary parenchymal cell of the liver and perform most of the metabolic and biochemical processes associated with the liver. In a fed state, the liver is responsible for carbohydrate and lipid metabolism and distribution throughout the body. Any excess carbohydrates and lipids are then converted to glycogen and triglycerides for later use. In a fasted state, the liver conducts glycogenolytic, gluconeogenic, and lipolytic pathways to meet the body's energy demands. In a healthy individual, these anabolic and catabolic pathways are regulated by insulin and TH to appropriately respond to meals, exertion, starvation, and other physiological conditions.

NAFLD is a progressive disease that compounds upon itself due to the concomitance and the adverse effects of obesity and type 2 diabetes. The increasing prevalence and progression of NAFLD and the severity of its later stages (i.e., NASH and hepatocellular carcinoma) ought to be of greater concern to the public. While general lifestyle and dietary changes can effectively treat some aspects of NAFLD and NASH, therapies for more acute and severe cases still require further research. At best, the most recent studies only propose treatments with limited scopes, timeframes, treatment windows, and generalizability. Few treatments offered in research can proceed to clinical stages and be made available to the public despite their urgent need. This study found that the best potential treatments should attempt to address the systemic precursors and aspects of steatohepatitis. Nonetheless, it appears that there is no evident prescription or therapy for NAFLD

better than exercise and diet, therefore indicating that improvements in treatment may require further studies in demographics and public health to address the issue of obesity at a social level.

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