Liver cholesterol: is it playing possum in NASH?

Geoffrey C. Farrell and Derrick van Rooyen

Australian National University Medical School, and Gastroenterology and Hepatology Unit, The Canberra Hospital, Garran, Australian Capital Territory, Australia

Since nonalcoholic steatohepatitis (NASH) became an accepted entity in 1980, its pathogenic mechanisms have remained obscure. Strong associations with central obesity, type 2 diabetes, and dyslipidemia have always suggested NASH is a metabolic disorder (2, 7, 19, 23), and by 2003 the connection to insulin resistance was securely established (6, 21). It has remained unclear, however, why only 10–25% of all those with nonalcoholic fatty liver disease (NAFLD) (25–45% of American adults) have NASH (32). An early suggestion was that this required a separate (second) injury/proinflammatory process, additional to the metabolic factors linked to steatosis (8). While useful in its day to stimulate research, the “two-hit” concept does not explain the strong links between NASH and diabetes or metabolic syndrome, a nexus that infers that the more severe the “metabolic movers”, the more does NAFLD manifest as NASH (6, 19, 21). For this and other reasons recently reviewed (1, 2, 7, 19, 23), most now accept that hepatocyte accumulation of triglycerides (TG) leads to steatosis, but different lipid molecules mediate the pathogenesis of NASH. Such “toxic lipid species” cause hepatocellular injury and cell death (1, 7, 30), directly or indirectly inciting inflammatory and profibrotic responses. This set of pathophysiologic processes is collectively termed lipotoxicity (2, 7, 19, 23).

Lipotoxicity is now the accepted mechanism for pancreatic β-cell injury in type 2 diabetes, intimal damage in atheroma, and cardiac toxicity in metabolic syndrome. Some have even suggested renaming NASH as “liver lipotoxicity” (7). To date, however, the identity of the lipid molecule(s) that causes liver lipotoxicity has remained veiled (2, 7, 23). Perhaps, like the proverbial possum, the lipotoxic assassin is lying low (31). Enter the possum!

Or, more specifically, the North American opossum. Gray short-tailed opossums (Monodelphis domestica) have been used for decades for biomedical research (26). About 20 years ago, one partially inbred line was noted to develop impressive increases in serum lipoproteins in response to an “atherogenic” high-cholesterol, high-fat (HCHF) diet (26). Investigators termed this line “high responders”, and comparisons are made with otherwise similar animals, “low responders”, that do not develop diet-induced hypercholesterolemia. In this issue of American Journal of Physiology-Gastrointestinal and Liver Physiology, Chan and colleagues from the National Primate Research Center, Genetics and Pathology at UT San Antonio report three highly novel findings (5). First, the genetic basis for this type of dietary hypercholesterolemia is a mutation in ABCB4, a phospholipid and cholesterol transporter expressed on the canalicular domain of the hepatocyte plasma membrane, deficiency of which impairs biliary cholesterol excretion. Second, feeding ABCB4 mutant opossums a HCHF diet caused a 10-fold increase of hepatic total cholesterol, more impressive than the 5-fold increase in TG. Third, such hepatic cholesterol accumulation was associated with steatosis, an inflammatory cell infiltration and ballooning of hepatocytes, together with perisinusoidal fibrosis; these are collectively the key findings of NASH (ductular proliferation and extramedullary erythropoiesis were also observed, but are not features of human NASH). Liver histology was normal in low-responder opossums fed the same diet.

So the most salient feature of this novel animal model of NASH is that it does not depend solely on genetic predisposition (like db/db and ob/ob mice) nor on diet alone (e.g., 2% cholesterol HCHF diets in rats or mice). Rather, like humans with NASH (10, 19), it depends on two factors: a genetically predisposed host, who becomes exposed to one or more environmental factors that were not the norm before 1980. In this case, the HCHF diet contained 0.7% cholesterol, considerably less than the 2% cholesterol diets (often with cholic acid) that are toxic to livers of rats and mice (2, 11, 16, 20, 22, 23), not surprisingly as the amount of dietary cholesterol is roughly equivalent to 20 kg/day for humans (>100 hamburgers?) (11).

These are not the first data that incriminate cholesterol as a potential mediator of lipotoxicity in NASH (1, 4, 11, 22, 25). In fact, the few existing human lipidomic data are entirely consistent with one or more cholesterol fractions [free cholesterol (FC), oysterolys, cholesterol esters] associating with the NASH phenotype of NAFLD, in fact more consistently so than for any other lipid class (4, 25). Thus Puri and colleagues (25) found that total cholesterol was higher in NASH livers than in NAFLD without NASH; free fatty acids (FFA) and other lipid fractions were not. Using nonquantitative filipin fluorescence, Caballero et al. (4) also confirmed that FC is present in abundance in human NASH livers. Until recently, the favored “lipotoxic mediator” in NASH has been either FFA, or lysophosphatidylcholine, or other phospholipid metabolites (12); for example, exposing hepatocytes to palmitic acid causes cell death via formation of lysophosphatidylcholine (15). This made sense because the dominant source of fatty acids in livers of obese patients with NASH is from lipolysis of peripheral tissues, such as adipose (7, 9), whereas saturated FFA cause lipotoxic injury to many cell types, including those of hepatic lineage (15, 24). However, an “inconvenient truth” is that there is no difference in hepatic FFA between NASH and not-NASH NAFLD in the few studies in which such data are available (1, 25).

There is other evidence that cholesterol could be implicated in NASH pathogenesis, other than 2% cholesterol high-fat (HF) diet studies, which may, like methionine- and choline-deficient mice that we popularized 16 yr ago (10), really be a model of “toxic steatohepatitis”. Llacuna et al. (20) recently demonstrated that 2% dietary cholesterol causes mitochondrial GSH depletion, rendering mice highly susceptible to ischemia-reperfusion injury. Furthermore, Dutch workers from the lab-
Editorial Focus

and fibrosis. Specifically, hepatic inflammatory cell recruit-
receptors in contributing to hepatic inflammation, apoptosis,
macrophage modified LDL (oxidized and acetylated LDL)
others (16 –18, 27, 28).

tempered in the authors' laboratory and now being used in several
hypothesis neurons, which causes an appetite defect and
heterozygous opossums in that the “genetic predisposition” that under-
limitations are posed by
being a sine qua non for human NASH (6, 7, 19, 21). No such

model of NAFLD.

ApoE2ki mice, despite T0901317-induced elevations in hepatic
et al. (34) subsequently demonstrated that administration of
reduces cholesterol uptake and, in turn, its activation upregulates hepatic cholesterol uptake via
liver X receptor agonist, simultaneously lowered
sterol-regulatory element binding protein 2 (SREBP2)
induces sterol-regulatory element binding protein 2 (SREBP2)
(27). SREBP2 is also upregulated in human NASH livers (4).

cholesterol is now a prime target. Third, the type of model in which to develop mechanism-based
lipotoxic molecules. Cholesterol is now a prime target. Third,
therapies rather than to repeat the numerous false leads in this
model of NASH pathogenesis.

What else can we take away from this important study?
First, there is an urgent need for an agreed set of pathological
criteria for NASH in experimental studies if we are to rid
journal pages of the irrelevant. In our view, we do not under-
stand the objection as to why these should not be the same as
for human NASH. If so, the present model would be one of the
few acceptable ones for valid studies of NASH pathogenesis
in intact animals, whereas purely dietary approaches in mice
(including 2% cholesterol diets) rarely seem to cause the key
features of hepatocyte ballooning and apoptosis, substantial
mixed cell inflammation, and perisinusoidal fibrosis in 24 wk
(16). Second, the focus on human studies should be on rela-
tionships between the pathophysiological changes (such as
insulin resistance), genetic and environmental determinants,
with disease phenotype, and how this is linked to candidate
lipotoxic molecules. Cholesterol is now a prime target. Third,
this is the type of model in which to develop mechanism-based
therapies rather than to repeat the numerous false leads in this
area based on concepts of disease pathogenesis that may be

In the study by Chan et al. (5), changes in hepatic gene
expression provide clues to a different mechanism for disor-
dered hepatic cholesterol turnover in opossums with NASH.
Assuming that the ABCB4 mutation is what leads to hepatic
cholesterol accumulation (there is no evidence of insulin re-
sistance, and SREBP2 was not studied), one might expect
compensatory suppression of cholesterol synthesis via its rate-
limiting step of 3-hydroxy-3-methylglutaryl-CoA reductase,
reduced hepatic cholesterol uptake, and stimulation of choles-
terol biotransformation and canalicular pathways for secretion
of cholesterol and bile acids. Instead, 3-hydroxy-3-methylglu-
taryl-CoA reductase mRNA levels were 3.8-fold higher in higher
responders than low responders (enzyme activity would have
been of interest for this complexly regulated protein). On the
other hand, LDLR mRNA values were lower in high respond-
ners, as was CYP27A1, which encodes the second pathway of
cholesterol biotransformation to bile acids (values for CYP27A1
mRNA were not different). Also suppressed were genes
involved in canalicular secretion of cholesterol [ABCG8, Ni-
emann-Pick C1-like protein (NPC1L1), but not the bile salt
export pump]. Without study of the regulatory proteins involved
(SREBP2, liver X-receptor, farnesyl X-receptor, short
heterodimeric partner, and liver receptor homolog 1), these
data are incomplete [for a more comprehensive overview of
hepatic cholesterol physiology, the reader is referred to a recent
review (29)]. However, the general observation accords with
our findings in HF-fed foz/foz mice, that is, that physiological
responses to hepatic cholesterol accumulation in disease situa-
tions can be paradoxical (apparently inappropriate) and are
clearly not always sufficient to remove the hepatic cholesterol
load. In the case of ABCB4 mutant opossums consuming a
moderate-cholesterol, high-saturated-fat diet, this leads to
“dysregulation” of hepatic cholesterol homeostasis that excer-
bates cholesterol accumulation in the liver and also facilitates
steatosis. The mechanisms for such dysregulation of choles-
terol homeostasis in this and other models, and the way in
which cholesterol causes liver injury, inflammation, and fibro-
sis should provide fascinating insights into NASH pathogene-

oratory of Hofker and Shiri-Svedlov identified a strong asso-
ciation between steatohepatitis and dietary cholesterol in hu-
manized apolipoprotein E2 knock-in (ApoE2ki) and LDL
receptor (LDLR−/−) C57/B16 mice (33). Feeding such mice a
HF diet containing 0.2% cholesterol increases hepatic total
cholesterol, with concomitant elevations in hepatic CD11b-
positive cell infiltration and CD68 mRNA expression, as well
as significant increases in hepatic monocyte chemotactic pro-
tein-1 and tumor necrosis factor gene expression (33). Wouters
et al. (34) subsequently demonstrated that administration of
T0901317, a liver X receptor agonist, simultaneously lowered
hepatic cholesterol content and CD11b cell recruitment in
ApoE2ki mice, despite T0901317-induced elevations in hepatic
TG. Separately, the same workers demonstrated a role of
macrophage modified LDL (oxidized and acetylated LDL)
receptors in contributing to hepatic inflammation, apoptosis,
and fibrosis. Specifically, hepatic inflammatory cell recruit-
ment, apoptosis, and fibrosis are decreased in C36D and
macrophage scavenger receptor (Msr)-1 deficient (CD36−/−/
Msr1−/−) chimeric LDLR−/− mice fed HF 0.2% cholesterol
vs. CD36+/−/Msr1+/+ bone marrow controls (3). These data
suggest that macrophage-dependent uptake of modified LDL
cholesterol contributes to NASH severity in the LDLR−/−
model of NAFLD.

A limitation of the above studies is that the mice were
generally not obese, diabetic, or insulin resistant (16), the latter
being a sine qua non for human NASH (6, 7, 19, 21). No such
limitations are posed by Aims1 mutant (foz/foz) mice charac-
terized in the authors’ laboratory and now being used in several
others (16–18, 27, 28). Foz/foz mice differ from ABCB4 mu-
ant opossums in that the “genetic predisposition” that under-
lies NASH is not in lipid handling: it is in ciliol anchoring to
hypothalamic neurons, which causes an appetite defect and
leptin resistance (13). After early-onset obesity, foz/foz mice
develop insulin resistance, diabetes, and dyslipidemia associ-
ated with hypoadiponectinemia (18), the metabolic accompa-
niments of human NASH (7, 19). Like high-responder opos-
sums, foz/foz mice on an appropriate strain background (NOD-
B10 or C57/B16, but not Balb-c) develop fibrotic NASH (17,
18), but only when fed a 0.2% cholesterol HF diet (18, 27),
again reflecting the need for both genetic and environmental
factors in NASH pathogenesis.

In HF-fed foz/foz mice with NASH, hepatic total cholesterol
is elevated 200-fold, an order of magnitude higher than the
increase in TG and FFA, and FC is ~5-fold increased over
both genotype and dietary controls (27). We have been able to
attribute the mechanism of such hepatic cholesterol accumula-
tion to hyperinsulinemia, by showing directly that this is what
induces sterol-regulatory element binding protein 2 (SREBP2)
(27). SREBP2 is also upregulated in human NASH livers (4).
In turn, its activation upregulates hepatic cholesterol uptake via
LDLR and suppresses cholesterol biotransformation to form
bile acids (via CYP7A1 and 27A1) and secretion into bile (via
ABCG5/8 or ABCB11, the bile salt export pump) (27, 29). Our
laboratory has also shown that correcting liver cholesterol
fractions (total, FC, oxysterols) by dietary restriction (27) or
pharmacological therapy (28) reverses liver injury, with corre-
sponding reversal of liver pathology from inflammation, apop-
tosis, hepatocyte ballooning, and fibrosis (NASH) back to
simple steatosis. Conversely, other lipid fractions, such as TG,
diacylglycerides, total, saturated, unsaturated, and individual
FFA, and phospholipids do not change in response to the
dietary and drug interventions that modulate disease phenotype
(27, 28).
flawed. Finally, it is high time that investigators and journal editors paid more attention to the possum and to other models of NASH that incorporate all three key elements of NASH pathogenesis: a genetically predisposed host, an altered environment, and resultant metabolic disorder. Run possum run!

DISCLOSURES
No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS
G.C.F. and D.M.V.R. drafted manuscript; G.C.F. edited and revised manuscript; G.C.F. approved final version of manuscript.

REFERENCES