Lipid Metabolism and Liver Inflammation.

I. Hepatic fatty acid uptake: possible role in steatosis

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Hepatic steatosis, or the presence of significant amounts of triglyceride (TG) in hepatocytes, was long thought to be mainly a symptom of alcoholic liver disease. In recent years, however, steatosis has been found in the absence of alcohol abuse and led to the definition of a series of disorders ranging from nonalcoholic fatty liver (NAFL) to nonalcoholic steatohepatitis (NASH) (18). These conditions are being diagnosed more frequently, and NASH is the primary diagnosis of liver failure in a growing number of liver transplant patients. Hepatic steatosis is becoming a significant public health concern in Western societies.

Hepatic steatosis may progress to steatohepatitis, which leads to fibrosis and cirrhosis. This progression may be due to damage caused by lipid peroxidation and the production of reactive oxygen species. A necessary component of excess lipid peroxidation is excess substrate, the lipid stored as TG in the hepatocytes. The possible sources of this lipid must be considered and has been the focus of many investigations. These sources include lipolysis of TGs, dietary fat, and the liver's own TG stores. The hepatocyte membrane may be of key importance to the initiation of steatosis.

Lipid Flux in the Hepatocyte

The liver is a major regulator of metabolite flow in the body, receiving input from the intestine via the portal vein, the general circulation via the hepatic artery, and the lymphatic system. Hepatocytes remove many materials from circulation and release them or their products at a moderated rate. This suggests that the hepatocyte must have a high capacity for the removal of various materials from the circulation, much higher than is necessary if all fuels were present at a steady-state concentration. This capacity for uptake of materials, especially lipids, may be of key importance to the initiation of steatosis.

Lipids arrive at the hepatocyte surface in a number of forms (Fig. 1). Both FFA and lipoprotein particles provide lipids to the liver. Although some lipoprotein particles are taken up by receptor-mediated endocytosis (17), in others, the TGs may be broken down by hepatic lipase (HL), to produce FFA, which cross the hepatocyte membrane by a combination of facilitated transport and diffusion (1).

In hepatocytes, the FFA may be converted to TGs or oxidized as fuel. Mitochondrial β-oxidation of FFA will produce both energy for the cell and ketone bodies. Some may be used in the synthesis of phospholipids and mediators such as prostaglandins and leukotrienes, and parts of the carbon skeletons may be used to synthesize glucose, cholesterol, and other compounds. FFA may also be converted to TGs, which can be used for production of VLDL particles for export. The amount that can be exported will depend on synthesis of the protein components, as well as the availability of TGs, and excess TG may be stored in lipid droplets.

An important source of lipid for the hepatocyte is circulating FFAs. The various sources of these fatty acids include lipolysis of stored TGs in adipocytes and dietary fat. These hydrophobic substances travel in the circulation bound to albumin, which increases the concentration of FFA in the serum and allows them to carry and maintains a very low unbound concentration. The importance of FFAs may lie in the fact that their concentration in plasma is often significantly increased in the various disorders associated with hepatic steatosis. Once in hepatocytes, the fate of FFA is the same as that outlined above. However, physiological levels obviously can meet the FFA requirements for all of these processes, as is evident in normal healthy individuals.

Any excess FFA must be dealt with in another manner. Some may be transported out of cells by the same mechanisms that allow for uptake, but this is likely a minor fraction, because FFA are generally modified by fatty acyl-CoA synthetases almost immediately on entry into cells and thus are unavailable for transport or diffusion. The majority of excess FFA is likely converted to TG, which may be stored.

Fatty Acid Uptake by Hepatocytes

Because the plasma membrane is a lipid bilayer, many have argued that fatty acids would diffuse through the membrane at a rate sufficient to supply the needs of the cell (12). Experimental determinations of the rate of diffusion, or “flip-flop” of fatty acids between the two leaflets of the membrane, have often yielded very high rates of diffusion, obviating the need for specific transporters (12). However, these experiments were generally done using synthetic vesicles in the absence of...

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albumin, so the unbound FFA concentration is much higher than in physiological conditions. Furthermore, the rate of flip-flop appeared to be inversely related to the radius of the vesicles (1), suggesting that the radius of curvature is a determining factor. Because the vesicles were much smaller than cells, these higher rates would not be representative of what would be seen in cells.

Recently, an investigation to compare diffusion and facilitated transport found an unusual anomaly (8). When flip-flop was measured in the standard manner, the rate of diffusion was found to be dependent on the concentration of vesicles (1), suggesting that the radius of curvature is a determining factor. Because the vesicles were much smaller than cells, these higher rates would not be representative of what would be seen in cells.

Fig. 1. Free fatty acid (FFA) metabolism and transport. In top panel, the transport and metabolism of FFA is discussed. Lipoprotein lipase (LPL) on adipocytes releases FFA from lipoprotein particles (small circles). The FFA is transported into the adipocyte and esterified to triglyceride (TG) for storage. Stored TG undergoes lipolysis by hormone-sensitive lipase (HSL) to produce FFA for export into the plasma. At the hepatocyte, hepatic lipase (HL) produces FFA from lipoproteins, which is transported into the hepatocytes along with plasma FFA. In the hepatocyte, FFA may undergo oxidation or be esterified to TG, some of which may be stored in lipid droplets (small white circles) for later use. In the bottom panel, changes due to insulin resistance are shown. Changes in font sizes and arrow thickness indicate increased or decreased activities. LPL expression decreases, so fewer FFA are derived from lipoproteins, but the FFA uptake rate may be increased. TG stores are larger, and HSL activity is increased, resulting in increased export of FFA to the plasma. Along with increased HL activity, this increases the amount of FFA available for uptake into hepatocytes, allowing for increased oxidation and esterification to TG. The excess TG is stored in larger and more abundant lipid droplets, resulting in steatosis.

In Fig. 2, the contributions of the various kinetic constants to uptake are illustrated. Starting the same values for \( V_{\text{max}} \), \( K_m \), and \( k \) as are used in Fig. 2, each is doubled while leaving the other two at the initial value. As can be seen, a doubling of \( k \) has a significant effect on the rate of uptake only at higher FFA concentrations. An increase in \( V_{\text{max}} \) has the greatest effect. An increase in \( V_{\text{max}} \) would be seen when the number of transporter molecules is increased. This type of increase has been seen in

![Diagram of Fatty Acid Uptake](http://ajpgi.physiology.org/)
hepatoma cells exposed to ethanol (25) and in adipocytes of obese rodents (2, 3) and humans (20). A number of putative fatty acid transporters has been identified. The first, plasma membrane fatty acid binding protein, has been shown to be identical to mitochondrial aspartate aminotransferase (AST) (4). The enzymatic activity contributes to serum AST levels measured in liver function tests, and increased expression may lead to increased FFA uptake (16, 25) and export of enzyme from hepatocytes (25). Fatty acid translocase (FAT) is another putative transporter that is also known as CD36, which binds lipoproteins (15). The fatty acid transport polypeptide (FATP) family of genes (13) has also been implicated in FFA uptake.

These figures are based on the albumin binding constants derived by Spector et al. (23), which have been called into question by more recent studies. Because the Spector constants have been used in many studies, they allow for comparison of many data sets. The use of different constants will not change the shape of the curves, because they reflect unbound concentration, but what will change is the placement of the data points for a given total FFA concentration. With the use of the newer binding constants described by Richeri et al. (22), for example, the values used here, 0.45, 0.9, and 1.8 mM, will give values of ~5.1, 10.7, and 33.3 nM unbound FFA and 0.15, 0.3, and 0.78 pmol·s⁻¹·50,000 cells⁻¹ for uptake. Although the unbound concentrations and rates of uptake appear substantially reduced, this also has the effect of placing all of the values in the portion of the curve where uptake is almost entirely due to saturable transport; so any change in FFA concentration will have a large effect on the uptake rate. Thus any prolonged period of increased plasma FFA concentration will cause a substantial increase in the amount of FFA entering the hepatocyte and potentially being stored as TG.

Thus the total uptake of FFA by hepatocytes depends on both the concentration of FFA in plasma and the capacity of the cells for FFA uptake. To use a nonbiological analogy, how much FFA flows into the cell depends on both the pressure differential (due to external FFA concentration) and the size of the pipe (the capacity of the uptake system). The capacity can be increased somewhat by increased diffusion, such as by increased cell surface area or alterations in membrane composition. However, it would be more efficient to alter the saturable process, which has a greater capacity for uptake, by increasing the number of transporter molecules. Upregulation of one or a few genes (2, 3, 5, 25) should be inherently more responsive to external forces than altering membrane composition. Conversely, should a transporter be upregulated inappropriately, it could lead to increased hepatocyte FFA uptake and contribute to steatosis and subsequent clinical problems. Such is hypothesized to be the case in alcoholic steatosis, where it can be demonstrated that a putative transporter is upregulated by ethanol exposure (21, 25). However, in nonalcoholic steatosis, the cause is more likely an increase in FFA concentration rather than induction of a transporter, although such induction cannot be ruled out in all cases where serum AST levels are sometimes increased (6).

**Sources of Elevated Plasma FFA**

One obvious source of increased plasma FFA is dietary. Excessive fat in the diet, common in Western countries, will lead to increased plasma concentrations of TGs and FFA. The increased FFA concentration will allow for increased uptake into hepatocytes, as will increased TGs, whether through the action of lipases to produce FFA from TG and lipoproteins, increased uptake of chylomicron remnants, or both. However, the definition of excessive must be considered. Because FFA are a source of energy, it would be feasible, if not desirable, to obtain all the calories necessary for metabolism from fat. However, excessive fat can be defined as fat in excess of caloric needs, and that is all too common. Furthermore, because carbohydrates and proteins can serve as sources of material for synthesis of fatty acids, excess caloric intake per se is another source of concern. The result of such intake is an increase in adiposity, and eventual obesity. Hepatic steatosis can occur in the presence of obesity, but there are other related disorders that appear to increase the risk substantially.

These disorders, which often occur in obese individuals, are type II diabetes and the metabolic syndrome, which may be part of a continuum that includes obesity. Type II diabetes usually occurs in a setting of obesity but with the added problem on insulin insensitivity or insulin resistance. Peripheral tissues fail to respond to insulin in a normal manner, leading to lowered utilization of glucose as an energy source. This increases plasma glucose and increases the amount available for other metabolic processes, including fatty acid synthesis. What may be more significant is the effect on the adipocyte. Insulin is normally a stimulus for adipose tissue to store fat. Insulin has the dual effects of stimulating adipocyte lipoprotein lipase (LPL), increasing the capacity of adipocytes to break down TGs in lipoprotein particles to FFA for uptake and storage as TGs. Insulin also decreases the activity of hormone sensitive lipase (HSL), which converts stored TG to FFA and glycerol for export. The net effect of insulin on adipocytes is normally to take up more FFA from lipoproteins and to export less FFA. Insulin resistance depresses both of these functions, as shown in Fig. 1.

The result of insulin resistance in adipocytes is lowered conversion of lipoprotein TGs to FFA for uptake and increased
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HSL activity to break down stored TGs for export. In truth, this might be beneficial to the adipocyte, because it occurs when adipose tissue is storing excessive amounts of fat. However, there are other effects on both obese adipocytes and the body in general that are less than beneficial. As to the adipocytes themselves, those from obese rodents (2, 3) and humans (20) have been shown to have an increased capacity for FFA uptake. Thus, although there are less FFA available due to decreased LPL activity, the cells have a greater capacity to transport what is there. Also, increased lipolysis leads to increased FFA export into the circulation. Increased uptake will cause some to be returned to adipocytes, but the plasma FFA concentration will rise. This increased concentration may be maintained by the change in adipocyte physiology, because the system will be unresponsive to insulin and there is substantial substrate for lipolysis stored by the time insulin resistance is observed. This may lead to a more constant rate of lipolysis and continually elevated plasma FFA levels. Thus, not only are hepatocytes exposed to more FFA for uptake, but they are exposed for longer periods of time. The net result will be a substantial increase in FFA flux across the hepatocyte membrane.

Another group of pathological conditions that have been seen to be associated with hepatic steatosis is the dyslipidemias. Conditions such as lipodystrophy, where adipose depots are severely reduced, or absent, prevent the normal storage of fat in adipocytes. Therefore, the plasma will have elevated levels of FFA and TG and the liver will be one organ capable of removing them from circulation. Indeed, any condition that abnormally elevates plasma FFA and TG levels appears to predispose a person to hepatic steatosis, including rapid weight loss. As FFA are released from adipocytes by lipolysis, they will pass through the liver via either the portal or general circulation and may be removed by the hepatocytes. Because uptake rate is dependent on FFA concentration, any condition that chronically elevates plasma FFA concentrations will lead to increased uptake of FFA by hepatocytes.

The question arises as to whether such an increased influx can result in enough of an increase in FFA in the hepatocyte to produce steatosis. Because the units of uptake used here are picomoles per second per 50,000 cells and there are 86,400 s in a day, then a single cell will take in >1.7 pmol/day for every “unit” of uptake measured. A fat droplet of 10-μm diameter, large in a typical hepatocyte, would have a volume of 524 fl and a mass of 482 pg using a density of 0.92 g/ml for fat. Oleate, a common fatty acid in the body, has a molecular weight of 282.5; so 1.7 pmol oleate would be sufficient for such a droplet. Thus an increase in uptake by 1 U over the course of 1 day, or 0.1 U for 10 days, would provide sufficient excess fatty acid to produce a fat droplet of significant size. Even with a substantial overcapacity for fat metabolism and/or export over that needed for optimal functioning, long-term elevation of plasma FFA concentrations may easily lead to fat accumulation in hepatocytes.

FFA LEVELS IN NAFLD AND NASH

The question arises whether patients with hepatic steatosis show evidence of altered plasma FFA levels. After all, patients may not present until their disease is advanced and may be in a very different physiological state than during earlier stages of progression. Some studies have addressed these matters. In an examination of patients with NAFLD, Donnelly et al. (10) found that plasma FFA were significantly elevated in patients compared with controls. Furthermore, their studies indicated that plasma FFA were the primary source for production of hepatic TGs, with an estimated 59% of the lipid coming from FFA. This indicates that not only are plasma FFA increased, but that they are important in the hepatic TG load. Patients with NASH have also been studied. It has been shown (9) that patients had higher plasma FFA concentrations and slightly different proportions of various FFA present in plasma compared with controls.

ANIMAL MODEL STUDIES

Animal models have also shown that increases in FFA levels correlate with hepatic steatosis. In a study using rats, a high-fat diet increased plasma FFA significantly, along with hepatic TG and steatosis, and all of these alterations were decreased by exercise (11). Dietary supplementation with a specific conjugated linoleic acid (7) causes decreases in adipose tissue mass with significant hepatic steatosis and upregulation of genes including peroxisome proliferator-activated receptor γ and FAT, a putative FFA transporter, which may increase uptake of the FFA no longer being stored in adipose tissue. Overexpression of one form of sterol-responsive element binding protein in adipose tissue produced increased adipose fatty acid synthesis and secretion (14), resulting in increased plasma FFA and hepatic steatosis. Transgenic mice overexpressing human ApoC1 on a leptin-deficient ob/ob background (19) show decreased adipose tissue, increased plasma FFA, and hepatic FFA uptake, with hepatic steatosis and insulin resistance. Mice lacking hormone-sensitive lipase, in contrast, have lowered plasma FFA and no steatosis (24). They also show increased hepatic insulin sensitivity, indicating that hepatic TG stores may be a factor in insulin resistance, which could exacerbate the situation in type II diabetes and the metabolic syndrome.

In conclusion, it is apparent that hepatocytes possess the capacity to take in excess FFA and that the amount in the cell depends on numerous factors, including plasma FFA levels. Elevated plasma levels of FFA may not necessarily cause steatosis but, in concert with other disruptions commonly found with increased FFA concentration, may play an important role in hepatic steatosis. In general, glucose and/or TG levels may be increased along with FFA levels. Not only do these materials provide more substrate for TG synthesis in hepatocytes, but they provide fuel to other tissues as well, reducing the demand on hepatocytes to provide energy by export of glucose, ketone bodies, and other metabolites. Lowering the demand for energy export produces a positive energy balance in the hepatocyte, which has the net effect of TG storage. Unlike glycogen, which has a limit on the amount stored in hepatocytes, lipid may accumulate in droplets that may grow in size to a significant fraction of the cytoplasmic volume. The lack of a physiological limit on this accumulation may seem an unsound evolutionary strategy, but this point in time is the first one where any selective disadvantage might be evident. A primitive diet generally was much closer to starvation than those today that can produce significant numbers of obese individuals. The growing epidemic of obesity, diabetes, and the metabolic syndrome could theoretically exert selection...
pressure on any number of physiological systems to compensate, including hepatic fatty acid uptake mechanisms.

Selection pressure on the phenotype, however, is not a solution to the immediate problem. The real pressure is on scientists and society to find solutions for patients. Although the old remedies of diet and exercise would go a long way to alleviating many of these problems, some cases are resistant to such interventions or are already too far advanced, resulting in pathological changes that will not easily be reversed. Prevention is always preferable, and if methods were available to limit fat accumulation by the liver, the number of NAFL and NASH cases might decrease substantially. One possible method of decreasing hepatic steatosis lies in decreasing hepatic FFA accumulation by the liver, the number of NAFL and NASH cases might decrease substantially. One possible method of decreasing hepatic steatosis lies in decreasing hepatic FFA uptake. Better understanding of the mechanisms and consequences of excessive FFA uptake could yield both important scientific data and possible avenues of therapy and prevention.

REFERENCES