Studies of human mutations have paved the way toward understanding elevated plasma cholesterol levels, both by exposing the mechanisms of lipid transport and by focusing hypotheses for further studies (1, 2). In this issue, Pullinger et al. (3) studied a kindred with hypercholesterolemia and identified a mutation in CYP7A1, which encodes cholesterol 7α-hydroxylase, the first and rate-limiting step in the classical bile acid synthetic pathway (4). As has been the case before, this new mutation has simultaneously shed light on mechanisms of lipid metabolism and raised a welter of issues for future investigation. The study by Pullinger et al. (3) must have been supremely gratifying for the authors: They started with an imaginative hypothesis about hypercholesterolemia — one rooted in simple clinical findings — and, after some serious screening efforts, hit pay dirt. They hypothesized that CYP7A1 deficiency would reduce the conversion of cholesterol to bile acids, resulting in elevated liver cholesterol levels, downregulated LDL receptors, and hypercholesterolemia. They further hypothesized that the increased hepatic cholesterol levels would render these patients resistant to the hypocholesterolemic effect of statins and that CYP7A1 deficiency would cause premature gallstone disease, a result of reduced bile acid secretion rates. By screening appropriate patients from their lipid clinic, they identified two brothers, both with premature gallstone disease and statin-resistant hypercholesterolemia, who were homozygous for a […]

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While the guiding hypothesis of Pullinger et al. (3) is attractive, we believe that a cautionary note should be sounded about the hyperlipidemic phenotype. Because the authors identified a hyperlipidemic kindred within a tertiary care lipid clinic, the possibl-
ity of ascertainment bias must be con-
considered, particularly since their kind-
dreds was not particularly large and
since the conclusions were based on a
limited number of lipid measure-
ments. More kindreds with CYP7A1
mutations will be needed if the pheno-
type is to be defined conclusively.

Assuming that the hypercholes-
terolemic phenotype is upheld by
future studies, it will be crucial to
explore mechanisms. Their hypothe-
sis holds that CYP7A1-deficient sub-
jects have elevated liver cholesterol
levels leading to reduced LDL recep-
tor activity and retarded removal of
LDL from the bloodstream. This
hypothesis must be tested. The VLDL
and LDL turnover rates in CYP7A1-
deficient subjects should be meas-
ured and compared with those in
both normal controls and subjects
with the heterozygous form of famil-
ial hypercholesterolemia.

CYP7A1 and triglycerides
A surprising and unexplained finding
in the current study was the observa-
tion that two of the three homozy-
gotes were hypertriglyceridemic. For
years, lipidologists have observed
hypertriglyceridemia in patients on
bile acid sequestrants, where bile acid
synthesis rates are high (7, 8). It seems
curious that low levels of bile acid syn-
thesis, as in CYP7A1 deficiency, should
also be associated with hypertriglyc-
eridemia. Several conceivable mecha-
nisms for hypertriglyceridemia could
be proposed. Low levels of bile acids
might downregulate farnesoid X
receptor–responsive gene products,
including apoC-II, an activator of
lipoprotein lipase, the enzyme that
removes triglycerides from the plasma
(9). Alternatively, it is possible that ele-
vated oxysterol levels in the liver might
induce liver X receptor–responsive
genes, leading to increased SREBP-1C
expression and increased triglyceride
synthesis (10, 11). Consistent with the
latter model, Pullinger and colleagues
(3) found higher levels of an oxysterol-
producing enzyme (sterol-27 hydroxyl-
ase) in a liver biopsy from one of their
homozygotes. However, it is not clear
that elevated levels of the enzyme
would necessarily cause higher steady-
state levels of oxysterols in hepato-
cyes, as the oxysterols can be convert-
ed rapidly to bile acids via an alternate
pathway not involving CYP7A1.

Qualitative and quantitative effects
on bile acids
Pullinger et al. (3) report a 94% reduc-
tion in bile acids excretion in one of
their homozygotes. In a healthy per-
son, cholic acid synthesis exceeds syn-
thesis of chenodeoxycholic acid by a
factor of two. In their patient, fecal bile
acid analysis showed that the major bile
acid formed was chenodeoxycholic
acid. The fact that CYP7A1 deficiency
did not abolish bile acid formation is
not surprising, since there are several
pathways, differing in their initial
steps, for conversion of cholesterol
into bile acids (Figure 1) (12, 13). The
classical neutral pathway involves
CYP7A1, which directly converts cho-
lesterol into 7α-hydroxycholesterol
(Figure 1a). In the alternate acidic
pathway, cholesterol is first converted
by sterol hydroxylases into oxysterols,
the major one being 27-hydroxychole-
steryl. These oxysterols then under-
go 7α-hydroxylation by an oxysterol
7α-hydroxylase, either CYP7B1 or
CYP39A1 (Figure 1b). Subsequent
enzymatic steps lead to the preferential
conversion of 7α-hydroxycholesterol
into cholic acid and the preferential
conversion of 7α-hydroxy-oxysterols
into chenodeoxycholic acid.

The shift in primary bile acid synthe-
sis toward chenodeoxycholic acid is
entirely consistent with decreased
CYP7A1 activity and compensation by
the acidic pathway. Of note, a low bile
acid synthetic rate and preferential
formation of chenodeoxycholic acid were
observed previously in two hypercho-
lesterolemic patients whose bile acid
synthesis profile was characterized by
Einarsson et al. (14) a quarter century
ago. We wonder whether those patients
might have had CYP7A1 mutations.

The 94% reduction in fecal bile acid
excretion (equivalent at steady state
to hepatic bile acid synthesis) was
observed in a single 24-hour stool col-
collection in a single subject. A longer
stool collection would seem essential
for quantifying accurately the de-
creased rate of bile acid synthesis.
Future studies should be directed at
clarifying this issue as well as deter-
mining the rate of bile acid secretion,
which can be measured by duodenal
intubation and isotopic tracer studies.
Better characterization of bile acid
biosynthesis in human CYP7A1-defi-
cient patients is particularly important
given a distinct lack of evidence for
cholesterol or vitamin malabsorption
in those individuals. Given that ade-
quate intraluminal concentrations of
bile acids are essential for the absorp-
tion of cholesterol and fat-soluble vita-
mins, it seems possible that increased
intestinal reclamation of bile acids in
CYP7A1-deficient patients may have
compensated for their decrease in bile
acid biosynthesis. In the guinea pig,
low concentrations of bile acids within
the lumen of the intestine upregulate
the ileal bile acid transport system
(15).

Differences in CYP7A1-deficient
mice and humans
The normal cholesterol absorption
and normal vitamin E levels in humans
with CYP7A1 deficiency con-
trasts with findings in Cyp7a1-defi-
cient mice, where the bile acid pool
size is reduced by 80%. In those mice,
cholesterol absorption is almost abol-
ished (16), and tissue vitamin E levels
are markedly reduced (17). Pullinger et
al. (3) also found triglyceride malab-
sorption in one of the homozygotes —
a very unexpected finding given that
triglyceride absorption is normal in
the Cyp7a1-deficient mice (16).

A single liver biopsy in one subject
suggested that liver cholesterol levels
might be increased in CYP7A1-defi-
cient humans. That observation,
although in accordance with the authors’ hypothesis, would not have
been predicted from animal models
(16). In chow-fed Cyp7a1-deficient
mice, liver cholesterol levels are not
increased, and cholesterol synthesis
rates actually increase, likely secondary
to reduced absorption of cholesterol
in the intestine (16). When additional
humans with CYP7A1 deficiency are
identified — if liver biopsies are possi-
ble — it will be very interesting to
determine if elevated liver cholesterol
levels are a consistent feature of
CYP7A1 deficiency. It will also be desir-
able to examine the expression level of
the LDL receptor and other gene prod-
ucts regulated by SREBPs.

Additional mysteries are apparent
when one compares Cyp7a1-deficient
mice and the CYP7A1-deficient
humans. The humans were hyperlipi-
demic, but the mice were not (17), at
least when originally characterized
(although see refs. 18–20 for evidence
of hypercholesterolemia in some genet-
ic backgrounds). Also, in the case of
the mice, Cyp7a1 deficiency abolished cho-
lesterol 7α-hydroxylase activity in the liver, but in the humans, the liver biopsy study suggested that cholesterol 7α-hydroxylase activity was reduced by only ~70%. This difference raises the possibility that some other enzyme, perhaps an oxysterol 7α-hydroxylase, can hydroxylate the C-7 position of cholesterol in humans. Finally, increased sterol-27 hydroxylation levels in a liver biopsy from one of the CYP7A1-deficient patients suggest that the oxysterol pathway is upregulated in humans, a response that was not observed in Cyp7a1-deficient mice (13).

Cyp7a1 expression is critical for mice in the postnatal period because Cyp7b1 expression is not turned on until 3 weeks of age. Most Cyp7a1-deficient mice die before weaning unless they are supplemented with cholic acid and fat-soluble vitamins (21). In contrast, as noted above, there was no indication that the CYP7A1-deficient patients had significant fat malabsorption or fat-soluble vitamin deficiencies as children. Thus, the activity of alternate bile acid synthesis pathways together with intestinal conservation of bile acids was apparently sufficient to prevent overt lipid malabsorption.

Humans and mice also exhibit different phenotypes for mutations in other bile acid metabolism–related proteins. A human infant lacking CYP7B1, who died with severe biliary cholestasis, has been described (22). In contrast, Cyp7b1-deficient mice appear perfectly healthy (23). Mice deficient in the canalicular bile salt export pump have mild cholestasis (24), whereas humans with this defect develop progressive familial cholestasis, a merciless pediatric disease causing early cirrhosis (25). Loss of sterol 27-hydroxylase in humans causes a striking deficiency of bile acids, cerebrotendinous xanthomatosis, and an accumulation of cholestanol in tissues (26–28). Mice with the same enzyme defect have a bile acid synthesis defect but do not develop cerebrotendinous xanthomatosis. Instead, they manifest elevated triglyceride levels in liver and plasma (29). Thus, when it comes to disorders of bile acid and cholesterol metabolism, clinical investigators would be wise to study both mice and humans.


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