Epidemiology of the American Indians' Burden and Its Likely Genetic Origins

Martin C. Carey[1] and Beverly Paigen[2]

It was not known until recently whether the endemic of cholesterol gallstones among certain southwestern American Indian tribes was unique among this ethnic group. With use of ultrasonography of the gallbladder and standard diagnostic criteria, gallstones are now found in epidemic proportions in 13 diverse American Indian tribes and communities living in Arizona, Oklahoma, and the Dakotas. We speculate that this predisposition is polygenic involving "thrifty" genes that conferred survival advantages when Paleo-Indians migrated from present-day Siberia to the Americas during the last Great Ice Age approximately 50,000 to 10,000 years ago. A reasonable hypothesis is that functioning of these genes promoted more efficient calorie utilization and storage in the form of adipose tissue. Beneficial results would have been operative during the isolation of Paleo-Indians in the Bering Strait land bridge (Beringia) when thrifty genes would have ensured sufficient fat reserves for survival of prolonged winters, successful pregnancy outcomes, and extended lactation periods. The authors' conjoint work on genetics of experimental cholesterol cholelithiasis in inbred mice promises help in pinpointing orthologous genetic loci (*LITH* genes) in the human genome. Moreover, the shared environments and homogeneity of American Indian tribes and communities should facilitate discovery of the ensembles of their common and rarer cholesterol gallstone genes. It is anticipated that knowledge of expression, polymorphisms, and functionality of *LITH* genes will help resolve the molecular mechanisms of this complex heterogeneous trait and thereby provide targets for novel therapies to prevent cholesterol cholelithiasis worldwide. (HEPATOLOGY **2002;36:781-791.**)

In 1962, Sievers and Marquis[1] at the US Public Health Service Indian Hospital in Phoenix labeled gallstone disease the "American Indian's burden." They were not alone in being impressed by the frequent clinical evidence of both gallstones and their ominous complication, gallbladder cancer, among American Indian tribes living in southwestern Arizona.[1] Franz J. Ingelfinger,[2] the doyen of American gastroenterology at the time, became acquainted with the high prevalence of gallstones among southwestern American Indian tribes from Dr. John S. Fordtran, now of Dallas, TX. For the several years preceding his arrival at Boston University Medical Center in 1960 to begin his fellowship in gastroenterology, Fordtran was in the Public Health Service at the Navajo Medical Center in Fort Defiance, AZ. He recalls[3] that he saw an astonishing number of men and women with gallstones among Navajo, Zuni, and Hopi Indians who lived in surrounding mesas. He recollected that while most American Indians were obese, many were not, and "you did not have to be obese to have gallstones." Fordtran told his former mentor about the extraordinarily high prevalence of gallstones among the Fort Defiance Indians. Ingelfinger did not believe him and decided to see for himself.[3] So he and Fordtran traveled to Fort Defiance, where they spent several days mulling over patients' charts, interviewing two surgeons who did the gallbladder surgery, and seeing patients. It was only then that Franz Ingelfinger became convinced of the enormity of the American Indian gallstone problem. As he said of himself, ". . .but sinners, when they see the light, feel with greater violence than do saints."[4] With the obsession of a convert, Ingelfinger vigorously promulgated gallstone research for-

Abbreviations: CYP7A1, cholesterol 7α-hydroxylase; LXRα, liver-X-receptor α; FXR, Farnesol-X-receptor; BP, before present; QTL, quantitative trait locus; PRARγ, peroxisome proliferator-activated receptor gamma.

From the [1]Department of Medicine, Harvard Medical School and Digestive Diseases Center, Gastroenterology Division, Brigham and Women's Hospital, Boston, MA; and [2]The Jackson Laboratory, Bar Harbor, ME.

Received March 26, 2002; accepted August 12, 2002.

Supported, in part, by the National Institute of Diabetes, and Digestive and Kidney Diseases (U.S. Public Health Service) grants DK51568, DK51553, DK36588, DK34854, and DK52911.

Address reprint requests to: Martin C. Carey, M.D., D.Sc., Gastroenterology Division, Thorn 1430, Brigham & Women's Hospital, 75 Francis St., Boston, MA 02115. E-mail: mccarey@rics.bwh.harvard.edu; fax: 617-730-5807.

0270-9139/02/3604-0002$35.00/0

doi:10.1053/jhep.2002.36545

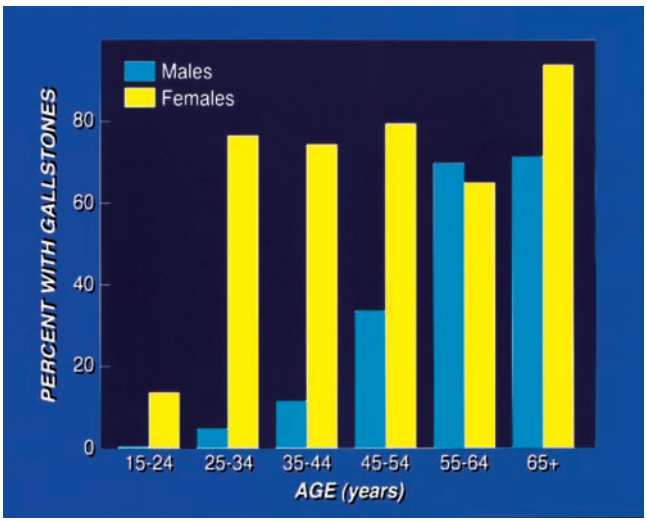

Fig. 1. True prevalence rates of gallstones as functions of increasing age in male and female Pima Indians, redrawn from the classic 1970 study of 596 subjects,[8] wherein ascertainment was based on prior cholecystectomy and oral cholecystography.

ever after[2]; he even carried around an American Indian gallstone, "a large cholesterol solitaire," which he brought to Bethesda, MD, to highlight the enormity of the American Indian problem to the officials at the NIH.[5] Dr. Donald M. Small[5] recalls that this is what got Franz Ingelfinger "his first big gallstone grant."

In his 1968 position paper,[2] Ingelfinger penned a bold manifesto: "The study of gallstones has been grossly neglected." He also noted that gallstones were an illness characteristic of four recent US presidents and that removing the gallbladder was big business, sometimes with unhappy results such as the "double cystic duct" mishap involving a well-known British Foreign Secretary. Moreover, Ingelfinger called for precise surveys of both asymptomatic and symptomatic gallstone patients, claiming that such an exercise would define the magnitude of the problem and identify risk factors such as habits, diet, and genetics.[2] Thinly veiled in his editorial was the fact that he had already catalyzed such a study in Pima Indians through the NIH, employing an English rheumatologist turned diabetologist named Dr. Peter H. Bennett.[6] In addition, he had also sent Dr. Donald Small to the Pasteur Institute in Paris (1963-1965)[5] to Dr. Dikran Dervichian, head of the Service de Biophysique, to learn by means of a thorough phase equilibrium study of model biliary lipid systems[7] how gallbladder bile could become supersaturated with cholesterol.

The classic Pima study was an age- and sex-stratified randomized sample of 596 American Indians, with prevalence rates of gallstones inferred from history of cholecystectomy plus positive oral cholecystography.[8] True prevalence rates (Fig. 1) showed that, although infrequent during adolescence, gallstones appeared explosively during the early 20s reaching a maximum in the third and fourth decades in women and somewhat later in men, with as many as 80% of women and 70% of men cumulatively affected. Clinical prevalence, *i.e.*, symptoms or prior cholecystectomy,[8,9] in the same population was 50% in women and 20% in men. This landmark study found no association of gallbladder disease with obesity (present in 84%), diabetes mellitus (34%), or parity (median 3-4 children per family).[8] Since at about the same time it was established that relative, as opposed to absolute, content of cholesterol was the quotient that resulted in supersaturation of bile,[10] an enormous flowering of studies on the pathophysiology of "lithogenic" bile as well as risk factors and true prevalence rates of gallstones followed worldwide (summarized in Paigen and Carey[11]). However, the lithogenic potential of the Pima appeared unique; the biles of these individuals, irrespective of gender, became markedly supersaturated with cholesterol during puberty.[12] In another American Indian population (Chippewa),[13] lithogenic bile was more common and severe among adolescent American Indian women with normal cholecystograms than in matched white controls. It was established in the Pima that the physical stages of stone disease, *i.e.*, nucleation, crystal formation, and stone growth, were delayed approximately 7 to 10 years after puberty.[8] Symptoms, if they occurred, did not appear for another 5 to 10 years.[9]

Several important demographic insights on gallstones are revealed by these and later epidemiologic studies.[11] Gallstone prevalence rates among Hispanic subgroups[14] and the white population of the United States[15] were surprisingly high, as were annual health care costs for treating gallstone patients,[16] amounting to $6.5 billion for the 20 million Americans with gallstones.[16] Gallstones are also remarkably common in all western European countries and countries to which Europeans migrated.[11] However, the disease is rare in much of sub-Saharan Africa, in the Middle and Far East, and in rural Asia.[11] Smaller clinical studies of gallstones in other North American Indians suggested that Chippewas, Micmacs, and Cree-Ojibwas most likely would express high true prevalence rates based on the ratio of true to clinical prevalence rates in the Pima.[8,9,11] Among US Hispanics, Mexican Americans are most notable for their high gallstone and gallbladder cancer prevalence rates compared with Cuban Americans and Puerto Ricans,[14] a fact attributed to the Mexican Americans' 30% to 50% admixture with North American Indian genes.[17] A true gallstone prevalence study among American Indians in South America showed that the Mapuche, a Chilean tribe,[18] displayed a gallstone prevalence rate of ≈50% in 50-year-old women and 13%

in men. Nonetheless, apart from the Pima, no true gallstone prevalence data for other North American Indian tribes or communities were available until recently.

In an earlier issue of this Journal, James Everhart et al.[19] determined true prevalence rates together with associated risk factors of gallbladder disease among American Indian subjects who participated in the Strong Heart Study, in which gallstones were detected by ultrasonography plus self-reporting of cholecystectomy.[20] This study involved 13 American Indian tribes and communities at three sites in Arizona, Oklahoma, and the Dakotas. Participants numbered 3,296 subjects and included Pima, Maricopa, and T'ohono O'otham (Arizona); Apache, Caddo, Comanche, Delaware, Fort Sill Apache, Kiowa, and Wichita (Oklahoma); Oglala Sioux and Cheyenne River Sioux (South Dakota); and Spirit Lake (North Dakota) tribes and communities, with cohorts evenly divided among sites. Ascertainment of the degree of American Indian heritage was based on self-reporting of the heritage of the participants' grandparents. Although this method has been validated independently against genetic markers,[21] it contrasts with the Chilean study[18] in which investigators determined ABO blood group distributions and mitochondrial DNA polymorphisms in Chilean Mapuche, Hispanic, and Maori tribes. In the recent study,[19] female and male participants with 100% American Indian heritage varied from 46% and 44% in the Dakotas, to 74% and 71% in Oklahoma, to 93% (both genders) in Arizona. Overall, 100% American Indian heritage was present in 72% of women and 68% of men.[19]

Because of selection bias in the Strong Heart Study,[20] all subjects were 45 years or older. But, as inferred from the original Pima study (Fig. 1), this age range should provide maximum gallstone prevalence rates in women and a close approximation in men. Intersite differences were small, and cumulative prevalence rates increased to 74% in women 65 years or older (age-standardized prevalence, 66%) and 44% in men 65 years or older (age-standardized prevalence, 31%). These frequencies, which were found across the three geographically and culturally diverse regions, are significantly higher than the maximum gallstone prevalence rates in Chilean Mapuche women (50%) and especially Mapuche men (13%)[17] despite their 88% American Indian heritage. In the study by Everhart et al.,[19] a strong positive correlation existed between risk of gallstones and the degree of North American Indian heritage when other factors were simultaneously controlled for. The authors' conclusion that gallstone disease is found in epidemic proportions in American Indian populations and "not limited to a small group of related tribes of a single region"[19] (Fig. 1) is timely and important information.

Apart from the association of gallstones with American Indian heritage and advancing age, multivariate logistic regression analysis revealed that waist circumference was associated with gallstones among American Indian men, whereas diabetes mellitus (type 2) and parity were associated in women.[19] However, body mass index was associated only with prevalence of gallstone disease in women in an age-adjusted analysis.[19] Similar to the original Pima study,[8] the prevalence rates of diabetes mellitus and obesity were so high that they may be very important determinants of gallstone risk at the genetic or phenotypic levels, but the association is not easily quantified because of their high frequencies.[8,19] In addition to the sizable morbidity and mortality rates associated with gallbladder surgery,[16] it is known that among Pima the death rate attributable to malignancies, especially gallbladder cancer, is 7 times higher in tribal members with gallstone disease.[22] However, similar information is not available for the other tribes and communities in the latest study.[19]

Pathophysiology of Gallstone Formation in American Indians

One clearly would like to know details of the gallstone phenotypes in American Indians and how their high prevalence rates of diabetes mellitus and severe obesity might contribute. Several interesting epidemiologic features concerning biliary cholesterol lithogenicity in American Indians compared with whites have been noted. (1) Cholesterol "solitaires" are much more common[6,18] than the $\approx$8% found in whites[11] (hence the "typicality" of Franz Ingelfinger's litho-talisman). (2) Pigment gallstones are very rare,[2,6,18] possibly related to the American Indians' high degree of cholesterol supersaturation,[12,13] which engenders phase-separated vesicles in bile that act as calcium and bilirubin binders and decrease biliary saturation with calcium bilirubinate.[23] (3) Symptomatic gallstones are considerably more frequent not only in female but also in male American Indians.[8,9,19]

After recruitment in the early 1970s from the Rockefeller University in New York, Dr. Scott Grundy and his colleagues moved to Phoenix, AZ, to quantify the pathophysiology of cholesterol gallstone formation in American Indians of the US Southwest.[6] Employing an innovative duodenal marker-perfusion technique, they measured individual secretion rates of all 3 biliary lipids in subjects with and without gallstones. They found major increases in biliary cholesterol secretion and significant decreases in bile salt secretion rates in American Indian gallstone patients.[24] Both abnormalities combined to result in the production of the most marked cholesterol supersatura-

tions observed in human bile.[24] Reuben et al. in London, UK,[25] and Shaffer and Small[26] in Boston, MA, performed similar studies in obese whites with this *in vivo* technique. Their findings revealed that the major abnormality in obese subjects, both with and without gallstones, was hypersecretion of cholesterol that was not compensated for by increased bile salt secretion rates, implying that there was a fixed level of bile salt synthesis and enterohepatic cycling of the bile salt pool in obesity.[25,26] In mice, increased hepatic cholesterol synthesis drives cholesterol 7α-hydroxylase (CYP7A1), the rate-limiting enzyme in bile salt synthesis (via the neutral pathway), through the action of the oxysterol nuclear receptor liver-X-receptor α (LXRα).[11] In obese humans, however, the increased total body synthesis rate of cholesterol[11] is not necessarily coupled with increased CYP7A1, as shown by measurement of bile salt kinetics in obesity; so it is not surprising to find that the promoter region of human *CYP7A1* does not contain a response element for LXRα. Differential gender frequencies of cholesterol gallstones in men and women may be related to CYP7A1 activity in that its gender-related expression may explain the male predominance of cholesterol gallstones in most susceptible inbred mice.[11] In contrast, in humans there is an appreciably higher female-to-male gender frequency of gallstones, as in American Indians and especially in whites.[8,11,18,19] In mice, gallstones form only in response to massive cholesterol intake; the sterol level in a lithogenic diet corresponds to an amazing 140-g intake per day in a 70-kg human, whereas a normal human diet contains approximately 350 mg of cholesterol per day.[27] Moreover, this diet also contains cholic acid, a major ligand for Farnesol-X-receptor (FXR), the bile salt nuclear receptor, that prevents increased cholesterol catabolism into *de novo* bile salt synthesis catalyzed by CYP7A1. Yet, despite this enormous cholesterol challenge, our strain survey of genetically diverse mice in The Jackson Laboratory showed that approximately 50% of mouse strains are totally resistant to gallstones and do not form lithogenic bile,[11] which affords persuasive evidence of the validity of a novel lithogenic hypothesis, which might be phrased "No cholesterol gallstone (*Lith)* alleles—no gallstones," at least in the mouse. A vivid demonstration of the importance of CYP7A1 in hepatic cholesterol homeostasis is provided by a transgenic experiment[28] in which an overexpressed *Cyp7a1* prevents cholesterol gallstones in gallstone-susceptible C57BL/6J mice. Moreover, it has been documented recently that CYP7A1 deficiency in humans[29] is associated not only with early formation of cholesterol gallstones but also with a hypercholesterolemic phenotype.

Whether the prediabetic state or diabetes mellitus (type 2) increases the risk of gallstones in American Indians is a vexed question since the literature is highly contradictory with some studies indicating an association of diabetes and gallstones and other studies suggesting no association.[11] However, those studies showing an increased risk of gallstones in diabetic subjects have failed to answer the question as to the precise mechanisms involved. One possible link is hyperinsulinemia, which occurs for decades prior to onset of clinically significant glucose intolerance. The appreciable up-regulation of *de novo* cholesterol synthesis by insulin[11] may contribute to the increased biliary cholesterol saturation index so often seen in diabetic patients. However, there may also be a decrease in gallbladder motility from both neurologic and defective smooth muscle signal transduction mechanisms due to absorbed cholesterol from gallbladder bile.[11] Another possibility is that gallstones and lithogenic bile could be a manifestation of insulin resistance in the metabolic syndrome (syndrome X), which is appreciably more common in Mexican Americans and Pima Indians compared with whites.[30]

In summary, there is modest evidence that all "thrifty" genes of American Indians for obesity, diabetes mellitus, and gallstones could combine strategically to augment lithogenicity of bile, but as we will show below, new data in mice suggest that at least obesity *per se* plays only an indirect role in cholelithogenesis via shared obesity and lithogenic genes.

Possible Genetic Origins of Cholesterol Gallstones Involving "Thrifty" Genes

A mid-19th century monograph on cholelithiasis by a notable French physician, Victor Albans Fauconneau-Dufresne[31] claimed that cholelithiasis is often "hereditary"; half a century later, Lord Moynihan of Leeds articulated a similar opinion with only anecdotal documentation.[11] In 1966, R. B. McConnell,[32] an astute Liverpudlian physician, analyzed clinical incidences of gallstones in families, twins, and the relatives of young gallstone patients and concluded that liability to gallstone formation was most likely *polygenic*. This view was articulated by several others in the intervening years to explain the hepatic origins of lithogenic bile,[11,33] but a genetic component was first proven by the elegant studies of Willem van der Linden[34] on an extensive series of families, especially those with young probands with gallstones in northern Sweden. He opined that the genetic basis of gallstones could not get an airing among contemporary (1980s) "cholanologists," because studies on the physical chemistry and pathophysiology of bile were so glamorous![34] In recent years, abundant evidence has accrued, especially from family and population studies, for a strong $\approx$30% genetic component to cholesterol gallstone disease

(summarized in Paigen and Carey[11]). For example, German, Mexican American, Danish, Swedish, Finnish, Israeli, and north Indian, and especially the extensive Italian studies (summarized in Paigen and Carey[11]), which controlled for confounding variables, have verified that cholesterol gallstones are from 2 to 5 times more common in first-degree relatives of gallstone patients. Furthermore, the apparent role of heredity is particularly evident in family members of children or young adolescents with gallstones.[34] In addition, concordance for gallstones in twin studies is significantly greater in monozygotic than in dizygotic pairs.[11] At both biophysical and chemical levels, abnormal bile has been found consistently in the siblings of stone-prone young women[35] and in each of identical twins[11] with gallstones.

Because of the study by Everhart et al.[19] and other American Indian epidemiologic studies,[11,18] a frame of reference has now been developed to dissect out both "nature and nurture" issues that cause such high prevalence rates of gallstones in the American Indians. Of particular relevance to this genetic predisposition are the ethnohistory and geographic origins of American Indians, which are among the most intensely studied topics concerning any native population,[36] with their transit to the new world often being called "the last great migration of our single species."[37] Based on linguistic, cultural, and anatomic evidence, the ancestors of today's American Indians were Central Asians from the interior of Siberia. During the last Great Ice Age, ≈50,000 to 10,000 years before present (BP), they entered the New World via the Bering Strait land bridge (Beringia) and adjacent portions of northeastern Asia and western Alaska.[38] Paleo-Indians were big game hunters who became trapped by mountainous cliffs on one side and glaciers on the other within the frozen tundra of an isolated Beringia for an amazing 20,000-year period, spanning the nadir of the last glacial epoch.[36-39] In this secluded area several times the size of present-day Alaska there was scant food, and survival depended on hunting-gathering strategies. It has been suggested that this bleak, desolate, and inhospitable environment saw 10 to 11 months of harsh winter[38,39] and was unlike any climate known on Earth today.[37] Apparently for a few weeks of the year, some vegetables, arctic plants, grasses, and sedges pushed through the thin ice.[38] Many wild animals were also isolated by glacial Beringia, and with no snow or arboreal cover, big game such as mastodon, wild horse, caribou, mammoth, steppe bison, and musk ox roamed in search of food,[38] and being obligate herbivores, contested aggressively for vegetation as a food staple. However, using stratagems unimaginable by modern-day societies, Paleo-Indians developed ingenious methods for trapping and killing these large animals to feed their families.[39] For a large family, victuals may not have been secured by male hunter-gatherers more than once a year![39]

As extrapolated from pre-Columbian skeletal remains, average life expectancy in these prehistoric times was no longer than late adolescence to early adulthood.[36] Moreover, current thinking is that the highly seasonal nature of food sources led to the likelihood that successful survival, and indeed reproduction, depended on genes that produced a favorable advantage principally by efficient fat storage.[40,41] The hypothesis that "thrifty" genes facilitated survival in the Pleistocene era, whereas today the same genes cause cholesterol gallstones, obesity, and non–insulin-dependent diabetes mellitus, is intriguing.[41,42] Certainly genetic evolution is most likely to have been accelerated by "scattered families, a population bottleneck favoring genetic drift, possibly a founder effect; and relatively sparse food supply and stringent environmental conditions."[42] As articulated elegantly by Weiss et al.,[42] these "thrifty" genes appear primarily to have involved lipid physiology, hepatobiliary function, and metabolism. Since digestion and absorption is so highly efficient in all *Homo sapiens*, it is likely that the major effect of those "thrifty" genes was to promote calorie storage in the form of adipose tissue for energy needs during extended winters.[42] A key event for survival of a population with a shortened life span would naturally be early puberty, possibly facilitated by leptin from adipocyte secretion, and sufficient fat reserves to go through a successful pregnancy and nursing period as well.[11] Good evidence that the scarcity of food favored the accumulation of "thrifty" (gallstone) genes in Paleo-Indians during their Beringeal seclusion is the infrequent occurrence of cholesterol gallstones in present-day Siberians.[36,43] Recent prevalence rates of cholelithiasis by ultrasonography in a sample of 6,676 Europeoids (Russians and Ukrainians) and 997 Mongoloids (Evenks and Hakases) revealed 2.9% in southern and 1.5% in northern Mongoloids, compared with 4.5% and 8.8% for Europeoids.[43] These prevalence rates are orders of magnitude less than in any North or South American Iindian tribe or community today.[8,18,19]

With Beringeal deglaciation, which probably began 20,000 to 12,000 years BP, an inviting, narrow ice-free corridor opened between the Cordilleran and Laurentine ice sheets in northwest Canada.[36,38] As the sea level rose as much as 300 feet,[36] Paleo-Indians fled from their vast domain in Beringia, and within approximately 1,000 to 2,000 years, had populated all of North, Central, and South America.[37] This rapid expansion into the Americas occurred principally by land, but there is emerging paleoarcheological evidence that there was also a western sea route, because settlers appeared in coastal and inland

Monte Verde, a part of southern Chile, as early as 12,500 BP, preceding settlement of some more proximal regions of Central and South America.[36,44] Early American Indians experienced a sustained hunting-gathering lifestyle in the Americas for many millennia during which they diverged culturally and linguistically.[36] However, in the case of the southwestern Indians, most notably the Pima, their subsistence depended on an agricultural economy for at least the last 2,000 years.[6,45] Moreover, throughout the continent, their diets were apparently invariant for thousands of years, yet the diet changed radically in the mid-20th century,[45] possibly with the appearance of the US Department of Agriculture's (USDA's) Commodity Food Distribution Program on Indian Reservations (http://www.commodityfoods.org), which included prepackaged high-caloric diets composed of meat, poultry, fruits, vegetables, grains, vegetable oils, and peanut and dairy products.[45]

One well-documented but not unique exception to this is the case of the Tarahumara Indians, who inhabit the Sierra Madre Mountains in the state of Chihuahua, Mexico. The diet of these Uto-Aztecan people, who are closely related to the Pima,[11,45-48] still consists largely of legumes, tubers, berries, fruits, and nuts. Moreover, both men and women value running and exercise constantly, often traveling over rugged mountainous terrain for distances of 10 to 30 miles per day.[48] The Tarahumares (the name means "fleet of foot") are remarkably free of obesity and diabetes mellitus and reputedly do not suffer from gallstones.[49] The NIH have also been studying the Mexican Pima, also called Mountain Pima (nearby cousins of the Tarahumara) residing in the Sierra Madre Mountains of northern Mexico.[6] They also live on a subsistence economy with traditional dietary habits (corn and beans) and are lean and extremely active. Dr. Peter Bennett[6] believes that they, like the Tarahumara Indians, probably do not have gallstone disease. Possibly the best evidence for the "sensitivity" of American Indian "thrifty" genes to the dietary environment is an on-site experiment performed on Tarahumara Indians by Dr. William E. Connor and colleagues over a decade ago.[47,49] These investigators challenged 13 fit and lean Tarahumara Indians (5 women and 8 men including one adolescent) with an affluent Western diet (4,100 kcal/d) for a short period of 5 weeks.[47] The recipients were encouraged to continue their normal lifestyle, but they gained weight rapidly (average 7%) and elevated their serum low-density lipoprotein cholesterol levels by 39% and their very-low-density lipoprotein triglyceride levels by 18%. Although not investigated, we would wager that their biles became lithogenic! Dr. William E. Connor[49] has voiced the opinion, "I suspect that they (Tarahumares) will remain a population without the diseases that their US counterparts are developing because of their poverty. When the crops failed over the past couple of years (pre-1997), there was outright starvation."

Fig. 2. Photograph of Solon and Melissa Jones with family—Pima, Sacaton, Arizona, from the Churchill collection (1904); photograph courtesy of The Heye Foundation and reproduced with kind permission of the National Museum of the American Indian, New York City, negative No. 26428. Photo by Col. Frank C. Churchill.

An anecdotal report[50] suggests that obesity may have been sporadic in American Indians for some centuries. In the early 18th century when the Jesuit missionary, Padre Eusebio Francisco Kino (born "Chini" in the Tyrolean village of Segno in the Italian Alps) made his sixth visit to the Pima (1702), his American Indian friends had become so "stocky" (*rechoncho*)[51] that he "hardly recognized them."[50] Moreover, by the end of the 19th and early 20th century, Pima obesity was documented frequently in photographs, especially of Pima women. They can be found in a classic work, "The Annual Report of the Bureau of American Ethnology to the Secretary of the Smithsonian Institution for 1904-05"[45] (see also Fig. 2). In a science feature that appeared in the New York Times in 1980,[50] Ms. Jane Brody made the fanciful prediction that if Father Kino could see the Pima today, "he would be even more astonished," which is certainly true according to Peter Bennett,[6] who has studied the Arizona Pima for over 40 years. Although the caloric intake of the Pima is not much different from American whites,[52] ≈80% are grossly obese, presumably because of the aggressive nature of their many "thrifty" obesity genes that cause efficient use of calories.[42]

Paleopathological evidence from pre-Columbian Indian burial sites is the most persuasive available that gallstones were probably once quite rare in American Indians.[53] These excavations and autopsies at several sites in North and South America have described infrequent

"small, multiple and pearly cholesterol stones."[53] Stone prevalence rates in mummified remains range from 2 of 75 Chileans, dating from 1,900 to 1,700 years BP,[54] to 6 of 1,000 Ohioans at a late Woodland site, dating from 1,000 to 800 years BP;[53] and autopsies in over 300 Peruvians dating from 2,600 to 320 years BP (the latter date being in the late 17th century) failed to show even one example of gallstones.[53] An ethno-botanical compendium of medicines used by Mesquakie Indians, which contains a total of 300 herbal remedies,[46] mentions neither gallbladder disease nor any remedy for it. Moreover, at the beginning of the 20th century, a published list of the 28 diseases commonly seen in the Pima made no mention of gallstones, nor biliary pain, nor jaundice.[45] Furthermore, in their vast repertoire of "medicine songs," which covers every conceivable ailment afflicting the Pima tribe, and indeed is their largest class of folksongs, not one refrain refers to gallstones or gallbladder disease.[45] Therefore, the preponderance of clinical and ethnologic evidence[55] supports the concept that "thrifty" human cholesterol gallstone alleles (*LITH* genes) lay dormant for thousands of years in the ancestors of today's American Indians. However, in the middle of the 20th century, possibly the decade immediately preceding and the decade during and after World War II,[42,48] the genes became unmasked by the radical environmental change previously alluded to, possibly the donation of USDA prepackaged commodity foods, and the American Indians' lipid metabolism responded explosively to this perturbation. This stands in contrast to whites, who putatively evidenced milder responses to a similar environmental challenge possibly during the Industrial Revolution a century earlier.[11] Most American Indians became very obese and acquired diabetes mellitus, and cholesterol gallstones formed rapidly and frequently.[8,18,19] The "cholelithogenic" environment of the latter 20th century Western diets seems to have been specifically consumption of foods rich in refined sugar and fat-containing foods with very low fiber content. Moreover, since hardship farming became less common, physical activity markedly decreased.[55] In his Bureau of American Ethnology Report (1908), Russell[45] opined that even then some young Pima men acquired a degree of obesity that "is in striking contrast with the tall and sinewy Indian conventionalized in popular thought." More recent evidence from large-scale surveys in whites has suggested that sedentary lifestyles in both genders correlate positively with risk of cholecystectomy,[56,57] a surrogate for symptomatic cholelithiasis, as well as the possibility of new gallstone formation. In the case of "thrifty" American Indian genes, the new diseases may be not only at the genetic level, but also at the metabolic level: in humans, diabetes mellitus and obesity are factors predisposing to gallstones, and marked obesity predisposes to diabetes mellitus.[11]

Genetics of Experimental Cholelithiasis in Inbred Mice

Our conjoint work for nearly a decade has focused on identifying cholesterol gallstone susceptibility loci (*Lith* alleles) in inbred mice.[11] As is the case for the human disease, cholesterol gallstones in the mouse are a complex polygenic trait. Although there is no assurance that *LITH* genes in humans and *Lith* genes in mice will be the same, the conservation of mammalian genomes for tens of millions of years is reassuring.[58] In 1995, we published use of quantitative trait loci (QTL) mapping, a powerful genetic technique, to locate the first cholesterol gallstone gene (*Lith1*) on mouse chromosome 2.[59] Subsequent studies determined its canalicular transporter expression on hepatocytes, fine mapping of the locus, and hepatobiliary phenotypes, resulting in the bile salt export protein (BSEP, officially ABCB11) being a candidate gene for *Lith1*.[60] In the same cross, *Lith2* was discovered on mouse chromosome 19 and may be identical to the multidrug resistance-related protein, isoform 2 (MRP2, officially ABCC2),[60] which is the conjugate organic anion transporter responsible for much of bile salt–independent bile flow.

Since summarizing our work on 9 QTLs located on 19 murine autosomes plus the X chromosome in January 2001,[61] we have completed an extensive strain survey involving most of the genetically diverse mouse strains (Paigen B, Bouchard G, Carey MC, http://aretha/jax.org pub-cgi/phenome/mpa cgi? rtu= studies&details id = 29). We found that somewhat less than half of the strains were susceptible, often favoring males in contrast to the female gender predominance in American Indians[8,18,19] (Fig. 1) and whites.[15] We then chose 4 of the most genetically diverse strains to outcross with 4 gallstone resistant strains, which were arranged (on paper) with alternating susceptible and resistant strains in a "daisy chain" configuration; experimentally each strain was outcrossed with both of its neighbors. Although these experiments are still ongoing, it appears that *Lith* genes (either main or interacting genes) may be present on essentially all murine chromosomes. With the use of modern genetic strategies to define the genetic regions, the entire ensemble of *Lith* loci in the mouse is capable of being located. Table 1 lists main QTL (*Lith*) loci numbers 1 and 5-9 from different crosses in inbred mice[60-65] and whose candidate genes for *Lith* alleles appear relevant to American Indian cholesterol gallstone formation. The table also suggests how the orthologous genes in humans could explain dysfunction of several key proteins in forming abnormal bile both chemically and pathophysiologically in post-pubertal Ameri-

Table 1. Selected Quantitative Trait Loci (QTLs of *Lith* Alleles) for Cholesterol Gallstones in Inbred Mice That Might Be Relevant to American Indian Cholesterol Gallstone Formation

QTL	Chr	Ref	Candidate Genes*	Candidate Function	Predicted Pathophysiological and Biochemical Events
Lith1	2	60-63	i. *Nr1h3* (*Lxra*) †	Oxysterol nuclear transcription factor: activates transcription of many cholesterol homeostatic genes (*e.g., Cyp7a1, Abca1, Abcg5/Abcg8, Lpl*)	i. Unlikely to influence bile salt synthesis via CYP7A1 but may augment cholesterol secretion rate.
Lith5	9	64	i. *Lipc*	Hepatic lipase: involved in both chylomicron remnant and high density lipoprotein cholesterol uptake	i. May increase exogenous and endogenous cholesterol delivery to liver.
			ii. *Scap*	SREBP-cleavage activating protein: regulates proteolytic activation of SREBPs, transcription factors whose targets include cholesterol and bile acid synthetic genes (*e.g., Hmgcr* and *Cyp8b1,* respectively)	ii. May up-regulate cholesterol synthesis and contribute to defective bile salt synthesis via acidic pathway.
Lith6	6	63-65	i. *Pparg*	Peroxisome proliferator-activated receptor-γ: ligand-activated nuclear transcription factor involved in lipid synthesis and storage; associated with hyperlipidemia, obesity, insulin resistance, type 2 diabetes, and CYP7A1 activity indirectly	i. May contribute to diabetes type 2 and obesity, thereby increasing cholesterol synthesis. May inhibit CYP7A1 and block bile salt synthesis via the neutral pathway.
			ii. *Apobec1*	Apolipoprotein B mRNA editing complex 1 converts full-length $ApoB_{100}$ to $ApoB_{48}$	ii. May contribute to more rapid delivery of chylomicron cholesterol to liver and hence biliary cholesterol hypersecretion.
Lith7	10	65	i. *Nr1h4* (*Fxr*)	Bile acid nuclear transcription factor: controls feedback inhibition of bile acid synthesis via CYP7A1, activates transcription of bile salt transporters including *Abcb11, Abcc2, Slc10a1* (*Ntcp*), and *Fabp6* (*Illbp*)	i. May lead to inhibition of bile salt synthesis as well as hepatic secretion and intestinal uptake of bile salts.
Lith8	4	65	i. *Nr0b2* (*Shp1*)	Short heterodimer partner 1: nuclear transcription factor that inhibits bile acid synthesis by interaction with LRH-1, the competence factor for *Cyp7a1* transcription	i. May inhibit bile salt synthesis via the neutral pathway.
			ii. *Scp2*	Sterol carrier protein 2: involved in intracellular cholesterol transport, and possibly biliary lipid secretion	ii. May promote biliary cholesterol hypersecretion.
			iii. *Lepr*	Leptin receptor: may influence biliary secretion of plasma-derived cholesterol	iii. May promote coupling of cholesterol to other biliary lipids.
Lith9	17	65	i. *Abcg5/Abcg8*	Canalicular (and intestinal) half-transporters for cholesterol and neutral sterols	i. May greatly augment biliary cholesterol secretion.

Abbreviations: Chr, chromosome on which the QTL is located; Ref, reference.

*Official gene symbol with common gene symbol in parentheses.

†Although *Abcb11* (Bsep) remains a viable candidate gene for *Lith1*, the gene listed here is also a candidate gene within the *Lith1* locus and is further ruled in by an independent intercross.[63]

can Indians. In one of these crosses[65] (Table 1), three major *Lith* QTLs (*Lith7, Lith8, Lith9)* were found. They may be caused by polymorphisms of genes leading to up-regulation of ABCG5 and ABCG8 (*Lith9*), the putative canalicular half-transporters responsible for transporting much of cholesterol into bile, and up-regulation of FXR (*Lith7*), the bile salt nuclear receptor that down-regulates cholesterol 7α-hydroxylase (CYP7A1), the rate-limiting enzyme in *de novo* bile salt synthesis via the neutral pathway.[11] With respect to *Lith8*,[65] the putative genes involved may lead to inhibition of bile salt synthesis, augment intracellular cholesterol traffic and hepatic uptake of lipoproteins, and couple cholesterol to biliary lipids for bile secretion (H. Wittenburg, MA Lyons, B. Paigen, M.C. Carey, unpublished observations, 2002). It will certainly be worth scrutinizing whether these genes are major gallstone genes in American Indians since many of the biochemical and pathophysiologic characteristics of the Pima[11,24] are similar to the phenotypes in these strains of mice.[62-65]

In connection with centripetal obesity typical of American Indians[11] (Fig. 1), we examined susceptibility to cholesterol gallstone formation in three polygenic and five monogenic strains of overweight mice.[66] Compared with background strains, some murine models of obesity increased while others decreased cholesterol gallstone susceptibility.[66] Therefore, cholesterol gallstone formation in overweight mice is not simply a secondary result of obesity *per se*, but rather, certain obesity genes impact cholesterol gallstone risk while others have no effect. Thus, it is possible that in American Indians obesity genes and gallstone genes are agonist or antagonist in the stone diathesis, irrespective of the magnitude of obesity *per se*. Indeed, among American Indian gallstone populations,[19] we are provided with a rich population base to perform such studies to differentiate effects of obesity genes from cholesterol gallstone genes on the gallstone phenotype, as well as gene-environmental interactions. We hope to be guided by results of inbred mouse studies, which will, in the interim, have revealed the entire *Lith* allele ensemble.[11]

Will all this work eventually have any relevance to gallstone prevention and new treatments? We believe the answer is an unequivocal yes. The late James V. Neel,[67] originator of the "thrifty" gene hypothesis[40] and a leading founder of modern human genetics, wrote, "Much more effective and cost-efficient improvement to human well-being can be made by tailoring the environment to the genome rather then the genome to the environment: Lifestyle interventions are more practical then genetic ones."[67] It is highly unlikely that this admonition can be applied easily to American Indians to alleviate their cholesterol gallstone epidemic, because the environmental changes that interacted with thrifty genes to produce the gallstone diathesis are not readily identified.[39,42,58] Moreover, lifestyle changes are notoriously difficult to implement and sustain, especially among impoverished peoples.[2] Besides, such incursions would depend critically on much fundamental and difficult-to-obtain information of gene-gene as well as gene-environmental interactions.[8,41,55] However, the new science of "nutrigenomics" might stimulate a careful search for micronutrients in the original American Indian diets that may bring unexpected results to reverse or ameliorate this rocky state of affairs. Nonetheless, we are more inclined to think that it is the surfeit of both micro- and macronutrients in American Indian diets for over half a century that is at least partially responsible for the gallstone epidemic and other "thrifty" gene dysfunctions; it was a salutary lesson to appreciate that it took only 5 weeks for gene-environmental interactions to become evidenced phenotypically by plasma lipid profiles in the Tarahumara Indians following consumption of a high-calorie Western diet.[47]

On the other hand, targeting genes to normalize their function would not necessarily involve making "transgenic humans," especially for a disease that is not often fatal. A good recent example is the widespread use of thiazolidinediones for treating type 2 diabetes mellitus,[68] which has heralded a new era of clinical pharmacogenomics. These drugs activate the peroxisomal proliferator-activated receptor (PPARγ), a nuclear receptor that regulates the expression of multiple genes involved in lipid metabolism. PPARγ has been considered the "ultimate thrifty gene,"[69] and it has been proposed that a maladapted "thrifty" response coordinated by PPARγ contributes to the recent rise in the prevalence among whites of obesity, atherosclerosis, insulin resistance, type 2 diabetes mellitus, and perhaps gallstones[63] (at least in the inbred mouse; see *Lith6* in Table 1). This fits well with the possibility that PPARγ or another master regulator, such as FXR as suggested by our mouse studies,[62] may also be involved in the gallstone epidemic among American Indians (Table 1). Hence, because mapping of regulatory genes in the inbred mouse allows us to look for the orthologous loci on the human genome, then clearly American Indian *LITH* alleles[11] will be discovered. It is not too far-fetched to believe that one day physicians responsible for the health of American Indian peoples will be prescribing agonists or antagonists for several hepatic lipid transporters (Table 1) such as ABCG5 and ABCG8,[62] or nuclear receptors (LXRα, FXR, PPARγ)[62,63] and other transcription factors and rate-limiting enzymes (*e.g.*, CYP7A1),[11] to prevent gallstones totally and hence eradicate a major contributor to the American Indian's burden.

Acknowledgment: The authors are grateful to Drs. Henning Wittenburg and Malcolm Lyons for critically reading the manuscript and providing information; Drs. John Fordtran, Donald Small, and Peter Bennett for taking the time to be interviewed and for their careful and constructive reviews of the final draft; Jack Eckert for bibliographic support; Monika Leonard for editorial clarification; and Maya Kavtaradze and Megan Raspa for word processing assistance.

References

1. Sievers ML, Marquis JR. The Southwestern American Indian's burden: biliary disease. J Am Med Assoc 1962;182:570-572.
2. Ingelfinger FJ. Digestive disease as a national problem, V. Gallstones. Gastroenterology 1968;55:102-104.
3. Fordtran JS. Telephone interview by MC Carey. Dallas, TX: May 24, 2002.
4. Ingelfinger FJ. Annual discourse—Swinging copy and sober science. N Engl J Med 1969;281:526-532.
5. Small DM. Telephone interview by MC Carey. Boston, MA: July 19, 2002.
6. Bennett PH. Telephone interview by MC Carey. Phoenix, AZ: July 29, 2002.
7. Small DM, Bourgès M, Dervichian DG. Ternary and quaternary aqueous systems containing bile salts, lecithin and cholesterol. Nature 1966;211: 816-818.
8. Sampliner RE, Bennett PH, Comess LJ, Rose FA, Burch TA. Gallbladder disease in Pima Indians: demonstration of high prevalence and early onset by cholecystography. N Engl J Med 1970;283:1358-1364.
9. Comess LJ, Bennett PH, Burch TA. Clinical gallbladder disease in Pima Indians: its high prevalence in contrast to Framingham, Massachusetts. N Engl J Med 1967; 277:894-898.
10. Admirand WH, Small DM. The physical-chemical basis of cholesterol gallstone formation in man. J Clin Invest 1968;47:1043-1052.
11. Paigen B, Carey MC. Gallstones. In: King RA, Rotter JI, Motulsky AG, eds. Genetic Basis of Common Diseases. 2nd ed. New York: Oxford University Press, 2002:298-335.
12. Bennion LJ, Knowler WC, Mott DM, Spagnola AM, Bennett PH. Development of lithogenic bile during puberty in Pima Indians. N Engl J Med 1979;300:873-876.
13. Thistle JH, Schoenfield LJ. Lithogenic bile among young Indian women. N Engl J Med 1971;284:177-181.
14. Maurer KR, Everhart JE, Ezzati TM, Johannes RS, Knowler WC, Larson DL, Sanders R, et al. Prevalence of gallbladder disease in Hispanic populations in the United states. Gastroenterology 1989;96:487-492.
15. Everhart JE, Khare M, Hill M, Maurer KR. Prevalence and ethnic differences in gallbladder disease in the United States. Gastroenterology 1999; 117:632-639.

16. Sandler RS, Everhart JE, Donowitz M, Adams E, Cronin K, Goodman C, Gemmen E, et al. The burden of selected digestive diseases in the United States. Gastroenterology 2002;122:1500-1511.
17. Mitchell BD, Williams-Blangero S, Chakraborty R, Valdez R, de la Villa I, Neto E, Gasper MJ, et al. A comparison of three methods for assessing Amerindian admixture in Mexican Americans. Ethn Dis 1993;3:22-31.
18. Miguel JF, Covarrubias C, Villaroel L, Mingrone G, Greco AV, Puglielli L, Carvallo P, et al. Genetic epidemiology of cholesterol cholelithiasis among Chilean Hispanics, Amerindians, and Maoris. Gastroenterology 1998; 115:937-946.
19. Everhart JE, Yeh F, Lee ET, Hill MC, Fabsitz R, Howard BV, Welty TK. Prevalence of gallbladder disease in American Indian populations: findings from the Strong Heart Study. HEPATOLOGY 2002;35:1507-1512.
20. Lee ET, Welty TK, Fabsitz R, Cowan LD, Le NA, Oopik AJ, Cucchiara AJ, et al. The Strong Heart Study. A study of cardiovascular disease in American Indians: design and methods. Am J Epidemiol 1990;132:1141-1155.
21. Williams RC, Steinberg AG, Knowler WC, Pettitt DJ. Gm 3;5,13,14 and stated-admixture: independent estimates of admixture in American Indians. Am J Hum Genet 1986;39:409-413.
22. Grimaldi CH, Nelson RG, Pettitt DJ, Sampliner RE, Bennett PH, Knowler WC. Increased mortality with gallstone disease: Results of a 20 year population based survey in Pima Indians. Ann Intern Med 1993;118: 185-190.
23. Cahalane MJ, Neubrand MW, Carey MC. Physical-chemical pathogenesis of pigment gallstones. Sem Liver Dis 1988;8:317-328.
24. Grundy SM, Metzger AL, Adler RD. Mechanism of lithogenic bile formation in American Indian women with cholesterol gallstones. J Clin Invest 1972;51:3026-3043.
25. Reuben A, Maton PN, Murphy GM, Dowling RH. Bile lipid secretion in obese and non-obese individuals with and without gallstones. Clin Sci 1985;69:71-79.
26. Shaffer EA, Small DM. Biliary lipid secretion in cholesterol gallstone disease. The effect of cholecystectomy and obesity. J Clin Invest 1977;59: 828-840.
27. Dietschy JM, Turley SD. Control of cholesterol turnover in the mouse. J Biol Chem 2002;277:3801-3804.
28. Miyake JH, Duong-Polk XT, Taylor JM, Du EZ, Castellani LW, Lusis AJ, Davis RA. Transgenic expression of cholesterol-7-alpha-hydroxylase prevents atherosclerosis in C57BL/6J mice. Arterioscler Thromb Vasc Biol 2002;22:121-126.
29. Pullinger CR, Eng C, Salen G, Shefer S, Batta AK, Erickson SK, Verhagen A, et al. Human cholesterol 7α hydroxylase (CYP7A1) deficiency has a hypercholesterolemic phenotype. J Clin Invest 2002;110:109-117.
30. Gower BA. Syndrome X in children: influence of ethnicity and visceral fat. Am J Human Biol 1999;11:249-259.
31. Fauconneau-Dufresne VA. Traité de l'affection calculeuse du foie et du pancréas. Paris: Masson, 1851:1-515.
32. McConnell RB. The Genetics of Gastro-intestinal Disorders: Gall-stones. London: Oxford University Press, 1966:184-193.
33. Small DM. Management of gallstones, particularly the silent variety. Advantages of a varied and individualized approach. In: Ingelfinger FJ, Ebert RV, Finland M, Relman AS, eds. Controversy in Internal Medicine. Philadelphia: WB Saunders, 1974:545-559.
34. van der Linden W. Genetics of cholelithiasis. In: Rotter JT, Samloff IM, Rimoin DL, eds. Genetics and Heterogeneity of Common Gastrointestinal Disorders. New York: Academic Press, 1980:313-320.
35. Danzinger RG, Gordon H, Schoenfield LJ, Thistle JL. Lithogenic bile in siblings of young women with cholelithiasis. Mayo Clin Proc 1972;47: 762-766.
36. Crawford MH. The Origins of Native Americans: Evidence from Anthropological Genetics. New York: Cambridge University Press, 1998:1-308.
37. Anderson DD. Reconstucting a migration. Science 1980; 210:1339-1340.
38. Fagen BM. The Great Journey: The Peopling of Ancient America. New York: Thames and Hudson, 1989:1-288.
39. Laughlin WS, Harper AB, eds: The first Americans. Origins, Affinities and Adaptations. Papers from a conference. Deerfield Beach, FL: Verlag Chemie, 1980;1-340.
40. Neel, J. Diabetes mellitus: a "thrifty" genotype rendered detrimental by "progress"? Am J Hum Genet 1962;14:353-362.
41. Lowenfels AB. Gallstones and glaciers: the stone that came in from the cold. Lancet, 1988;1:1385-1386.
42. Weiss KM, Ferrell RE, Hanis CL. A new world syndrome of metabolic disease with a genetic and evolutionary basis. Yearbk Physical Anthropol 1984;27:153-178.
43. Tsukanov VV, Shtygasheva OV, Gorskovskaya IA, Tonkich DL, Zuev VV, Grichenko NN. Cholelithiasis epidemiology in Siberian populations [Abstract]. Digestion 1998;59(Suppl 3):560.
44. Meltzer DJ. Monte Verde and the Pleistocene peopling of the Americas. Science 1997;276:754-755.
45. Russell F. The Pima Indians. Washington, DC: Government Printing Office,1908:3-389 plus Plates I-XLVII.
46. Shaheb S. Cholelithiasis among American Indians (letter). Gastroenterology 1990;98:251-252.
47. McMurry MP, Cerqueira MT, Connor SL, Connor WE. Changes in lipid and lipoprotein levels and body weight in Tarahumara Indians after consumption of an affluent diet. N Eng J Med 1991;325:1704-1708.
48. Pennington CW. The Tarahumara of Mexico. Salt Lake City: University of Utah Press, 1963.
49. Connor WE. Private written communication to MC Carey. Portland, OR: May 27, 1997.
50. Brody JE. Tending to obesity, inbred tribe aids diabetes study. The New York Times, February 5, 1980:C1, C5.
51. Del Castillo FF, ed. Las misiones de Sonora y Arizona. Comprendiendo: la crónica titulada: "Favores celestiales" y la "Relación díaria de la entrada al norueste" por el Padre Eusebio María Kino (Kuhne). Vol. 8. Mexico: Editorial "Cultura," 1913-1922;3-413.
52. Reid JM, Fullmar SD, Pettigrew KD, Burch TA, Bennett PH, Miller M, Whedon GD. Nutrient intake of Pima Indian women: relationships to diabetes mellitus and gallbladder disease. Am J Clin Nutr 1971;24:1281-1289.
53. Steinbock RT. Studies in ancient calcified soft tissues and organic concretions. III Gallstones (Cholelithiasis). J Paleopathol 1991;3:28-36.
54. Munizaga J, Allison MJ, Paredes C. Cholelithiasis and cholecystitis in pre-Columbian Chileans. Am J Phys Anthropol 1978;48:209-212.
55. Weiss KM, Ferrell RE, Hanis CL, Styne PW. Genetics and epidemiology of gallbladder disease in New World Native Peoples. Am J Hum Genet 1984;38:1259-1278.
56. Leitzmann MF, Giovannucci EL, Rimm EB, Stampfer MJ, Spiegelman D, Wing AL, Willett WC. The relation of physical activity to risk for symptomatic gallstone disease in men. Ann Intern Med 1998;128:417-425.
57. Leitzmann MF, Rimm EB, Willett WC, Spiegelman D, Grodstein F, Stampfer MJ, Colditz GA, et al. Recreational physical activity and the risk of cholecystectomy in women. N Engl J Med 1999;341:777-784.
58. Paigen K. A miracle enough: the power of mice. Nat Med 1995;1:215-220.
59. Khanuja B, Cheah Y-C, Hunt M, Nishina PM, Wang DQ-H, Chen HW, Billheimer JT, et al. *Lith 1*, a major gene affecting cholesterol gallstone formation among inbred strains of mice. Proc Natl Acad Sci U S A 1995; 92:7729-7733.
60. Paigen B, Schork NJ, Svenson KL, Cheah Y-C, Mu J-l, Lammert F, Wang DQ-H, et al. Quantitative trait loci mapping for cholesterol gallstones in AKR/J and C57L/J stains of mice. Physiol Genomics 2000;4:59-65.
61. Lammert F, Carey MC, Paigen B. Chromosomal organization of candidate genes involved in cholesterol gallstone formation: a murine "gallstone map." Gastroenterology 2001;120:221-238.
62. Lammert F, Wang DQH, Cohen DE, Paigen B, Carey MC. Functional and genetic studies of biliary cholesterol secretion in inbred mice evidence for primary role of sister to P-glycoprotein, the canalicular bile salt export pump in cholesterol gallstone pathogenesis. In: Paumgartner G, Stiehl A, Gerok W, Keppler D, Leuschner U, eds. Bile Acids and Cholestasis. Dordrecht: Kluwer, 1999:224-228
63. Lyons MA, Wittenburg H, Korstanje R, Walsh K, Carey MC, Paigen B. Evaluation of the cholesterol gallstone susceptibility locus *Lith* 6 in CAST/Ei

and DBA/2J inbred strains of mice: evidence for a role of PPRG in murine cholelithiasis [Abstract]. HEPATOLOGY 2002:36(Part 2) (in press).
64. Wittenburg H, Lammert F, Wang DQ-H, Churchill GA, Bouchard G, Carey MC, Paigen B. Interacting QTL's for cholesterol gallstones and gallbladder mucin in AKR and SWR stains of mice. Physiol Genomics 2002;8:67-77.
65. Wittenburg H, Lyons MA, Li R, Carey MC, Paigen B. New cholesterol gallstone susceptibility (*Lith*) loci with attractive positional candidate genes in an intercross of PERA/Ei and I/LnJ strains of mice [Abstract]. Gastroenterology 2002;122:A543.
66. Bouchard G, Johnson D, Carver T, Paigen B, Carey MC. Cholesterol gallstone formation in overweight mice establishes that obesity per se is not linked directly to cholelithiasis risk. J Lipid Res 2002;43:1105-1113.
67. Weiss KM, Ward RH. Obituary: James V. Neel, M.D., Ph.D. (March 22, 1915-January 31, 2000): founder effect. Am J Hum Genet 2000;66:755-760.
68. Schoonjans K, Auwerx J. Thiazolidinediones: an update. Lancet 2000;355:1008-1010.
69. Auwerx J. PPARγ, the ultimate thrifty gene. Diabetologia 1999;42:1033-1049.