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A theoretical study of lipid accumulation in the liver—implications for nonalcoholic fatty liver disease

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ABSTRACT

A hallmark of the nonalcoholic fatty liver disease is the accumulation of lipids. We developed a mathematical model of the hepatic lipid dynamics to simulate the fate of fatty acids in hepatocytes. Our model involves fatty acid uptake, lipid oxidation, and lipid export. It takes into account that storage of triacylglycerol within hepatocytes leads to cell enlargement reducing the sinusoids radius and impairing hepatic microcirculation. Thus oxygen supply is reduced, which impairs lipid oxidation. The analysis of our model revealed a bistable behavior (two stable steady states) of the system, in agreement with histological observations showing distinct areas of lipid accumulation in lobules. The first (healthy) state is characterized by intact lipid oxidation and a low amount of stored lipids. The second state in our model may correspond to the steatotic cell; it is marked by a high amount of stored lipids and a reduced lipid oxidation caused by impaired oxygen supply. Our model stresses the role of insufficient oxygen supply for the development of steatosis. We discuss implications of our results in regard to the experimental design aimed at exploring lipid metabolism reactions under steatotic conditions. Moreover, the model helps to understand the reversibility of lipid accumulation and predicts the reversible switch to show hysteresis. The system can switch from the steatotic state back to the healthy state by reduction of fatty acid uptake below the threshold at which steatosis started. The reversibility corresponds to the observation that caloric restriction can reduce the lipid content in the liver.

1. Introduction

The liver is the central organ of metabolism in higher animals including humans, it deals with the metabolism of carbohydrates and lipids and performs gluconeogenesis and ketogenesis in times of starvation. However, this fine-regulated metabolic system can be easily disrupted. The lifestyle, especially diet composition, in Western societies often leads to chronic inflammation [1] and an increased incidence of metabolic disorders attributable to diet (e.g. obesity, type II diabetes mellitus [2,3]). In this context, it is not surprising that a serious health problem in Western populations are liver diseases [4] such as the nonalcoholic fatty liver disease (NAFLD). NAFLD is a common chronic liver disease with prevalence between 3% and 24% in Western countries (reviewed in [5]). Understanding the underlying mechanisms of this disease is a key for the successful development of diagnostic markers and a successful therapy.

The primary form of NAFLD can be attributed to diet conditions [6], while the secondary form is caused by drugs [7]. A hallmark of NAFLD, regardless of the form, is the increased lipid accumulation in the cytosol of hepatocytes (steatosis) exceeding 5% of liver weight, this is generally used as the primary characteristic of this disease [8]. Besides lipid accumulation, NAFLD is often associated with insulin resistance (metabolic syndrome [9,10]), mitochondrial dysfunctions [11,12], and oxidative stress [13]. It has been found that aging does not influence the development of a fatty liver [14]. A simple steatosis is clinically innocuous but can progress to serious liver diseases including nonalcoholic steatohepatitis (NASH) and cirrhosis. Patients with end-stage liver diseases have an increased risk of death [15]. Lifestyle modifications, such as reduction or modification of the diet and increased physical activity, were shown to attenuate steatotic liver disease and are currently recommended to patients with simple steatosis (reviewed in [16] and [17]).

Steatosis in patients with diet-induced NAFLD is the result of an impaired lipid metabolism, mainly caused by the increased delivery of fatty acids to the liver (for summary see [18,19]). The detailed alterations of the hepatic lipid metabolism in patients with steatosis, however, are not well understood because of the complexity of liver metabolism and the interconnection between different pathways (e.g. gluconeogenesis and lipid metabolism; [18]). In this context, tools of mathematical modeling are a very useful instrument to explore the interconnection and regulation of liver metabolism. For example, a kinetic model of the

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tricarboxylic acid cycle (TCA cycle) and closely related metabolism was used to determine the importance of different regulatory enzymes [20]. More recently, modeling of the hepatic carbohydrate and lipid metabolism [21] was used to predict metabolite concentrations and fluxes under different perfusion mediums. Kaleta et al. [22] have established a network model to show that gluconeogenesis from fatty acids is, in principle, feasible. With these models the relationship between carbohydrate and lipid metabolism can be investigated.

Here, we will apply mathematical modeling of biochemical systems [23] to study the development of steatosis under a high-fat diet. We focus on simple steatosis and exclude inflammatory phases of NAFLD and cirrhosis. Ijaz et al. [24] stated that lipid accumulation in hepatocytes must be the result of an imbalance between fatty acid oxidation and triacylglycerol synthesis and secretion. This reasoning is the basis of our model. We hypothesize (1) that the contribution of different pathways in lipid metabolism is altered in the healthy and the diseased state (NAFLD) and (2) that the amount of stored lipids and, thus, the volume of hepatocyte cells does trigger the shift between pathways. We further point out (3) that a major factor is the change in the availability of oxygen because the small blood vessels in the liver lobules (sinusoids) are compressed by the swelling of hepatocytes. A mathematical model of the hepatic lipid dynamics should be helpful to understand the importance of each pathway in the healthy and diseased livers.

2. Theory

2.1. Physiological background of the model

The delivery of oxygen to the liver affects hepatic metabolism due to the importance of oxygen in mitochondrial oxidative phosphorylation. It has been shown that hepatic oxygen consumption is constant over a wide range of oxygen delivery values ("oxygen supply independence"), but below a critical threshold value of oxygen delivery the consumption decreases proportionally; this is called "oxygen supply dependence" (see [25]). This relationship between oxygen delivery and oxygen consumption plays an important role for several hepatic processes, e.g. for the NAD redox state [26], for a shift between lactate consumption and production [27], and for galactose elimination [28].

"Oxygen supply dependence" may also play an important role in liver diseases. Mantena et al. [29] reported increased hypoxia in the liver of mice fed a high-fat diet and they concluded that hypoxia may play a critical role in liver diseases associated with steatosis. In the state of "oxygen supply dependence" the liver suffers from oxygen paucity which can have fatal consequences for liver functioning and cell apoptosis. Indeed, it was shown that decreased oxygen availability can switch the oxidative phosphorylation pathway from a low to a high reactive oxygen species (ROS) production state; this high production state sustains even if the availability of oxygen increases again (hysteresis, [30]). Thus, under hypoxic conditions a high amount of ROS is produced, causing cell damage and lipid peroxidation. In addition, steatosis is associated with an increased activation of hypoxia-inducible transcription factors, which stimulate lipid storage and inhibit degradation of lipids [31,32].

The reduction of oxygen consumption under steatotic conditions can be explained by the influence of hepatocyte swelling on the hepatic microcirculation: An excess of fatty acids is directed to the synthesis of triacylglycerol (TAG), which is partly stored in lipid droplets within the cytosol of hepatocytes. The increased storage causes a swelling of hepatocytes [33–35] and this cell enlargement compresses the hepatic sinusoids space (Fig. 1, [24,33,35]). This compression impairs hepatic microcirculation [35–37] leading to reduced oxygen supply to hepatocytes [35].

The impairment of hepatic lipid metabolism under reduced oxygen availability (Fig. 1; [24]) in the steatotic liver is likely mediated by the mitochondrial oxidative phosphorylation pathway. Uptake of fatty acids in hepatocytes stimulates fatty acid degradation pathways such as β-oxidation and TCA cycle and this increased activity can exceed the capacity of the respiratory chain and, consequently, NADH accumulates. NADH fluorescence studies confirmed this assumption by showing a higher level of NADH in hepatocytes of obese than of lean animals [35,36]. In turn, a lack of reducing agents (NAD+) inhibits the activity of β-oxidation and TCA cycle and, consequently, fatty acids cannot be catabolized and are directed to TAG synthesis. A reduced rate of the respiratory chain under hypoxic conditions is supported by the observation that patients with NAFLD feature mitochondrial dysfunctions [29,38,39]. Vial et al. [39] showed that under a high fat diet the consumption rate of oxygen was lower, the mitochondrial redox state was more reduced (lower and more reduced quinone pool 9) and the activity of β-oxidation was depressed. In accordance with these studies, it has been reported that an inhibition of the TCA cycle [40] or the oxidation of fatty acids [41] leads to steatosis.

In conclusion, two mechanisms can be found out to be involved in the development of a fatty liver (Fig. 1):

(1) An overload with fatty acids may exceed the capacity of the pathways of β-oxidation, TCA cycle, and respiratory chain and, thus, fatty acids are directed to the synthesis of TAG [42]. The storage of

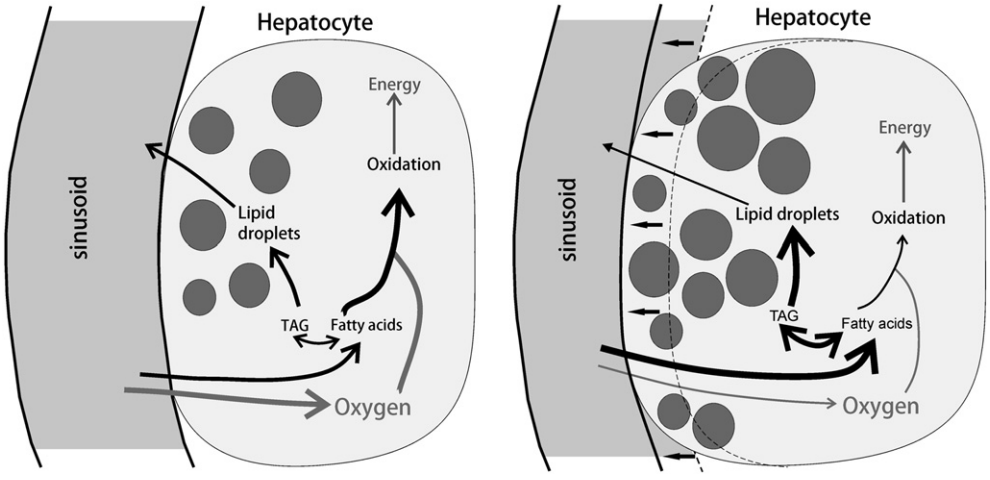

Fig. 1. Degradation and synthesis of triacylglycerol (TAG) under normal feeding conditions (left) and under conditions of increased fatty acid uptake (right). For simplicity's sake, the space of Disse, lying in between hepatocytes and sinusoids, is neglected. Increased fatty acid delivery to the hepatocyte exceeds the capacity of degradation and the overload is directed to TAG synthesis. Synthesized TAG can either be exported via very-low-density-lipoproteins or stored in lipid droplets within the cytosol of the hepatocyte, causing swelling of the cell. This swelling compresses the sinusoid leading to a reduced blood flow and, thus, a decreased oxygen supply. The reduced oxygen availability impairs lipid oxidation.