From whole body to cellular models of hepatic triglyceride metabolism: man has got to know his limitations

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Green CJ, Pramfalk C, Morten KJ, Hodson L. From whole body to cellular models of hepatic triglyceride metabolism: man has got to know his limitations. Am J Physiol Endocrinol Metab 308: E1–E20, 2015. First published October 28, 2014; doi:10.1152/ajpendo.00192.2014.—The liver is a main metabolic organ in the human body and carries out a vital role in lipid metabolism. Nonalcoholic fatty liver disease (NAFLD) is one of the most common liver diseases, encompassing a spectrum of conditions from simple fatty liver (hepatic steatosis) through to cirrhosis. Although obesity is a known risk factor for hepatic steatosis, it remains unclear what factor(s) is/are responsible for the primary event leading to retention of intrahepatocellular fat. Studying hepatic processes and the etiology and progression of disease in vivo in humans is challenging, not least as NAFLD may take years to develop. We present here a review of experimental models and approaches that have been used to assess liver triglyceride metabolism and discuss their usefulness in helping to understand the aetiology and development of NAFLD.

hepatocyte; NAFLD; fatty acid; triglyceride

THE LIVER IS THE CHIEF METABOLIC visceral organ that performs over 500 different functions (168), including: metabolism of lipids, carbohydrates, and vitamins, detoxification of foreign and metabolic poisons, and production and secretion of vital substances such as bile, albumin, fibrinogen, and globulin (209). The liver lobule is composed of parenchymal cells (hepatocytes) and nonparenchymal cells (Kupffer cells, hepatic stellate cells, and sinusoidal endothelial cells), with the latter localized in the sinusoidal compartment of the tissue (114). Hepatocytes occupy ~80% of total liver volume, and their use as in vitro models to explore aspects of liver function and metabolism has escalated in recent years. Primary hepatocytes are considered the “gold standard” in vitro model, as they appear to have the closest resemblance to the in vivo liver. However, they do not come without challenges; thus, a variety of other in vitro liver models have been utilized, including human and rodent liver cell lines.

Diseases of the liver are a global public health issue and burden (77). Nonalcoholic fatty liver disease (NAFLD) is one of the most common liver diseases in developed countries (77) in individuals who do not consume significant amounts of alcohol, that is, greater than 30 and 20 g of alcohol daily for men and women, respectively (29, 169). A well-documented risk factor for NAFLD is obesity (29). Depending on the assessment method used and the ethnicity of the cohort studied, it has been estimated that between 6 and 51% of the population have NAFLD (29, 156, 169); prevalence is reported to be higher (~70%) in individuals with type 2 diabetes (25). NAFLD represents a spectrum of conditions ranging from simple fatty liver (hepatic steatosis), where intracellular lipid exceeds 5% of the hepatic tissue (56, 203), more severe steatosis coupled with marked inflammation [nonalcoholic steatohepatitis (NASH)] through to severe liver diseases such as cirrhosis and potentially hepatocellular carcinoma (HCC) (25, 29, 32, 156, 169).

The hallmark of hepatic steatosis is a net retention of lipids, particularly triglyceride (TG) but also cholesteryl esters, within the liver (3), and the factors influencing the partitioning of fatty acids between esterification and oxidation pathways may play an important role. When fatty acids enter the hepatocyte, they are rapidly “activated” by acyl-CoA synthases to form fatty acyl-CoAs, which may enter either esterification or oxidation pathways. Fatty acids may come from exogenous sources or be produced endogenously via the de novo lipogenesis (DNL) pathway, which occurs within the cytoplasm via a series of sequential steps. Briefly acetyl-CoA is synthesized to malonyl-CoA via acetyl-CoA carboxylases (ACC), and then the fatty acid synthase (FAS) complex mediates the synthesis of saturated long-chain fatty acids (200). The initiation of TG synthesis is the esterification of fatty acyl-CoA with glycerol 3-phosphate [via glycerol-3-phosphate acyltransferases (GPAT)]. TG formation is governed by a series of reactions in the Kennedy pathway that involve acylglycerophosphate acyltransferase (AGPAT), phosphatidic acid phosphohydrolase (PAP), and diacylglycerol acyltransferases (DGATs). There are two DGATs, both of which are abundantly expressed within hepatocytes: DGAT1 esterifies exogenous fatty acids, and DGAT2 incorporates endogenous fatty acids (200, 244). TG enters secretory or storage pools, which have distinct rates of
turnover. The formation and metabolism of lipid droplets within hepatocytes has been comprehensively reviewed (200). The lipases responsible for TG hydrolysis from the storage pool are suggested to be TG hydrolase (71) and arylacetamide deacetylase (AADA) (225), but adipose triglyceride lipase (ATGL) and hormone-sensitive lipase (HSL) may also play a role (192).

TG in the secretory pool is utilized in very-low-density lipoprotein (VLDL) assembly and requires interaction with microsomal triglyceride transfer protein (MTP) for lipids to be added to the particle (177). The assembly of VLDL has been well reviewed and is highly dependent on the amount of TG in the liver, the expression of enzymes [e.g., DGATs, MTP, and secretory proteins such as apolipoprotein (apo)B] along with fatty acid availability and insulin (70, 174, 177, 245). For fatty acyl-CoA to enter the mitochondria, it is transported by carnitine palmitoyltransferase-1 (CPT1) (98). Within the mitochondrion, β-oxidation produces acetyl-CoA, which may enter either the ketogenic pathway or the tricarboxylic acid (TCA) cycle for oxidation to CO₂ (98). The ketone body 3-hydroxybutyrate (3OHB) is often used as a surrogate marker of hepatic fatty acid oxidation (98).

To date, work has been undertaken in a variety of models to understand the development and progression of NAFLD. In this review we discuss in vivo and in vitro models that have been used to assess the development of NAFLD, with a specific focus on models that report intrahepatic TG metabolism and fatty acid oxidation to investigate hepatic steatosis and discuss their usefulness and potential application in providing insight into the etiology and development of NAFLD. Although hepatic steatosis is often thought of as being the histological hallmark of NAFLD, it may also occur with other liver diseases, including alcoholic liver disease, viral hepatitis, cystic fibrosis liver disease, human immunodeficiency virus, and genetic lipodystrophies along with being a manifestation of drug toxicity (191); although interesting, these models will not be discussed as they are outside the scope of this review.

IN VIVO MODELS

Studying the initiation, development, and progression of hepatic steatosis using in vivo models is challenging to say the least, as it may take years to occur, and the primary events may not be well reflected or are difficult to define and/or measure. In addition, it is likely that a combination of other factors, including genetic susceptibility, over- and inappropriate nutrition, and a sedentary lifestyle may also play a role. Therefore, a variety of approaches have been taken, using human and animal in vivo models, to try to elucidate how alterations in liver fatty acid metabolism may lead to development of hepatic steatosis.

Human In Vivo Studies

Fatty acids within the liver are derived from multiple sources, and their removal occurs by two processes; one is secretion as VLDL-TG, and the other is oxidation (97, 98). It has been proposed that the liver stores TG to accommodate fatty acids that have accumulated in excess of requirement. Thus, accumulation of intrahepatocellular lipid represents an imbalance between input and removal pathways. The factors proposed to influence the development of NAFLD have been well reviewed (6, 12, 19, 25, 26, 98, 110, 127, 156, 162, 163, 207, 222, 242).

Although the amount of TG stored within an individual’s liver can be determined via imaging modalities or a liver biopsy (56), little insight is gained about mechanisms involved in the development or progression of the disease. Histological examination of a liver biopsy provides quantitative and qualitative information (50), as the proportion of hepatocytes containing lipid droplets and the size of the lipid droplets (i.e., steatotic pattern) can be determined. The steatotic pattern is either high-grade microsteatosis or combined macromicrosteatosis; there is no pure macrosteatosis (51). Microsteatosis consists of fatty microvesicles measuring less than 1 μm filling the hepatocyte cytoplasm, where the nucleus remains centrally located. In contrast, macrosteatosis is when hepatocytes contain a single, large vacuole of fat, which displaces the nucleus to the periphery of the cell (51). The factors regulating the development and progression of the steatotic pattern and how this may influence or is influenced by intrahepatic fatty acid partitioning have yet to be elucidated.

A variety of different approaches have been taken to try to elucidate the mechanisms involved in the development of hepatic steatosis. Ideally, liver fatty acid metabolism would be determined directly, which could be best achieved by arteriovenous difference measurements; few studies have employed this methodology, and even fewer have focused on subjects with NAFLD. Havel et al. (89), using radioabeled tracers, reported on the transport of free fatty acids from the blood into the splanchnic region and their conversion to VLDL-TG, together with estimates of splanchnic oxidation of free fatty acids to ketones, carbon dioxide, and water in normolipidemic and dyslipidemic individuals after a prolonged (18-h) fast. More recently, Westerbacka et al. (236) reported the splanchnic flux of free fatty acids, glycerol, ketone body, and TG in nine individuals with varying degrees of steatosis in the fasting state and during a low-dose insulin infusion; it is difficult to draw conclusions regarding differences in metabolism with increasing liver fat due to the small number of participants and no comparison group. Aside from being invasive, using this methodology requires specialist skills and equipment; thus, using arteriovenous difference methodology across the splanchnic bed is impracticable to do on a large scale or repeatedly over time in individuals. An alternative approach was taken by Iozzo et al. (106) who directly assessed hepatic fatty acid metabolism using positron emission tomography (PET) in control and obese subjects. They found hepatic fatty acid oxidation to be significantly higher in obese than in control subjects (106). To our knowledge, similar studies have not yet been undertaken in individuals with and without NAFLD.

More recently, a number of studies have used stable-isotope tracers to “label” different fatty acid sources that contribute to the hepatic fatty acid pool. Stable-isotope tracers offer the opportunity to probe TG metabolism in subjects with and without NAFLD in the fasting (57, 58, 126, 217) and postprandial (44, 126) states. Using a multiple-tracer approach in subjects with NAFLD, Donnelly et al. (44) demonstrated that the contribution of fatty acid sources to liver and VLDL-TG was very comparable; these data provide support for the use of VLDL-TG as a surrogate marker of the liver TG pool. Fabbri et al. (57, 58) demonstrated that individuals with NAFLD have...
the role of PNPLA3 in liver TG accumulation, the exact demonstrated to be strongly associated with liver fat content; of note this is the only gene consistently recently (5, 100). In 2008, Romeo et al. (195) reported a single genetic modifiers; the genetics of NAFLD have been reviewed result of exposure to environmental factors acting on specific individuals undergoing bariatric surgery, as they may have been on appropriate control group appears to be challenging. Consideration with control subjects (108). Obtaining samples from an appro-

Another approach has been to compare gene expression in liver biopsies taken from individuals with and without NAFLD (42, 49, 117, 159, 160). Liver tissue is often obtained from either individuals undergoing surgery for suspected NAFLD (42, 117, 159, 160) or those undergoing bariatric or abdominal surgery (49). These studies have reported that genes involved in the lipogenic pathway, such as ACC, FAS, stearoyl-CoA desaturase (SCD), and carbohydrate-responsive element-bind-

ANIMAL MODELS

As the development of NAFLD is likely to be multifactorial, animal models are often utilized, as specific factors that may influence initiation and/or progression can be examined in a controlled setting over a short time frame (6, 111, 140, 203, 218). An ideal animal model should reflect the histopathology and pathophysiology of human-related liver lipid metabolism; no single animal model, at this point in time, appears to display these attributes (6, 111, 140, 203, 218). The use of animal models to study NAFLD has been extensively reviewed (6, 93, 108, 123, 140, 165, 167, 203, 218).

Typically, rodent models have been utilized to investigate hepatic TG metabolism and NAFLD. Anstee and Goldin (6) noted that mouse models have been widely adopted as there are many standardized and well-characterized inbred strains that allow factors such as genetic heterogeneity, sex, and dietary variation to be eliminated. An important consideration should be the background strain used when utilizing a murine model. For example, C57BL/6 mice have a higher liver TG content than 129S6/SvEvTac mice when on a chow diet; this difference is exacerbated when the mice are placed on a high-fat diet (HFD) (18). Cohen et al. (33) reported that lean (ab/ab) and obese (ab/ab/ob/ob) asebia mice have significantly lower levels of liver TG than ob/ob mice. Notably asebia mice carry mutations in SCD1, the enzyme that catalyzes the biosynthesis of monounsaturated fatty acids, which may play an important role in hepatic TG synthesis (96).

Both genetic and environmental rodent models have been used to delineate many aspects of NAFLD. Given that only a small proportion of individuals acquire NAFLD due to genetic reasons, the use of dietary models to induce changes in liver TG could be considered a more relevant approach. When translating between species, consideration needs to be made for the habitual diet, which varies greatly. For example, mice on a chow diet have a low-fat intake (4% fat by weight) (187), whereas humans typically consume ~35% total energy (TE) as fat (189); therefore, the contribution of DNL fatty acids may be
of greater importance for TG production (96) and steatosis development in animals than that observed in humans (44).

To induce steatosis, a variety of dietary regimens have been used, including a methionine-choline-deficient (MCD) diet. This diet depletes the liver of antioxidants and is typically used to induce NASH; however, as part of this process steatosis may develop (107, 193). Rinella et al. (193) fed db/db, insulin-sensitive heterozygote db/m, and wild-type mice a MCD or control diet for 4 wk. Higher amounts of liver TG were noted in animals fed the MCD compared with the control diet, which was due to greater hepatic uptake of fatty acids and decreased VLDL-TG secretion (193). It has been suggested that an MCD diet causes reduced hepatic β-oxidation (229), although direct evidence of this is sparse. Kirsch et al. (113) compared species, strain, and sex differences in response to an MCD diet. They found that MCD diet-fed male rats (Long-Evans, Sprague-Dawley, and Wistar) had more pronounced steatosis than female rats, and between species, Wistar rats had greater hepatic TG accumulation than the other two; there were no strain or sex differences in the control-fed groups (113). A comparison of MCD diet fed Wistar rats and C57/BL6 mice found the mouse to have less steatosis than the rat model (113). As an MCD diet causes inflammation and fibrosis, it may not be the most appropriate dietary model to study the induction of steatosis. Moreover, translation to human disease is debatable due to the fact the majority of humans do not consume diets deficient in methyl groups.

High-energy, typically high-fat, diets have been utilized to investigate the effects of obesity and its associated metabolic complications (e.g., insulin resistance) on the development of steatosis. By use of this dietary regimen, animals are typically fed greater than 50% TE as fat (133, 175, 232). Nishikawa et al. (175) compared the effects of an HFD on young C57/BL6 and BALB/c male and female mice and found that young BALB/c female mice were resistant to HFD-induced obesity and, hence, did not accumulate hepatic TG compared with the other animals. Middle-aged male and female C57/BL6 mice fed an HFD had a similar level of hepatic TG accumulation (175). Gao et al. (63) compared the responses of feeding chow and HFD in male Syrian gold hamsters and male C57/BL6 mice. Hepatic TG content was significantly increased in both hamsters and mice to a similar extent when fed an HFD (63). HFD feeding increased most, but not all, lipogenic genes in both hamsters and mice; however, there was an opposite effect of HFD on MTP expression, which increased in hamsters and decreased in mice compared with chow-fed controls (63). In line with the change in MTP expression, the hepatic TG secretion rate significantly increased in hamsters and decreased in mice compared with controls (63). These studies highlight how choice of species or background strain may effect development of the model and interpretation of results; therefore, selection of an appropriate background strain is necessary when designing and carrying out studies in rodents.

Feeding C57/BL6 male mice an “American fast-food” diet (45% TE fat) with or without trans fats, and/or with high-fructose corn syrup (HFCS) for 16 wk, resulted in significantly greater hepatic TG accumulation in all groups except animals consuming the diet devoid of trans fats compared with animals fed a control diet (14% TE from fat) (223). Wang et al. (232) investigated metabolic factors that might play a role in the development of hepatic steatosis in Sprague-Dawley rats either fed a HFD (60% TE from fat, 73% being from trans fats) or with diabetes induced by either a high dose of streptozotocin [type 1 diabetes mellitus (T1DM)] or a low-dose of streptozotocin [type 2 diabetes mellitus (T2DM)]. The HFD and diabetic groups developed marked steatosis and had a significant reduction in expression of mitochondrial biogenesis genes despite increased expression of genes related to fatty acid oxidation compared with controls (232). Studies such as these provide the opportunity to “tease apart” the specific effects of diet, in this case overconsumption of trans fatty acids and/or metabolic factors that may play pivotal roles in the development of NAFLD.

Rodent models have investigated the effects of changing from chronic consumption of a HFD (45% TE fat, 35% TE carbohydrate) to a low-fat diet (LFD) (10% TE fat, 70% TE carbohydrate) and found that, although progression of NAFLD was halted, steatosis remained (178). A plausible explanation for the lack of change in steatosis was that the LFD was carbohydrate rich, which maintained hepatic fatty acid synthesis via DNL. The DNL pathway is stimulated under conditions of carbohydrate excess via ChREBP and insulin (59). In support of the DNL pathway playing a role in the development of hepatic steatosis, studies in mice deficient in SCD1 and fed a fructose-rich diet (20% weight of diet) had a lower amount of liver TG than wild-type animals (154). The authors were able to demonstrate this was due to a lack of DNL as sterol regulatory element-binding protein (SREBP-1c), a transcription factor responsible for the enzymes involved in fatty acid synthesis, was not induced (154). Indeed, liver-specific SCD1 knockout mice are protected from high-carbohydrate- but not high-fat-induced liver steatosis and obesity (155). It has been proposed that the de novo synthesis of monounsaturated fatty acids via SCD1 may be important for TG production; however, in humans, due to the abundance of dietary monounsaturated fatty acids, endogenous synthesis may not be so critical (96). Feeding C57/BL6 male mice a high-sucrose diet (65% by weight) for 16 wk resulted in significantly higher body weight and liver TG content than mice fed a standard chow diet (40). It was also noted that genes involved in the DNL pathway were significantly upregulated (40). The effects of a compound (GW3965) that stimulates hepatic expression of a member of the long-chain acyl-CoA family (ACSL3) was investigated in Syrian golden hamsters fed a high-fructose (60%) diet or normal chow (43). Animals treated with the compound had reductions in hepatic TG levels (43). The authors suggested that ACSL3 might play a pivotal role in directing fatty acid entry into catabolic pathways thus lowering liver TG accumulation (43). Taken together, these studies glean insight into the potential factors that may regulate and stimulate the process of DNL.

Genetic manipulation of rodent models has helped elucidate some of the key molecular mechanisms involved in the regulation of hepatic fatty acid partitioning. Surprisingly little is known about the regulation by which hepatic intracellular TG is mobilized and fatty acids are then made available for other processes such as oxidation or reesterification in another lipid pool. Animal work has played a role in providing insight. For example, although hepatic ATGL expression is relatively low compared with other tissues, studies in ATGL knockout mice suggest it is functional (247). Using a murine model, Reid et al. (192) demonstrated that overexpression of HSL or ATGL
within the liver ameliorated steatosis, increased free fatty acid concentrations, and plasma 3OHB concentrations while TG secretion rates remained unchanged. GPAT catalyzes the first committed step in TG (and phospholipid) biosynthesis, and in the liver, mitochondrial GPAT (mtGPAT) comprises up to 50% of total GPAT activity (136). By use of C57BL/6 mice consuming a chow diet, it was demonstrated that liver-directed overexpression of mtGPAT resulted in significantly greater liver TG content, TG secretion, and lower fatty acid oxidation compared with control animals (136). DGATs are also important in the TG synthesis pathway. Injecting C57BL/6 mice with recombinant adenovirus containing either DGAT1 or DGAT2 increased liver TG significantly more than vector alone, with no change in VLDL-TG production rates (152). Villanueva et al. (230) reported no difference in liver TG between DGAT1 knockout and wild-type mice fed a chow diet; however, feeding an HFD resulted in significantly lower liver TG in knock-out compared with wild-type animals. That work went on to demonstrate that pharmacological inhibition of DGAT1 mRNA prevented the accumulation of liver TG in HFD mice, suggesting that pharmacological inhibition of DGAT1 may be a potential strategy for treatment of steatosis (230).

By studying hepatic metabolism of rats in a fed state, McGarry and Foster (147) provided a key link between carbohydrate and fat metabolism with their discovery that malonyl-CoA is a potent inhibitor of CPT1. Under conditions of carbohydrate excess, malonyl-CoA concentrations are high; thus, fatty acid oxidation is suppressed via inhibition of CPT1. Using adeno-associated viruses to mediate long-term hepatic gene transfer of CPT1α or an active mutant form (insensitive to malonyl-CoA) it was demonstrated that upregulation of fatty acid oxidation protected against hepatic TG accumulation in animals fed a HFD (60% TE fat) (180). These data suggest that disposal of fatty acids via oxidation pathways may be important in the prevention of steatosis.

A number of studies have investigated the effects of both genetic and environment manipulation, for example the effect of an MCD diet on a peroxisome proliferator-activated receptor-α (PPARα) knockout model was found to accelerate progression and severity of NAFLD (109). Additionally, the use of animal models allows for investigation into the consequences of NAFLD on hepatic microcirculation, an area difficult to investigate in humans. Feeding a HFD to rats induced steatosis, which resulted in impaired microcirculation due to associated hepatocellular ballooning (196). It has been reported that in rats fed L-arginine there were significant improvements in hepatic arterial and portal venous blood flow, hepatic microcirculation, and tissue oxygenation in both fatty and control livers (105). An emerging methodology that may overcome species differences is the humanizing of animal models to make them more reflective of human metabolism. A potentially interesting and useful model may be the Fah/Rag2/Il2rg mouse, as when transplanted with primary human hepatocytes, a more typical human lipidprofile and bile acid composition was reported (54). However, hepatic fat was not measured in those mice, and future experiments need to evaluate the usefulness of this model in studies of hepatic steatosis. Intriguingly, Solinas et al. (212) compared the ultrastructural features of NAFLD in a rat model fed an HFD to that of humans and found that rat and human NAFLD shared overlapping ultrastructural features. This area warrants further investigation.

Studying the development and progression of NAFLD using animal models offers the opportunity of a markedly reduced length of time for disease initiation and progression to occur. Additionally, the models are genetically and phenotypically consistent, providing a platform in which the manipulation of a specific or multiple factors can be undertaken in a controlled manner. However, translation to the development of NAFLD in humans may be more challenging, as dietary composition, lifestyle factors, and environmental effects on genes may differ. Consideration also needs to be given in regard to hepatic TG metabolism, which varies between species. For example, in rodents, extensive apolipoprotein (apo)B editing also occurs in the liver; they lack cholesteryl ester transfer protein (CETP) activity in plasma, have more efficient clearance of chylomicrons and VLDL remnants from the circulation (181) and have major differences in bile acid metabolism and composition that may influence the secretion of TG-rich lipoproteins.

**IN VITRO MODELS**

A wide variety of in vitro and cellular models have been used to investigate liver fat metabolism. Historically, the majority of studies have focused on cholesterol or lipoprotein metabolism; thus, the use of in vitro/cellular models to investigate factors involved in NAFLD development and progression is in its infancy. For cellular models to start making, secreting, and/or storing fat, there is a requirement that key cellular machinery and enzymes be present; after review of the literature, the phenotype of cells/cell lines and gene expression profile of the cell is often not described. As both factors will influence the usefulness of a model to study NAFLD, a more complete characterization of in vitro models is required to identify the most suitable platform to assess disease development. As the accumulation of intrahepatocellular lipid in humans represents an imbalance between input and removal pathways, an ideal cellular model would take up, synthesize, and secrete TG and oxidize fatty acids, thus to some degree recapitulating what occurs in humans in vivo.

**Liver Slices**

Rodent liver slices were first used in the 1920s by Otto Warburg (234) and then in the 1930s by Hans Krebs, who, in a seminal set of experiments, uncovered the urea cycle (121, 122). Precision-cut tissue slices resemble the organ from which it is prepared; thus, all cell types in the original tissue matrix are present with the cytoarchitecture/structure (i.e., cell-cell and cell-matrix interactions) retained. Tissue slices can be obtained from liver fragments and needle biopsies (81), and the development of a precision tissue slicer has led to less tissue trauma (38, 81). In order to achieve uniform tissue slices, a recent modification is to first drill a cylindrical core of tissue trauma (38, 81). The preparation, incubation, viability, and functionality of tissue slices have been reviewed (38). Liver slices from humans have not been utilized to investigate TG metabolism, and to date no study has utilized liver slices to investigate the mechanisms responsible for the development of NAFLD, al-
though liver slices isolated from diet-induced obese rats have been reported to retain elevated TG compared with age-matched controls (48), suggesting they could be useful in understanding NAFLD progression. Liver slices have been used by only a few to investigate other aspects of hepatic fatty acid metabolism. For example, Nassir et al. (170) demonstrated reduced VLDL (TG and apoB) secretion when comparing isolated incubated liver slices from ob/ob mice with a whole body deletion of the CD36 transporter (ob-CD36) and wild-type animals. Notably, ob-CD36 animals also had increased liver TG compared with ob/ob mice; inhibition of VLDL secretion resulted in a microvesicular steatotic pattern in ob-CD36 compared with wild-type animals (170). The degree and pattern of steatosis may be influenced by the “origin” of the slices. Accumulation of TG into cytosolic and microsomal pools in rat and calf liver slices was shown to be notably different when exposed to increasing concentrations of oleate; TG was significantly greater in rat compared with calf liver (74). The higher accumulation of TG in rat compared with calf liver cannot be explained by lower VLDL production or secretion rate, or differences in fatty acid oxidation, rather a lower/slower uptake of fatty acids (74). The ability of liver slices from eight species (cattle, sheep, pig, guinea pig, rat, fish, rabbit, and chicken) to synthesize and secrete TG from fatty acids added to the media was investigated (190). It was found that the overall rate of liver TG synthesis from fatty acids was similar among species, but in species where lipogenesis is minor (e.g., sheep, cattle, pig, and guinea pig) there was a lower amount of TG synthesized compared with species where lipogenesis is higher (e.g., chicken, fish, rabbit, and rat), with the authors suggesting that the ability of liver slices to secrete VLDL-TG is proportional to the lipogenic capacity (190). These data highlight the need for consideration and characterization of species-specific traits when selecting a model to investigate human NAFLD. Microarray analysis of liver slices from eight species (cattle, sheep, pig, guinea pig, rat, fish, rabbit, and chicken) has been linked to cytoplasmic remodeling during culture, starting after one week, leading to a gradual loss of liver-specific enzyme activities (197). Dedifferentiation (in primary rat hepatocytes) has been reported by 10.220.33.5 on October 21, 2017 http://ajpendo.physiology.org/ Downloaded from http://ajpendo.physiology.org/ by 10.220.33.5 on October 21, 2017

Primary Hepatocytes

The technique for isolating primary hepatocytes was first described in the 1960s (15, 102, 103), with Berry and Friend (15) being the first to report the collagenase perfusion method, which was then adapted in the 1970s (208). Since the inception of this methodology, a variety of work has been undertaken in isolated human and animal hepatocytes. Once isolated, hepatocytes can be used in suspension but have a limited lifespan (4 h) (28). Alternatively, hepatocytes adhere to extracellular matrices such as collagen and can then be cultured for a number of days (23) (Table 1). Conditions to improve hepatocyte function and survival in vitro have been extensively reviewed (64, 80, 81, 83, 94). When using primary hepatocytes, consideration needs to be given to the batch variability, as this may vary greatly between preparations (168). Notably, the level of steatosis, the type of liver disease the sample is obtained from (i.e., resected tissue where patients have received preoperative chemotherapy, biliary cirrhosis, autoimmune hepatitis, etc.), and the pharmacological or nutritional agents that patients are exposed to prior to surgery, appear to influence hepatocyte yield, viability, and functionality (17). Other factors to consider are reported in Table 1.

A wide variety of cell models have been utilized to investigate various aspects of liver metabolism. Cellular models offer the opportunity to study specific mechanistic details. However, these models, particularly primary cells, are inherently challenging to work with, not least the first step of obtaining tissue samples and then isolating cells. Many early studies used radiolabeled tracers to investigate TG secretion in primary hepatocytes and some cell lines (47, 68, 69, 201), with only a few studies investigating intracellular TG accumulation. When making cells steatotic, the majority of studies have employed fatty acid incubations, most often comparing saturated and unsaturated fatty acids in isolation or in combination (72) for acute periods of time; notably, steatotic patterns or images of steatotic cells are rarely reported. In the majority of studies, exogenous fatty acids have been added to the culture media; typically, oleic acid alone has been given at varying concentrations and usually over a short time course (36, 135, 150, 204, 233). The rationale for why oleic acid rather than other fatty acids have been used is not clear but may be because monounsaturated fatty acids are thought to be essential for TG production (96) and palmitic acid appears to be lipotoxic when given in isolation (72). Culturing cells in a variety of concentrations of exogenous fatty acids for periods between 8 and 24 h resulted in a dose-dependent TG accumulation (72, 142, 158). Steatosis in these situations is defined as TG accumulation within the cells above control, often measured by fluorescence or Oil red O staining. Thus, cellular models have the potential to provide a platform for investigations into possible treatments of hepatic steatosis/NAFLD, as specific compounds such as pharmacological agents can be added to the media to investigate the reversal of intracellular TG accumulation. A study of primary hepatocytes to investigate intracellular TG accumulation and secretion (where reported by authors) is shown in Table 2. In this table, we report the lipoprotein particle in which TG is secreted, as this pathway may play an important role in determining whether cells start to accumulate TG. Of note, studies appear to not measure markers of fatty acid oxidation.

Human hepatocytes. Bhogal et al. (17) reported “the one hundred liver experience” using the collagenase perfusion method. Although this method is considered the gold standard, once isolated, viability in culture, metabolic activity, and hepatocyte function are all factors that require consideration (Table 1). Many challenges exist with the use of primary human hepatocytes, including availability, phenotypic instability, limited lifespan, and lack of robust, reproducible cryopreservation techniques (79, 82, 85). Human hepatocytes, either fresh or preserved, are commercially available. In culture, primary hepatocytes lose the activity of the diverse group of enzymes [cytochrome P-450s (CYPs)] that catalyze the oxidation of organic substances after 72 h (23, 138) and dedifferentiate after one week, leading to a gradual loss of liver-specific functions (197). Dedifferentiation (in primary rat hepatocytes) has been linked to cytoplasmic remodeling during culture,
leading to a 50\% decrease of mitochondrial number and changes in mitochondrial morphology (194).

A steatosis model in human primary hepatocytes showed similar intracellular TG accumulation to human liver when a 2:1 ratio of oleate to palmitate was used (72). As accumulation of intracellular TG is dependent on input and removal pathways, it is important that cells secrete lipoproteins. Isolated human hepatocytes have been shown to secrete nascent VLDL.

### Table 1. General characteristics and factors to consider for specific in vitro liver models

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<th>Model</th>
<th>Origin</th>
<th>Factors to Consider</th>
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<td>Liver slices</td>
<td>Human and rat</td>
<td>• Studies limited to 1–3 days as liver function may rapidly decrease</td>
<td>38, 79, 81, 88</td>
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<td>• Slices must be fresh as cells are not equally preserved in sections if frozen</td>
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<td>• Diffusion of oxygen and nutrients from media may not be equal across tissue</td>
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<td>• Abundance of CYP decreases approximately 50% of initial amount after 24 h</td>
<td></td>
</tr>
<tr>
<td>Primary Cells</td>
<td></td>
<td>• Do not proliferate</td>
<td>16, 23, 52, 82, 85, 168, 194, 197, 221</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• Secrete albumin</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>• Can be cultured in suspension for hours and as a monolayer for up to a week</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Human</td>
<td>• Inter-donor variability including: age, sex, liver disease, genetic polymorphisms, premedication</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>• Express CYP2E1, 3A4, 1A2, 2C9, 2A6 but lose some activity after 72 h in culture</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>• May dedifferentiate after 1 wk</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>• 3D models increase culture time to 1 m</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>• Poor viability after cryopreservation</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Rodent</td>
<td>• Express CYP1, -2, -3 and -4 but lose expression over 72 h in culture</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>• CYP inducibility better retained when cells cultured in sandwich configuration, difference not observed in human hepatocytes</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>• During culture progressively lose mitochondria</td>
<td></td>
</tr>
<tr>
<td>IHH</td>
<td></td>
<td>• Proliferate</td>
<td>204</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• Grow in monolayers</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>• Polygonal in appearance</td>
<td></td>
</tr>
<tr>
<td>Hepatocyte cell lines</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HepG2</td>
<td>Human: 15-yr Caucasian American male</td>
<td>• Proliferate</td>
<td>23, 144, 150, 197, 221, 224</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• Secrete albumin</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>• Express CYP1A1, 3A3, UGT</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>• Epithelial in morphology</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>• Lack SER</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>• Resemble fetal liver cells</td>
<td></td>
</tr>
<tr>
<td>Huh-7/7.5</td>
<td>Human: tumor of 57-yr Japanese male in 1982</td>
<td>• Proliferate</td>
<td>150, 214</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• Secrete albumin</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>• Resemble HepG2 cells</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>• Adopt primary hepatocyte-like morphology and hepatocyte differentiation markers with addition of human serum</td>
<td></td>
</tr>
<tr>
<td>McA-RH7777</td>
<td>Rat: Morris hepatoma 7777–male Buffalo strain</td>
<td>• Proliferate</td>
<td>14, 55, 87, 148</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• Secrete albumin</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>• Synthesise ( \alpha )-fetoprotein</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>• Express a number of CYPs</td>
<td></td>
</tr>
<tr>
<td>Fetal liver cells/progenitor cells</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HepaRG</td>
<td>Human: biopotent progenitor cell line from Edmonson grade I well-differentiated liver tumor.</td>
<td>• Proliferate</td>
<td>4, 7, 76, 79</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• Secrete albumin</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>• Express CYP1A2, 2B6, 2C9, 2E1, 3A4</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>• Can differentiate into hepatocytes or cholangiocytes</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>• Retain expression of liver-specific glycolytic enzymes</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Rat: Morris hepatoma 7777–male Buffalo strain</td>
<td>• Proliferate</td>
<td>73, 84</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• Secrete albumin</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>• Express CYP3A4, 4A3, CYP reductase</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>• Can be cultured for ( \leq ) 14 days</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>• Express hepatocyte specific genes including albumin, AFP and HNF-4( \alpha )</td>
<td></td>
</tr>
</tbody>
</table>

Continued
particles within the range observed in human serum and with a comparable TG and phospholipid composition to those found in vivo (134, 138, 204). It has been shown that human hepatocytes respond to exogenous fatty acid supplementation by increasing TG synthesis and VLDL-TG secretion with no change in apoB abundance (135). Recently, it was demonstrated that cortisol caused a dose-dependent decrease in palmitate synthesis in primary human hepatocytes, an effect augmented in the presence of insulin (90). Although changes in intracellular TG content were not reported, these data demonstrate that the de novo pathway is functionally active in primary human hepatocytes. In drug discovery and development of novel agents for steatosis it may be important to use human hepatocytes. For example, changes in lipid metabolism induced by hypolipidemic drugs such as clofibrate have been shown to be different in human compared with rat hepatocytes, which may be due to enzymatic and metabolic differences between species (20). Consideration needs to be given regarding the genotype of the donor cells, as this may affect the amount and TG in isolated hepatocytes. For example, the PNPLA3 I148M variant has been shown to be related to liver fat content (195) therefore cells from these individuals may display different metabolic properties, such as lower TG turnover, compared with non-variant carrying cells.

Immortalized human hepatocytes. In an attempt to circumvent the availability, reproducibility, and storage problems associated with primary human hepatocytes, some laboratories have developed human hepatocytes that have been immortalized (IHH) using the SV40 large T antigen-expressing plasmid (204). As with the primary human cells, consideration regarding genotype (and potentially phenotype) needs to be taken into account. These cells have been reported to secrete TG-containing lipoproteins; however, unlike primary human hepatocytes, they mostly secrete lipoproteins in the LDL size range (204). Samanez et al. (202) further characterized IHH cells by culturing them under different glucose concentrations and found increased lipoprotein secretion at the higher glucose concentration. They also found expression of lipogenic genes to be increased in high-glucose conditions (202). In terms of NAFLD, it has been reported that incubating IHH cells with oleic acid results in steatosis and that this can be enhanced by activation of cannabinoid receptors (37). Further work using physiological ratios of saturated and unsaturated fatty acids is required to validate whether these are a useful model for studying NAFLD. Culturing IHH cells that express the PNPLA3 I148M variant in media containing exogenous fatty acid resulted in a greater TG accumulation compared with mock-transfected and wild-type cells (186). The increase in TG accumulation was thought to be due to a reduced rate of TG hydrolysis in the mutant IHH (186), an effect exacerbated by the addition of fatty acids in the media. This suggests that these cells would be useful in understanding the mechanisms and increased risk of NAFLD development and progression in human carriers of PNPLA3 I148M.

Rodent hepatocytes. In situ collagenase perfusion is often utilized to isolate rodent primary hepatocytes to study various aspects of liver metabolism. Rodent hepatocytes have similar limitations to human hepatocytes, as summarized in Table 1. Once in culture, rat hepatocytes progressively lose the expression of CYP enzymes over 72 h (194); CYP inducibility appears to be better retained when cells are cultured in sandwich rather than a conventional monolayer configuration, a difference not observed in human hepatocytes (82). Rat hepatocytes appear to progressively lose mitochondria when cultured, due to increased mitochondrial-specific autophagy (mitophagy) (194). In contrast to human, rat hepatocytes have been shown to recover some transcription abilities, including CYP-dependent activities following 2–3 days in culture (82).

Rat primary hepatocytes have been used to investigate the turnover of the intracellular TG pool, along with factors influence

---

Table 1.—Continued

<table>
<thead>
<tr>
<th>Model</th>
<th>Origin</th>
<th>Factors to Consider</th>
<th>Ref. No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Liver stem cells</td>
<td>Human</td>
<td>Stem cells represent 0.5–2.5% liver cell population across all ages</td>
<td>173</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Fetal liver stem cells have been isolated by digestion of human liver tissue in manner similar to isolation of primary hepatocytes</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Proliferate</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Secrete albumin</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Transitional cell type between hepatoblast and fetal hepatocyte</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Differentiation into hepatocytes poorly understood; can be differentiated into cholangiocytes</td>
<td></td>
</tr>
<tr>
<td>Pluripotent adult stem cells</td>
<td>Human</td>
<td>Proliferate</td>
<td>78, 86</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Secrete albumin</td>
<td>173, 213</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Express some CYPs, mostly resemble fetal hepatocytes or hepatoblasts</td>
<td>219, 228</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Time taken to grow and differentiated into hepatocyte-like cell up to 30 d</td>
<td></td>
</tr>
<tr>
<td>Embryonic stem cells</td>
<td></td>
<td>Isolated from blastocytes</td>
<td>78, 86</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Proliferate</td>
<td>173, 213</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Secrete albumin</td>
<td>228</td>
</tr>
<tr>
<td></td>
<td></td>
<td>May have chromosomal abnormalities</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Express CYP3A4, 3A7, 1A1, resemble fetal hepatocytes or hepatoblasts</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Transplantable into mice to form vascularized liver-like tissue</td>
<td></td>
</tr>
</tbody>
</table>

IHH, immortalized human hepatocytes; CYP, cytochrome P-450; apo, apolipoprotein; VLDL, very-low-density lipoprotein; 3D, 3-dimensional; SER, smooth endoplasmic reticulum; UDP-glucuronosyltransferase; AFP, α-fetoprotein; HNF4α, hepatocyte nuclear factor-4α.
ence the partitioning of fatty acids toward oxidation and ketogenic pathways (215, 226). By use of rat primary hepatocytes, it has been elegantly demonstrated that the mobilization of stored cytosolic TG for VLDL-TG production involved lipolysis followed by reesterification of fatty acids, with only part of the mobilized TG being utilized for VLDL assembly, the remainder being recycled back to the cytosolic pool (66, 237). Debate still remains as to whether any of these hydrolyzed fatty acids enter the oxidation pathways (13, 66). Hepatocytes isolated from obese rats had significantly reduced fatty acid oxidation and ketone production compared with lean rats (226). Wiggins and Gibbons (237) demonstrated that secretion of VLDL was dependent on intracellular TG content, whereas the synthesis and output of ketone bodies required the presence of extracellular fatty acids. Similar studies, investigating the partitioning of fatty acids into oxidation pathways in human

<table>
<thead>
<tr>
<th>Cell Type</th>
<th>Ref. No.</th>
<th>Glucose, mM*</th>
<th>Serum*</th>
<th>Insulin*</th>
<th>Fatty Acid Added*</th>
<th>Main Lipoprotein</th>
<th>Accumulation</th>
<th>Secretion</th>
</tr>
</thead>
<tbody>
<tr>
<td>Human Primary</td>
<td>135</td>
<td>11</td>
<td>10% FBS</td>
<td>4 mU/ml</td>
<td>± OA</td>
<td>N/R</td>
<td>VLDL</td>
<td>OA induces VLDL-TG secretion</td>
</tr>
<tr>
<td>Hepatocytes</td>
<td>134</td>
<td>11</td>
<td>10% FBS</td>
<td>4 mU/ml</td>
<td>No</td>
<td>VLDL</td>
<td>Accumulate</td>
<td>Comparable VLDL and TG to human plasma</td>
</tr>
<tr>
<td></td>
<td>138</td>
<td>11</td>
<td>10% FBS</td>
<td>4 mU/ml</td>
<td>± OA</td>
<td>N/R</td>
<td>VLDL</td>
<td>Comparable VLDL and TG to human plasma</td>
</tr>
<tr>
<td>Rat Primary</td>
<td>135</td>
<td>11</td>
<td>10% FBS</td>
<td>4 mU/ml</td>
<td>No</td>
<td>VLDL</td>
<td>Accumulate</td>
<td>OA increased VLDL-TG secretion</td>
</tr>
<tr>
<td>Hepatocytes</td>
<td>35</td>
<td>25</td>
<td>10% FBS</td>
<td>1 mg/l</td>
<td>± OA</td>
<td>N/R</td>
<td>VLDL</td>
<td>OA increased TG secretion</td>
</tr>
<tr>
<td></td>
<td>238</td>
<td>27.8</td>
<td>10% FBS</td>
<td>No</td>
<td>± OA</td>
<td>N/R</td>
<td>VLDL</td>
<td>Absence of OA rate of VLDL-TG secretion greater than amount of TG lost from cell secretion VLDL-TG secretion</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>27.8</td>
<td>No</td>
<td>± 78 nM</td>
<td>No</td>
<td>N/R</td>
<td>VLDL</td>
<td>Insulin promotes TG storage OA increased TG content</td>
</tr>
<tr>
<td></td>
<td>46</td>
<td>27.8</td>
<td>No</td>
<td>± 78 nM</td>
<td>± OA</td>
<td>N/R</td>
<td>VLDL</td>
<td>OA increased TG accumulation</td>
</tr>
<tr>
<td></td>
<td>66</td>
<td>27.8</td>
<td>No</td>
<td>± OA</td>
<td>N/R</td>
<td>VLDL</td>
<td>No accumulation</td>
<td>No synchronous relationship between extracellular FA concentration and VLDL-TG secretion</td>
</tr>
<tr>
<td>IHH</td>
<td>246</td>
<td>5.5</td>
<td>No</td>
<td>No</td>
<td>± OA</td>
<td>N/R</td>
<td>VLDL</td>
<td>OA increased TG secretion</td>
</tr>
<tr>
<td></td>
<td>204</td>
<td>11</td>
<td>10% FBS</td>
<td>20 mU/ml</td>
<td>± OA</td>
<td>LDL</td>
<td>N/R</td>
<td>OA increased VLDL-TG secretion</td>
</tr>
<tr>
<td></td>
<td>202</td>
<td>11 vs. 1</td>
<td>10% FBS</td>
<td>20 mU/ml</td>
<td>No</td>
<td>LDL</td>
<td>N/R</td>
<td>OA increased TG secretion</td>
</tr>
<tr>
<td>HepG2</td>
<td>36</td>
<td>NR</td>
<td>10% FBS</td>
<td>± 1 µg/ml</td>
<td>± OA</td>
<td>LDL</td>
<td>No accumulation</td>
<td>OA increased secretion of VLDL, LDL, and HDL</td>
</tr>
<tr>
<td></td>
<td>233</td>
<td>5.5</td>
<td>10% FBS</td>
<td>No</td>
<td>± OA</td>
<td>IDL</td>
<td>OA increased TG accumulation</td>
<td></td>
</tr>
<tr>
<td></td>
<td>12</td>
<td>10% FBS</td>
<td>No</td>
<td>No</td>
<td>± OA</td>
<td>IDL</td>
<td>OA increased TG accumulation</td>
<td></td>
</tr>
<tr>
<td></td>
<td>25</td>
<td>10% FBS</td>
<td>No</td>
<td>No</td>
<td>± OA</td>
<td>IDL</td>
<td>OA increased TG accumulation</td>
<td></td>
</tr>
<tr>
<td></td>
<td>150</td>
<td>N/R</td>
<td>10% FBS</td>
<td>No</td>
<td>± OA</td>
<td>LDL</td>
<td>OA increased TG accumulation</td>
<td></td>
</tr>
<tr>
<td></td>
<td>135</td>
<td>11</td>
<td>10% FBS</td>
<td>4 mU/ml</td>
<td>± OA</td>
<td>LDL</td>
<td>OA increased TG accumulation</td>
<td></td>
</tr>
<tr>
<td></td>
<td>239</td>
<td>N/R</td>
<td>10% FBS</td>
<td>No</td>
<td>± OA</td>
<td>LDL</td>
<td>OA increased TG accumulation</td>
<td></td>
</tr>
<tr>
<td></td>
<td>246</td>
<td>5.5</td>
<td>No</td>
<td>No</td>
<td>± OA</td>
<td>LDL</td>
<td>OA increased TG accumulation</td>
<td></td>
</tr>
<tr>
<td>Huh 7.5</td>
<td>214</td>
<td>25</td>
<td>10% FBS</td>
<td>20 mU/ml</td>
<td>± OA</td>
<td>LDL</td>
<td>OA increased TG accumulation</td>
<td></td>
</tr>
<tr>
<td>McA-RH 7777</td>
<td>216</td>
<td>N/R</td>
<td>10%-20%</td>
<td>No</td>
<td>± OA</td>
<td>VLDL</td>
<td>OA increased TG accumulation</td>
<td></td>
</tr>
<tr>
<td></td>
<td>9</td>
<td>N/R</td>
<td>10% FBS</td>
<td>No</td>
<td>± OA</td>
<td>VLDL</td>
<td>OA increased TG accumulation</td>
<td></td>
</tr>
<tr>
<td>HepaRG</td>
<td>202</td>
<td>11</td>
<td>10% FBS</td>
<td>135 mU/ml</td>
<td>No</td>
<td>VLDL</td>
<td>No accumulation</td>
<td>Expression of HL decreased VLDL-TG secretion</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>10% FBS</td>
<td>No</td>
<td>No</td>
<td>± OA</td>
<td>VLDL</td>
<td>No accumulation</td>
<td>High glucose increased TG secretion</td>
</tr>
<tr>
<td>hiPCs</td>
<td>65</td>
<td>17.3</td>
<td>20% KOSR</td>
<td>1% ITS</td>
<td>No</td>
<td>VLDL</td>
<td>No accumulation</td>
<td>Increased TG accumulation</td>
</tr>
</tbody>
</table>

*All study/culture conditions are in the format reported by the authors. FBS, fetal bovine serum; HS, human serum; HL, hepatic lipase; OA, oleic acid; VLDL, very-low-density lipoprotein; LDL, low-density lipoprotein; HDL, high-density lipoprotein; IDL, intermediate-density lipoprotein, TG, triglyceride; N/R, not reported; apoc-III, apolipoprotein C-III; KOSR, knockout serum replacement; hiPCs, human induced pluripotent cells; ITS, insulin-transferrin-selenium.
hepatocytes, have yet to be reported. Interestingly, rat hepatocytes have been reported to retain TG when isolated from HFD-fed animals (226) along with retaining enhanced reactive oxygen species (ROS) and mitochondrial defects observed in vivo (124); these observations suggest some level of cellular memory and therefore may be useful models for investigation into the reversal of NAFLD. In studies using rat hepatocytes, some (35, 135) but not all (183) have observed exogenous fatty acids such as oleic acid increased TG secretion; it is plausible that the discrepancy in results may be due to differences in culturing conditions or methodology differences (Table 2).

Rodent hepatocytes have been extensively utilized to better understand many aspects of TG metabolism, specifically the esterification process and how this may impact on the partitioning of fatty acids into oxidation pathways, along with factors that may influence the removal of TG from the hepatocyte via VLDL. Genetic knockout of specific GPAT isoforms in mouse hepatocytes revealed that GPAT1, and not GPAT4, reciprocally regulated hepatic DNL TG synthesis and fatty acid oxidation (235). Similar findings have also been shown in rat hepatocytes where adenoviral overexpression of mitochondrial GPAT led to decreased fatty acid oxidation and increased TG synthesis (137). Taken together, these data suggest that GPAT may be an important locus between esterification and oxidation pathways. Disposal of fatty acids via oxidation pathways may help protect from TG accumulation more so than increased secretion of VLDL-TG, as these particles may be recycled back to the liver.

Recently, the role of lipid droplet proteins, such as cell death-inducing DFFA-like effector B (CIDEB) in TG metabolism has been investigated. CIDEB mediates lipid droplet fusion and VLDL maturation; however, many hepatoma cell lines have limited expression. It has been noted that VLDL has a lower TG content without a change in apoB in CIDEB knockout mouse hepatocytes (243). This suggests that CIDEB is important for TG enrichment of VLDL. A plausible explanation for lower VLDL-TG is due to less hepatic TG being available for lipidation of particles. Hepatocytes from CIDEB knockout mice have a decreased TG content compared with cells from wild-type animals. Notably, lipid droplets were smaller and more clustered in the hepatocytes from the CIDEB knockout mice compared with controls (131). Turnover of the TG storage pool is important for providing substrate for VLDL-TG lipidation and potentially fatty acids to oxidation. Knockdown of ATGL in mouse hepatocytes resulted in the development of steatosis (179). Lipidation of VLDL via MTP is a critical step for intracellular TG removal. Inhibiting MTP in rat (92) and mouse hepatocytes (125) resulted in decreased VLDL-TG secretion. This was due to a large proportion of TG being reesterified and retained in membranes rather than packaged into VLDL (92), demonstrating that MTP is crucial for TG removal from the liver. Notably, the administration of the MTP inhibitor (lomitapide) in humans is reported to increase hepatic lipid accumulation and decrease fasting plasma TG concentrations; the latter may reflect a decrease in VLDL-TG secretion (34).

Hepatocytes isolated from fatty livers. Human hepatocytes may be isolated from surplus resection tissue from cancer patients, and it is likely that a large proportion of individuals have often undergone chemotherapy, which has been shown, in some cases, to cause hepatic steatosis (60, 112, 185). When one uses primary cells from a donor with steatosis, it may be technically more challenging, as it has been shown that the higher fat content is retained and the lipid droplets present may alter the morphology of hepatocytes (17). Unfortunately, the difficulties in culturing fat-laden cells are not often reported; however, some studies have highlighted potential issues. The rate of successful hepatocyte isolation from fatty livers seems to be dependent on the severity of steatosis (17) For example, when isolating cells from livers with a fat content more than 40%, one is advised to use double the starting weight of tissue due to an often less viable cell population (146). Livers having more than 30% steatosis affects the cells’ ability to survive a Percoll gradient, leading to decreased overall yield (231). It has been suggested the reduced viability of these cells is due to higher intracellular levels of ROS, making them more susceptible to damage (199). This may be overcome by the addition of N-acetylcysteine to the perfusion fluid, which has been reported to improve yield (199). Hepatocytes with high fat content can be buoyant and therefore do not attach to collagen-coated matrixes (i.e., they float despite being viable) (17). Steatotic cells have been reported to secrete their lipid content following a few days in culture, becoming more comparable to healthy hepatocytes (146). The added complications of working with hepatocytes isolated from fatty livers may in part explain why detailed mechanistic studies have not been undertaken in primary hepatocytes thus far. These data highlight the challenges that would occur in any hepatocyte model where there is a lot of fat, for example isolating hepatocytes from genetically modified animals such as the ob/ob mouse, which has fatty liver.

Other Primary Cells

Along with hepatocytes, other cells, including nonparenchymal Kupffer and stellate cells, have been isolated from perfused livers and may play an important role in the health and function of hepatocytes in vivo (115). Kupffer cells are resident macrophages located in the sinusoids of the liver and modulate the response to injury and sepsis. Stellate cells are fibroblasts that are the main matrix producing cell of the liver, playing important roles in regeneration and inflammation. Isolation of Kupffer cells has been hampered with difficulties including high adherence of cells to the culture substrate and resistance to conventional dissociation reagents. A new method of human liver tissue homogenization has been described for Kupffer cell isolation that circumvents previous isolation problems (1). Kupffer cells have been shown to play important roles in the TG, phospholipid, and cholesterol metabolism of hepatocytes, with Kupffer cell-derived prostaglandin E2 promoting decreased synthesis of these lipids in hepatocytes (171). This finding suggests cross-talk between the Kupffer cells and hepatocytes. As obesity is, in some cases, an inflammatory disease, it could be postulated that in the obese state Kupffer cells have a more inflammatory phenotype that promotes increased lipid accumulation/uptake or, alternatively, a reduced TG hydrolysis in neighboring hepatocytes through mediators such as cytokines. In line with this, both nonstimulated and lipopolysaccharide (LPS)-stimulated Kupffer cells were shown to release factors that resulted in an increased VLDL secretion from rat hepatocytes (11). LPS has been reported to promote a
more phospholipid-rich and TG-poor VLDL particle (8), thus potentially leading to a lower secretion and greater retention of TG within the hepatocyte. Additionally, hepatic steatosis in obese rats has been associated with lower phagocytic activity of the Kupffer cells (241). Lack of Kupffer cell activity by pharmacological inhibition resulted in hepatocyte TG accumulation without any inflammatory stimulus (172). Thus, it could be speculated that when TG accumulates without inflammatory stimulus it would lead to a benign phenotype with no dysregulation of other parameters. Understanding the role of Kupffer cells using in vitro models may be critical to understanding their role in the development and progression of NAFLD. This highlights the merits of developing coculture models with hepatocytes and Kupffer cells.

The major function of hepatic stellate cells, in vivo, is the exchange of lipids between blood and sinusoidal regions of the liver along with the regulation of extracellular matrix production and storage of lipids (200). Coculture of hepatocytes with stellate cells either in direct contact or by conditioned media has been reported to help maintain the differentiated function of hepatocytes; beneficial effects of stellate cell coculture are thought to come from soluble factors secreted by stellate cells (120). Thus, stellate cells may play a key role in the development and progression of NAFLD but further studies are required.

Human primary cells currently offer the best model in terms of recapitulation of the in vivo human situation, and work is ongoing to further improve the model using coculture techniques in vitro. In order to fully validate the translational nature of hepatocytes to humans, it would be useful to study cells in parallel with human experimentation. For example, to confirm the ability of cells to reflect in vivo human fatty acid metabolism it would be of interest to use stable isotopes and coculture models.

**Cell Lines**

Hepatocyte cell lines and hepatocyte-like cells derived from stem cells are emerging as alternative models to study the development and progression of NAFLD. Liver cell lines are often obtained by oncogenic immortalization or from hepatic tumors. Despite effort being put into the oncogenic immortalization of adult hepatocytes, results have been disappointing (79). Major advantages of these immortalized cells are their ability to proliferate and the relative ease of cryopreservation; factors to consider are summarized in Table 1.

**Carcinoma cell lines.** Hepatic carcinoma-derived cell lines are the most extensively used human hepatocyte cell lines, and their origin and characteristics are described in Table 1.

**HepG2 cells.** HepG2 is a human hepatoblastoma-derived cell line that has been shown to resemble fetal liver cells, making them unsuitable for investigating drug metabolism (82, 197). In regard to their usefulness as a model to study NAFLD, these cells have, by some, been shown to accumulate TG when exogenous fatty acids were added to the media (135, 139, 233), whereas others report TG accumulation only when oleate is used (128). Unlike with primary cells, culturing HepG2 cells in palmitate alone does not promote TG accumulation (128), although palmitate has been shown to induce insulin resistance through enhanced diacylglycerol (DAG) production (128). This suggests that palmitate is not being further metabolized via esterification, desaturation, or β-oxidation pathways, resulting in accumulation of toxic lipid intermediates. Discrepancies between findings may arise from differences in fatty acid concentrations and culturing times, with longer incubations potentially promoting TG accumulation.

A plausible explanation of why HepG2 cells avidly accumulate TG compared with other cell types is that they secrete relatively dense, TG-poor particles that are more like LDL than VLDL lipoproteins secreted in vivo by mammalian liver (36, 150, 233). The lack of TG-rich VLDL particles secreted (224) may be explained in part by a lack of smooth endoplasmic reticulum (SER), which is required for making TG-enriched globules that can be incorporated into primordial VLDL particles prior to secretion (144). Additionally, the lower VLDL-TG secretion may also be explained by the absence of TG hydrolase, which is involved in the mobilization of TG for VLDL secretion (129). Defective MEK-ERK signaling has also been suggested to contribute to defective VLDL secretion (227). The basal rate of TG secretion from HepG2 cells is low compared with rat primary hepatocytes (233), and very little newly synthesized TG is incorporated into VLDL contributing to intracellular TG accumulation (67). Secretion of VLDL-sized particles can be increased by supplementation with exogenous fatty acids (135, 150) (Table 2). Despite being a human cell line, HepG2 cells do not reflect primary hepatocytes with the lipid content, notably sphingomyelin and TG levels, being much higher than healthy human liver tissue (41, 233).

Despite their limitations, HepG2 cells are one of the few cell lines that have been used to investigate the role of DGAT1 and DGAT2 in TG metabolism. In an elegant piece of work, Wurie et al. (240) demonstrated that DGAT1 and -2 had different roles in TG metabolism, with DGAT2 lying upstream of DGAT1. It was demonstrated that DGAT2 is necessary for incorporation of newly formed (endogenous) fatty acids into TG, whereas DGAT1 incorporates exogenous (preformed) fatty acids and remodels TG acyl composition (240, 244).

It is possible that culture conditions also have an effect on TG accumulation and secretion; for example, increasing glucose concentrations in media was shown to increase TG secretion in HepG2 cells (233). Clavey et al. (31) reported that the batch of fetal calf serum used may influence lipoprotein and apolipoprotein (apoB, apoE, apoC-III) secretion from HepG2 cells. Therefore, both the glucose concentration and the serum batch of fetal calf serum used may influence lipoprotein and apolipoprotein (apoB, apoE, apoC-III) secretion from HepG2 cells. Thus, while the glucose concentration and the serum used may have an impact on the discrepancy in results seen between studies along with the usefulness of HepG2 (and other) cells as a model to study of human NAFLD.

**C3A cells.** C3A cells are a clonal derivative of HepG2, selected for strong contact inhibition of growth, high albumin production, high production of α-fetoprotein (AFP), and ability to grow in a glucose-deficient medium. Clarke et al. (30) have shown that C3A cells accumulate TG when exogenous fatty acids are added to the medium. Additionally, 72h oleate supplementation has been shown to increase TG accumulation without negative effects on mitochondrial respiration (141).

**Huh7/7.5 cells.** Huh7/7.5 cells are derived from a hepatocellular carcinoma (Table 1), are able to be differentiated, and have been extensively used in the study of hepatitis C. Work by Meex et al. (150) clearly demonstrated that Huh7 cells resembled HepG2 cells in the effects of exogenous lipids, MTP inhibition, and proteasome inhibitors of apoB-100 secretion.
recovery, and degradation. Like HepG2 cells, Huh7 cells also resemble fetal liver cells (164, 166), therefore they do not offer advantages over HepG2 cells as a model of NAFLD. Recently, it was reported that the addition of human rather than fetal bovine serum might bring about more human hepatocyte-specific functions, including VLDL secretion in Huh7.5 cells (214). Additionally, Huh7.5 cells are known to express negligible amounts of CIDEB; however, when cultured with human serum, CIDEB expression significantly increased, which resulted in the formation of larger lipid droplets (210). The importance of lipid droplet morphology and function in the development of hepatic steatosis is currently not well characterized; this model may provide a platform for future investigations. This work highlights the importance of culture conditions and has the potential to a useful model for studying NAFLD. If the addition of human rather than fetal bovine serum offers a more representative model of human metabolism, its use should be encouraged. Recently, Ampuero et al. (2) reported that Huh7/7.5 cells are mutant carriers for PNPLA3, which may influence TG accumulation and metabolism. Defining the genotype of other human cell lines would be useful to aid in the interpretation of studies investigating TG metabolism. Studies are needed to characterize and compare the changes in cell function, morphology, and metabolism under different culture conditions.

MCARDLE RAT HEPATOMA CELLS (MCA-RH 7777). Mca-RH 7777 cells were established as a stable cell line in 1974 (14). This cell line has been utilized to study NAFLD progression specifically from steatosis to NASH (75) and understanding the role of ER stress in steatosis (182). Ota et al. (182) demonstrated that incubation of Mca-RH 7777 cells with 1 mM oleic acid or 500 mg/dl Intralipid for 1–48 h increased cellular TG content in a duration-dependent manner. These data clearly demonstrate that this cell-line can be made steatotic with the addition of exogenous fatty acids. Mca-RH 7777 cells actively synthesize and secrete TG-enriched lipoproteins when exogenous fatty acids are added to media (9, 22, 216, 220), and the lipoproteins resemble those found in rat plasma (220). When exogenous fatty acids were removed, cells secrete more TG-poor containing particles, resembling high-density lipoproteins (HDL) (Table 2) (22). It should be noted that there is variation in the serum used to culture these cells, and this may help explain any discrepancies in findings (see Table 2). Recently, Pirazzi et al. (188) utilized this model to generate a cell line that overexpressed the PNPLA3 I148M variant. When incubated in oleate, there was a relatively higher intracellular TG content, lower apoB secretion, and fatty acid efflux in cells overexpressing PNPLA3 148M compared with cells overexpressing PNPLA3 148I wild-type; these data recapitulated the findings in vivo in humans (188). On the basis of these observations, the authors proposed that PNPLA3 148M promotes intracellular lipid accumulation in the liver by reducing the lipidation of VLDL (188). Olate-treated Mca-RH7777 cells overexpressing HSL or ATGL increased fatty acid oxidation and direct release of free fatty acids into culture medium, resulting in decreased intracellular TG (192). On the basis of these findings, the authors suggested that intracellular lipases could be targeted as a potential therapeutic pathway to help ameliorate hepatic steatosis (192).

Taken together, carcinoma-derived hepatic cell lines fail to recapitulate human hepatic fatty acid metabolism, although work to improve this by using different culturing conditions is ongoing. However, due to the high TG content of HepG2 cells, they may be important in understanding the reversal of steatosis. Additionally, Mca-RH 7777 cells are a robust model for understanding steatosis in rodents, although translation to humans may prove challenging.

Fetal liver cells/progenitor cells. Human hepatic stem cells obtained from either embryos (multipotent embryonic stem cells) or somatic adult tissues (pluripotent adult stem cells) are potentially a more reliable source of hepatocytes and overcome inter-donor variability and availability issues that may arise with the use of primary hepatocytes. We have recently been characterizing a human fetal liver hepatocyte cell line and have found it to exhibit many adult human hepatocyte qualities (unpublished data). In this cell line, we have also found that intracellular TG accumulates in a dose-response manner when exogenous fatty acids (a combination of saturated, mono- and polyunsaturated fatty acids) were added to the media (unpublished data). The usefulness of these cells as a model of investigating the development of NAFLD needs to be further explored.

HEPARG. HepaRG is a human biopotent progenitor cell line originally isolated from a grade I liver tumor (76, 143) that appears to express the majority of liver-specific functions, is functionally stable at confluence, gives reproducible and consistent data, and is suggested to be suitable for high-throughput screening. Although HepaRG cells have an indefinite growth potential, when purchased commercially they are terminally differentiated. This cell line has been mostly utilized for drug metabolism and toxicology studies and as yet appears not to be utilized as a model of steatosis; indeed, it remains to be elucidated whether these cells accumulate TG when exposed to exogenous fatty acids. HepaRG cells have been reported to secrete TG- and apoB-containing lipoproteins in a glucose-dependent manner and to express a number of lipogenic genes, which appear not to be glucose dependent (202). More work is required to fully characterize the usefulness of these cells as a model of NAFLD.

HEPATIC PROGENITOR CELLS. Hepatic progenitor cells (HPCs) were first identified in rats and represent a transitional cell type between hepatoblasts and fetal hepatocytes. In adult humans, liver stem cells account for 0.5–2.5% of hepatic cell population in all ages (173) and have been shown initially to express mesenchymal and epithelial liver markers (130). The differentiation of these cells into hepatocytes is poorly understood; they can also be differentiated into cholangiocytes. HPCs are thought to represent a reserve compartment in the liver that is activated only when the mature epithelial cells in the liver are continuously damaged or inhibited in their replication or in cases of severe cell loss (27). HPCs, when differentiated to hepatocyte-like cells, retain expression of liver transcription factors and have been shown to resemble fully differentiated hepatocytes after 14 d (73). Like other cell types described, HPCs lack the majority of CYPs or expression is at very low levels. Recently, HPCs have been suggested to be a source of adiponectin, which is negatively correlated with NAFLD progression (173). To our knowledge, this is the only study to use HPCs to investigate any aspect of hepatic fatty acid metabolism. Their usefulness should be explored further to establish their usefulness as models of NAFLD.
Embryonic and pluripotent stem cells. Both embryonic and adult stem cells (iPSCs) have been reported to be useful models of liver cells following differentiation, although some consideration is required (Table 1). Very recently, iPSCs cells have been used to develop a vascularized three-dimensional (3D) liver-like organ (219), with liver buds (formed from differentiated iPSCs) transplanted into mice becoming vascularized within 48 h reflect liver-like qualities. Hannan et al. (86) reported a culturing protocol for iPSCs to differentiate into hepatocytes. By collecting and culturing iPSCs from patients with different types of liver disease, including glycogen storage disease, Ghodsizadeh et al. (65) were able to develop iPSC models of liver-specific disease. These hepatocyte-like cells expressed a number of liver makers, stored lipid, and took up LDL particles, but removal pathways such as lipoprotein secretion and fatty acid oxidation were not investigated (65). The use of iPSCs is in its infancy; thus, further investigation is required to determine their usefulness as a model of NAFLD. One possible disadvantage this model may have over other cellular models is the very long (30 d) culturing protocol to get hepatocyte-like cells. Very recently, Zhu et al. (249) reported the generation of human fibroblast-derived hepatocytes that are able to repopulate mouse livers. These cells were generated using a modified protocol bypassing iPSCs to generate induced multipotent progenitor cells and subsequently hepatocytes, reducing culturing time (249). Further development and validation of fetal and progenitor cells needs to be carried out to confirm their usefulness as hepatocyte models.

Genetic Manipulation of Liver Cells

In vitro work with hepatocytes and liver cell lines offers the opportunity to genetically manipulate key genes involved in TG metabolism to further unravel their role in the development of steatosis. Expression of PNPLA3 I148M has been undertaken in a variety of cell models, and, in line with human findings, expression in human and rat liver cell lines leads to increased accumulation of TG (91, 186, 188, 198). The expression of PNPLA3 I148M resulted in a reduction of TG hydrolysis, which may help explain the increased TG accumulation (91, 186, 188, 198). Genetic manipulation of other important genes involved in lipid metabolism has also helped provide insight into factors that may influence the development of NAFLD (45, 91, 101, 119, 132, 136, 157, 186, 188, 198). For example, overexpression of FOXO1A in human hepatocytes has been shown to reduce steatosis through inhibition of gene transcription (157). Conversely, overexpression of DGAT1 in human and rat cell lines resulted in increased accumulation of TG (132).

Cell line limitations may be overcome through genetic manipulation. For example, McArdle cells lack phosphatidylethanolamine N-methyltransferase (PEMT), which catalyzes the conversion of phosphatidylethanolamine (PE) to phosphatidylcholine (PC). Noga et al. (176) demonstrated that hepatocytes from PEMT knockout mice secreted 50% less TG in VLDL compared with those of wild-type animals. However, when cells were transfected with PEMT, VLDL-TG secretion increased compared with cells lacking PEMT (176). HepG2 cells stably expressing HSL have increased rates of fatty acid oxidation leading to decreased TG accumulation compared with control cells (184). These studies highlight the potential use of genetic manipulation to improve cell models to resemble a more relevant model.

With all cells, the successful transfection of genetic material can vary and is largely dependent on the vehicle or vector used as a delivery system. Many vehicles for transfection are now commercially available, allowing increased transfection efficiency of previously difficult cells such as primary hepatocytes. Unfortunately, little information is reported regarding how easy individual liver cells or cell lines are for genetic transfection; typically transfection efficiency is reported as a percentage; thus, it is difficult to determine if this is the best obtainable result. The use of viral vectors such as lentivirus or adenovirus has increased over recent years and has significantly aided increased efficiency of genetic transfer to produce stable cell lines. Therefore, it is likely that modern techniques will aid this area of research and help to overcome some of the limitations when liver cell models are used.

EMERGING IN VITRO METHODOLOGIES

Coculture of Primary Hepatocytes

Interaction between hepatocytes and nonparenchymal cells has been shown to modulate cell growth and hepatocyte morphology and increase functionality (16). Studies have demonstrated that coculture of primary hepatocytes with other cell types (including Kupffer cells and fibroblasts) improves hepatocyte viability and function (111, 120). However, studies are still required to explore the potential cross-talk between hepatocytes and other hepatic cells in order to determine the role these may play in the development of steatosis/NAFLD. The coculture of primary hepatocytes with other resident liver cells may be the best way to recapitulate the in vivo liver ex vivo. Many different coculturing techniques exist where cell-cell interaction can be modified by various materials including porous membranes and 3D scaffolds. Culturing primary human and rat hepatocytes in 3D scaffolds significantly increased cell lifespan (151, 206), and culturing in a double extracellular matrix (EM) system has been shown to maintain morphology and polarity for 2 wk compared with the traditional single EM model (116). Coculture 3D models that represent the complex architecture of the liver might serve as important tools in the understanding of NAFLD as these models allow the study of cell-cell cross-talk; further research is still required to optimize the conditions. Of note, during macrovesicular steatosis, the ballooning of hepatocytes changed the way in which the cells interact with hepatic stellate cells, Kupffer cells, endothelial cells, lymphocytes, and fibroblasts (200). Thus, it is likely that coculture may be important to fully understand the progression of NAFLD as interactions and signals from other cell types may play a role.

Liver Organoids

An emerging methodology is the use of pluripotent stem cells to make artificial livers, termed organoids. These organoids are being developed mainly as transplantable cell therapies to treat disease and for regenerative medicine and have been recently reviewed (21, 104, 153). Organoids have been established using two different methodologies: either using a scaffold in which cells are seeded and grown in either bioreactors or in vivo or by the induction of cell regeneration.
As yet, there is no “perfect” in vitro model of human liver metabolism; once taken out of the natural environment, primary cells may alter or in some cases, lose aspects of their normal function. Conversely, cell lines may or may not have had those functions to start with. Given the work being undertaken in culturing techniques and methods (120, 151, 206, 219), some of these limitations may be overcome. Moreover, consideration of an in vivo or in vitro effect is required. For example, using primary hepatocytes derived from rats fed a diet enriched with an n-3 fatty acid versus culturing hepatocytes acutely with n-3 fatty acids resulted in different effects on hepatic TG metabolism between the two models (24). This work highlights the need to establish the physiological conditions in vitro that may reflect in vivo physiology to aid translation. Although there is no ideal cellular model of human liver metabolism, some aspects can complement in vivo investigations if the model chosen is justified for the hypothesis or question being addressed. Thus, on balance it would seem that “man has got to know his limitations” of the (cellular) model being used to aid in the understanding of the aetiology and progression of NAFLD in humans.

CONCLUSION

The etiology and progression of NAFLD in humans are, at this point in time, not well understood, which may be due to the fact these processes take time to develop and are likely to not be due to a single causative factor. As discussed, studying these processes in vivo in humans is challenging. Thus, many investigators have turned to animal and in vitro cellular models to address specific research questions related to human liver metabolism and disease. We have focused here on the cellular models, and on reviewing the literature, it is apparent that the use of these models to investigate NAFLD is in its infancy. More work is required in characterizing and optimizing the cells and cell-lines utilized; genotype information of cells would be advantageous to aid in understanding the models. If working with primary human hepatocytes, consideration for donor variation in genotype and phenotype is required.

When findings from animal studies are compared and they are related to humans, many aspects including species, background strain, sex, dietary regimen, and genetics need to be considered as these may markedly impact on translation. In line with this, in vitro culture conditions, such as the type of serum used (214), the presence of other liver cells (168), the amount of glucose in the media (202), along with the amount and type of fatty acid(s) that are added may alter functionality and lead to discrepancies in findings between studies. In regard to the type of fatty acid(s) added to media, it would seem reasonable to suggest the use of a mixture of fatty acids. The basis of this suggestion stems from the fact that in humans the liver is exposed to a mixture of fatty acids, oleate, palmitate, and linoleate being the most abundant (99).

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MODELS OF HEPATIC FATTY ACID METABOLISM


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