Fig. 1. Seasonal gallstone prevalence in wild deer mice as a function of seasonal fecal bile acid (BA) content. Seasonal fecal BA contents have been phase-advanced 3 months. Data derived from Ginnett et al.⁹ The seasons for collection of mice for gallstone prevalence determination are indicated.

prevalence strongly relates to fecal BAs (Fig. 1). From analysis of gastric contents it was concluded⁹ that the intake of dietary fiber may contribute to the fecal loss of BAs, providing a potential physiologic and nutritional mechanism for cholesterol gallstone formation.

Direct measures of the fecal excretion of BAs in human subjects with gallstone disease are now warranted to confirm if an increased fecal excretion of BAs in gallstone disease as reported¹⁰ remains when investigated on large series of patients.

MATS RUDLING, M.D., PH.D., PROFESSOR

Karolinska Institute, Center for Endocrinology, Metabolism, and Diabetes, Department of Medicine, Karolinska University Hospital, Huddinge, Stockholm, Sweden, and Molecular Nutrition Unit, Department of Biosciences and Nutrition

References

- Bertolotti M, Gabbi C, Anzivino C, Carulli L, Carulli N. Changes in bile acid synthesis in gallstone disease: cause, consequence, or neither? *HEPATOLOGY* 2007;46:1664; author reply 1664-1665.
- Galman C, Miquel JF, Perez RM, Einarsson C, Stahle L, Marshall G, et al. Bile acid synthesis is increased in Chilean Hispanics with gallstones and in gallstone high-risk Mapuche Indians. *Gastroenterology* 2004;126:741-748.
- Bertolotti M, Gabbi C, Anzivino C, Mitro N, Godio C, De Fabiani E, et al. Decreased hepatic expression of PPAR-gamma coactivator-1 in cholesterol cholelithiasis. *Eur J Clin Invest* 2006;36:170-175.
- Reihner E, Angelin B, Bjorkhem I, Einarsson K. Hepatic cholesterol metabolism in cholesterol gallstone disease. *J Lipid Res* 1991;32:469-475.
- Galman C, Arvidsson I, Angelin B, Rudling M. Monitoring hepatic cholesterol 7 α -hydroxylase activity by assay of the stable bile acid intermediate 7 α -hydroxy-4-cholesten-3-one in peripheral blood. *J Lipid Res* 2003;44:859-866.
- Muhrbeck O, Wang FH, Bjorkhem I, Axelson M, Einarsson K. Circulating markers for biosynthesis of cholesterol and bile acids are not depressed in asymptomatic gallstone subjects. *J Hepatol* 1997;27:150-155.
- Sauter GH, Munzing W, von Ritter C, Paumgartner G. Bile acid malabsorption as a cause of chronic diarrhea: diagnostic value of 7 α -hydroxy-4-cholesten-3-one in serum. *Dig Dis Sci* 1999;44:14-19.
- Sauter GH, Moussavian AC, Meyer G, Steitz HO, Parhofer KG, Jungst D. Bowel habits and bile acid malabsorption in the months after cholecystectomy. *Am J Gastroenterol* 2002;97:1732-1735.
- Ginnett DA, Theis JH, Kaneko JJ. Spontaneous gallstone formation in deer mice: interaction of cholesterol, bile acids, and dietary fiber. *J Wildl Dis* 2003;39:105-113.
- Mamianetti A, Garrido D, Carducci CN, Vescina MC. Fecal bile acid excretion profile in gallstone patients. *Medicina (B Aires)* 1999;59:269-273.

Copyright © 2008 by the American Association for the Study of Liver Diseases.

Published online in Wiley InterScience (www.interscience.wiley.com).

DOI 10.1002/hep.22168

Potential conflict of interest: Nothing to report.

Reply:

We deeply appreciate the interest of Dr. Rudling in our short comment. As correctly pointed out, the numbers of subjects described in individual studies where hepatic tissue sampling is required are necessarily limited. Studies using surrogate markers of bile acid synthesis such as 7 α -hydroxy-4-cholesten-3-one (C4) can allow for inclusion of patient numbers of an almost "epidemiological" size.

Such evidence leads to consideration of bile acid malabsorption, and consequently increased bile acid synthesis, as a risk factor for gallstone formation. We completely agree with this view, already supported by older evidence in the literature of patients with small bowel disease.¹

What we disagree on is the view of this as a general (and therefore, global) finding in patients with gallstones, and as a major, or only, cause of gallstone formation; the fact itself that such large numbers are required to disclose statistical significance on a 30%-40% difference points to an epidemiological association more than to a universal pathophysiological cause-and-effect relationship.

Indeed, a look at the literature reveals a body of evidence that is against an increase in bile acid synthesis as a prominent feature in gallstone disease. In older papers by Nilsell et al.² and Berr et al.,³ when bile acid synthesis was measured *in vivo* by isotope techniques, no difference in total primary bile acid synthesis was detected between patients with gallstones and control patients. Once again, in individual studies the numbers are not impressive, but this is the evidence that can be collected in human studies of such complexity.

Moving to *in vitro* studies, none of the pieces of evidence reported up to now, as far as we know, have shown increased CYP7A1 activity in gallstone disease, with some studies suggesting a reduced rate⁴⁻⁶ and others showing no differences.⁷⁻⁹

No significant differences were detected in tissue messenger RNA expression of CYP7A1 in our own data (Bertolotti et al.¹⁰, and unpublished data, summing up 13 control subjects and 16 patients with gallstones) and in a recent paper by Jiang et al. on 22 Chinese patients with gallstones compared with 13 matched controls.¹¹

Adding up all figures derived from these studies, we total nearly 40 patients in each experimental group in the two *in vivo* studies, and nearly 100 control subjects versus more than 130 patients with gallstones with data *in vitro*. Even keeping in mind the extreme heterogeneity among these studies and the fact that most of them were performed decades ago, such evidence is far more than anecdotal.

Is there any reason why indirect measurement of CYP7A1 activity by assay of plasma C4 levels should yield results which are discrepant with *in vitro* assay of CYP7A1 expression and activity, or with other measurements of bile acid synthesis? This cannot be excluded, but as far as we know there is no evidence supporting this view.

The data provided by Rudling on mice are interesting and thought-provoking; once again, other pieces of evidence where mice overexpressing CYP7A1 show reduced risk for gallstone formation reveals another side to this story.¹²

In the article by Muhrbeck et al.,¹³ quoted by Rudling, the authors themselves conclude that “the increased biliary cholesterol saturation and cholesterol secretion in patients with gallstones is not the result of a single monogenic defect but rather the aetiology of cholesterol gallstones is multifactorial”. A recent review on the genetic background of cholesterol cholelithiasis¹⁴ is also in line with this view.

We believe that, in close analogy with other chronic diseases (for example, atherosclerosis), the etiology of cholesterol gallstone disease is to be considered multifactorial; in this context, we all agree that some “risk factors” may be identified, among which are defects in bile acid absorption. Such conditions, much like dyslipidemia or hypertension for cardiovascular diseases, may certainly be relevant even if not universally present. It is not paradoxical that reduced bile acid synthesis may also represent a risk factor in specific situations, for example, patients receiving fibrate treatment or with the extremely rare condition of genetic deficit of CYP7A1 already mentioned in our letter.

Further research on the interrelationships underlying the control of bile acid recirculation along the enterohepatic axis is certainly required to provide clearer insight in this field. Because the observed changes, as pointed out by Dr. Rudling, may precede gallstone formation, proper identification of the subgroups of patients with higher risk for gallstone development might be relevant in the perspective of prevention.

MARCO BERTOLOTI

CHIARA GABBI

CLAUDIA ANZIVINO

LUCIA CARULLI

NICOLA CARULLI

*Dipartimento di Medicina, Endocrinologia, Metabolismo e Geriatria
Università degli Studi di Modena e Reggio Emilia
Modena, Italy*

References

1. Rutgeerts P, Ghooys Y, Vantrappen G, Fevery J. Biliary lipid composition in patients with nonoperated Crohn's disease. *Dig Dis Sci* 1986;31:27-32.
2. Nilsell K, Angelin B, Liljeqvist L, Einarsson K. Biliary lipid output and bile acid kinetics in cholesterol gallstone disease. Evidence for an increased hepatic secretion of cholesterol in Swedish patients. *Gastroenterology* 1985;89:287-293.
3. Berr F, Pratschke E, Fischer S, Paumgartner G. Disorders of bile acid metabolism in cholesterol gallstone disease. *J Clin Invest* 1992;90:859-868.
4. Salen G, Nicolau G, Shefer S, Mosbach EH. Hepatic cholesterol metabolism in patients with gallstones. *Gastroenterology* 1975;69:676-684.
5. Carulli N, Ponz de Leon M, Zironi F, Pinetti A, Smerieri A, Iori R, et al. Hepatic cholesterol and bile acid metabolism in subjects with gallstones: comparative effects of short-term feeding of chenodeoxycholic and ursodeoxycholic acid. *J Lipid Res* 1980;21:35-43.
6. Key PH, Bonorris GG, Marks JW, Chung A, Schoenfield LJ. Biliary lipid synthesis and secretion in gallstone patients before and during treatment with chenodeoxycholic acid. *J Lab Clin Med* 1980;96:816-826.
7. Reihner E, Angelin B, Björkhem I, Einarsson K. Hepatic cholesterol metabolism in cholesterol gallstone disease. *J Lipid Res* 1991;32:469-475.
8. Honda A, Yoshida T, Tanaka N, Matsuzaki Y, He B, Osuga T, et al. Hepatic cholesterol and bile acid synthesis in Japanese patients with cholesterol gallstones. *Gastroenterol Jpn* 1993;28:406-414.
9. Ito T, Kawata S, Imai Y, Kakimoto H, Trzaskos JM, Matsuzawa Y. Hepatic cholesterol metabolism in patients with cholesterol gallstones: enhanced intracellular transport of cholesterol. *Gastroenterology* 1996;110:1619-1627.
10. Bertolotti M, Gabbi C, Anzivino C, Mitro N, Godio C, De Fabiani E, et al. Decreased hepatic expression of PPAR- γ coactivator-1 in cholesterol cholelithiasis. *Eur J Clin Invest* 2006;36:170-175.
11. Jiang Z-Y, Parini P, Eggertsen G, Davis MA, Hu H, Suo G-J, et al. Increased expression of *LXR α* , *ABCG5*, *ABCG8* and *SRBI* in the liver from normolipidemic nonobese chinese gallstone patients. *J Lipid Res* 2008;49:464-472.
12. Moschetta A, Kliewer SA. Weaving β Klotho into bile acid metabolism. *J Clin Invest* 2005;115:2075-2077.
13. Muhrbeck O, Wang F-H, Björkhem I, Axelsson M, Einarsson K. Circulating markers for biosynthesis of cholesterol and bile acids are not depressed in asymptomatic gallstone subjects. *J Hepatol* 1997;27:150-155.
14. Kusters A, Jirsa M, Groen AK. Genetic background of cholesterol gallstone disease. *Biochim Biophys Acta* 2003;1637:1-19.

Copyright © 2008 by the American Association for the Study of Liver Diseases.
Published online in Wiley InterScience (www.interscience.wiley.com).

DOI 10.1002/hep.22260

Potential conflict of interest: Nothing to report.