JCI The Journal of Clinical Investigation

From fatty streak to fatty liver: 33 years of joint publications in the *JCI*

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J Clin Invest. 2008;118(4):1220-1222. https://doi.org/10.1172/JCI34973.

Personal perspective

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From fatty streak to fatty liver: 33 years of joint publications in the *JCI*

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The 100th anniversary of the American Society for Clinical Investigation coincides with nearly 85 years of its journal, *The Journal of Clinical Investigation*. The occasion calls for reflection upon the crucial role that both institutions played in shaping American medical science in the 20th century. In this article we pay tribute to the inspirational role that the *JCI* played in our collaborative career over the past 37 years.

We published our first joint *JCI* article in 1975, more than 30 years ago (1) and one year after our election to the ASCI. In that article, entitled "Role of the low density lipoprotein receptor in regulating the content of free and esterified cholesterol in human fibroblasts," we reported for the first time that LDL delivers cholesterol to cells and that this delivery is mediated by the cell-surface LDL receptor that we had described one year earlier (2). There was no cholesterol delivery to cells from patients with homozygous familial hypercholesterolemia (FH), which lack LDL receptors. This finding was a crucial advance in understanding how the LDL receptor functions, and it led directly to the subsequent demonstration that the LDL receptor internalizes the entire LDL particle through a process that we subsequently called receptor-mediated endocytosis (3). Over the ensuing years, we published an additional 25 joint papers in the *JCI*, including an opinion piece on clinical investigation entitled "The clinical investigator: bewitched, bothered, and bewildered — but still beloved" (4).

In addition to publishing in the *JCI*, both of us have been active members of the ASCI for 35 years. One of us (Goldstein) served as ASCI president in 1986. We presented our work on several plenary sessions in Atlantic City, including a memorable double-header presentation in 1974 — one at the ASCI and the other at the AAP — when we unraveled the basic defect in FH and revealed the evidence tying LDL receptor deficiency to hypercholesterolemia, atherosclerosis, and myocardial infarction. For us, the annual ASCI meeting was both intimidating and inspirational. Like others who have written for this series, we made a huge effort to tell our scientific story in a lucid, engaging, and convincing way. For this purpose, we had the unceasing and unerring advice of our father figure, Donald Seldin, chair of Medicine at UT Southwestern, who took an intense personal interest in all plenary session presentations. Other colleagues, such as Jean Wilson, Daniel Foster, and John Fordtran — all ASCI stalwarts — helped enormously as unabashed critics. It was an enormous thrill to stand on the podium looking out over an audience of hundreds of leaders of academic medicine, many of whom were long-standing heroes of ours.

Here, we focus on two of our *JCI* articles, both of which dealt with fatty deposits — one in atherosclerotic plaques (5) and the other in liver (6). The first article was entitled "Overloading human aortic smooth muscle cells with low density lipoprotein-cholesteryl esters reproduces features of atherosclerosis in vitro." This work was stimulated by a paradox. In studies of cultured fibroblasts, we had earlier found that the LDL receptor is downregulated when cholesterol begins to accumulate within the cell (7). This downregulation makes it impossible to overload fibroblasts with cholesterol by incubation with LDL, no matter how high the concentration. Yet in arterial fatty streaks that lead to atherosclerotic plaques, smooth muscle cells and macrophages are filled with cholesteryl ester droplets (8). These deposits are particularly severe in arteries of patients with homozygous FH despite their lack of LDL receptors. Clearly, some other uptake process must be responsible.

In the 1977 *JCI* article, we confirmed the downregulation of LDL receptors in a line of human aortic smooth muscle cells. We showed that we could overload these cells with cholesteryl esters in the absence of LDL receptors if we derivatized LDL with an adduct that created additional positive charges on the protein component (Figure 1, A and B). The positively charged LDL adhered nonspecifically to negatively charged cell-surface proteins and carbohydrates, and it entered the cells, albeit at a low efficiency, by nonselective endocytosis. Although this chemical modification is not physiologic, it provided the first opportunity to study the cellular response to cholesterol overloading. This study paved the way to subsequent studies in which we used acetylated LDL to deliver cholesterol to macrophages. These latter observations opened the door to the discovery of scavenger receptors (9, 10) and a plausible mechanism by which lipoprotein-derived cholesterol may deposit in artery walls, accelerating atherosclerosis in the absence of LDL receptors (11).

As a historical aside, it is interesting to note that the photograph of oil red O droplets in Figure 1B was the first color photograph published in the *JCI*. Our insistence on color in 1977 created a stir among the *JCI* editors, some of whom balked at our suggestion, invoking the argument that the color photo was "outlandish" and would set a bad precedent. Fortunately, the editors acquiesced, but only at the last moment, when the article was in the proof stage and only with the proviso that we pay the rather large charge in advance.

One other historical footnote about this article: it was the only one of our 26 *JCI* papers that was accepted without a single revision and published in what was record time 30 years ago, i.e., in

Conflict of interest: The authors have declared that no conflict of interest exists.

Citation for this article: *J. Clin. Invest.* **118**:1220–1222 (2008). doi:10.1172/JCI34973.

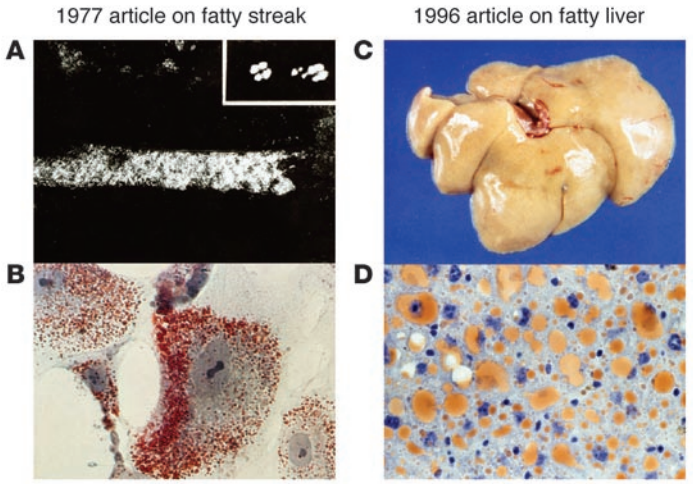

Figure 1
Photographs of a cholesterol-loaded smooth muscle cell (**A** and **B**) and a fatty liver (**C** and **D**) that were published in our 1977 and 1996 articles in the *JCI* (5, 6). **A** and **B** show human aortic smooth muscle cells that were incubated with LDL that had been given a net positive charge by covalent linkage with *N*,*N*-dimethyl-1,3-propanediamine. After 19 days in culture, the cells were examined either with polarizing microscopy (**A**) or stained with oil red O and hematoxylin (**B**). Note that the smooth muscle cell in **A** shows inclusions that exhibit birefringence with typical formée crosses indicative of liquid crystals of cholesteryl esters as shown in the inset. Parallel dishes of cells incubated with native LDL in the same experiment showed no birefringence and no oil red O staining. Original magnification: ×525 (**A**); ×3,520 (**A**, inset); ×600 (**B**). **C** and **D** show the pale color and fat-laden histology of the enlarged fatty liver from a mouse expressing a transgene encoding the nuclear form of SREBP-1a. The liver sample in **D** was stained with Sudan IV, which stains fatty inclusions orange. Original magnification, ×378.

less than 3 months after the date of submission. These were the days before FedEx, fax, and e-mail! Needless to say, not all of our papers received such swift attention.

Twenty years later (1996), we published another article on fatty overload (6). This time the overloaded organ was the liver, and the fat came not from exogenous uptake but from endogenous synthesis. These experiments followed upon our discovery of SREBPs, a unique family of membrane-bound transcription factors that activate genes encoding all of the enzymes required for synthesis of cholesterol, fatty acids, and triglycerides in animal cells (12, 13). In order to enter the nucleus, the active domains of the SREBPs must be released from membranes proteolytically after the protein is transported in vesicles from its site of synthesis in the ER to its site of processing in the Golgi complex. This vesicular transport process is highly regulated. When sterols build up in ER membranes, the SREBPs become trapped in the ER and lipid synthesis declines dramatically.

Our 1996 *JCI* article was entitled "Overproduction of cholesterol and fatty acids causes massive liver enlargement in transgenic mice expressing truncated SREBP-1a." In collaboration with our colleague Robert Hammer, we prepared a transgene encoding the nuclear fragment of SREBP-1a, the most potent of the three SREBP isoforms. The encoded protein was truncated so as to eliminate the membrane attachment domain. The truncated SREBP-1a is synthesized as a cytosolic protein, and it enters the nucleus without a requirement for proteolysis. The truncated SREBP-1a is therefore immune to downregulation by cholesterol. The transgene encoding the truncated SREBP-1a was driven by a promoter that gives high-level expression in the liver. We produced several lines of transgenic mice that carried one or more copies of this transgene.

The result of this experiment was dramatic. Livers of the transgenic mice became stuffed with fat (Figure 1, C and D), which consisted of a mixture of cholesteryl esters and triglycerides. Despite the enormous overloading with fat, the messenger RNAs encoding lipogenic enzymes were not downregulated. In fact, they were markedly increased. As a result, the liver continued to produce large amounts of cholesterol, fatty acids, and triglycerides at rates that were 5- to 25-fold higher than those observed in normal liver. In a subsequent *JCI* article, we showed that a similar phenotype could be elicited by eliminating the genes for Insigs, which are the regulatory proteins responsible for turning off the processing of SREBPs when intramembranous cholesterol rises (14). Thus, endogenous levels of SREBPs are sufficient to produce massive lipid overproduction if the mechanism that constrains them is eliminated.

Feedback regulation of hepatic lipid synthesis is crucial in the prevention of fatty liver. In humans, such fat deposition frequently leads to cirrhosis and liver failure. Recent experiments by several groups of investigators have demonstrated the crucial role of one isoform of SREBP, SREBP-1c, in synergizing with alcohol to produce fatty liver in mice (15, 16). Moreover, it is likely that excessive activity of SREBP-1c contributes to nonalcoholic steatohepatitis (NASH), which occurs frequently in subjects with insulin-resistant type 2 diabetes in the form of the metabolic syndrome (17). Experiments in mice suggest that excessive triglyceride accumulation in liver and blood is caused by high levels of insulin, which increase the mRNA for SREBP-1c in liver and enhance its proteolytic processing (18). These studies of SREBP in mouse liver may have implications for human disease.

In retrospect, it is remarkable that the 1977 article on smooth muscle cells and the 1996 article on liver both reported that lipid overaccumulation required use of artificial means to defeat normal feedback mechanisms. In 1977 we used a modified lipoprotein to circumvent the downregulation of LDL receptors, and in 1996 we used a truncated transcription factor to circumvent the downregulation of mRNAs encoding lipid biosynthetic enzymes. These studies illustrate the crucial importance of the elaborate mechanisms that mammalian cells use to guard against lipid accumulation from exogenous or endogenous sources.

Why was it essential that our two articles on fat overload be published in the *JCI*? The explanation lies in the unique status of this journal. The importance of both papers went beyond basic science. Editors and readers of elite basic science journals such as *Nature*, *Cell*, and *Science* would have difficulty comprehending the importance of studying fat accumulation in smooth muscle cells and hepatocytes. These articles are relevant to medical science because they address important diseases, namely, atherosclerosis and cirrhosis. They might have been published in subspecialty medical journals, but such journals have a narrow readership. By publishing in the *JCI*, we were able to reach a broad audience of medically oriented scientists. This audience relies upon the rigors of the *JCI* editorial process.

Articles published in the *JCI* carry an aura of prestige and importance that goes beyond individual medical disciplines. As investigative medicine progresses through the 21st century, it is crucial that we preserve this attribute of the *JCI*. Physician-scientists are drawn more and more to narrow specialties. We need to maintain a community of scholars who are willing and anxious to read and absorb articles of medical science outside their own field. By preselecting articles that merit wide readership, the *JCI* fulfills an essential function. May the ASCI and the *JCI* flourish for another 100 years.

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Atlantic City memories

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Fifty years ago, the Atlantic City meetings, held the first week in May of every year, were attended by all the elite of American academic medicine and all who wanted to join that group. Part of the magic of those meetings was that professors and neophytes took each other seriously and talked to each other.

In the 1950s and 1960s, Atlantic City was an informal meeting place attended, the first week in May, by all the elite of academic medicine and all who wanted to join that group. There were many fewer academic physicians then than there are today. At Yale, for example, the entire full-time faculty in the Department of Internal Medicine had no more than 20 members, comparable, perhaps, to the number in a division within such a department today. Specialists in one branch of medicine usually felt some obligation to keep up an interest in new advances in other branches, though one tuberculosis specialist once told me, "With me, everything below the diaphragm is strictly for pleasure." The Boardwalk, spacious and sunny, was the place to hang out, to meet your friends, and to catch glimpses of the leaders of scientific medicine in their relaxed moments. For many years, the headquarters of the three societies that met together in Atlantic City, dubbed "Young Squirts" (American Federation for Clinical Research [AFCR]), "Young Turks" (American Society for Clinical Investigation), and "Old Farts" (Association of American Physicians), was the Haddon Hall Hotel. Invitation to breakfast with a senior academic physician at Haddon Hall was a well-recognized recruitment maneuver.

To have an abstract accepted for oral presentation at the meeting of the Young Turks was a prized accolade that made the long days of a slow accumulation of data seem worthwhile. There were always questions afterward, sometimes by authorities whose names you recognized and whose papers or chapters you had actually read.

After the Young Turks' day of scientific papers (at Haddon Hall or, later, at the Steel Pier), the accepted procedure was to crowd into the bar of the Hotel Brighton for one or more Brighton Punches, a lethal rum–fruit juice combination designed to promote irreverence (Figure 1). Then one went to Hackney's or Cap't Starn's Seafood Restaurant at the extreme north end of the Boardwalk for a dinner that always included a dozen oysters and a grilled lobster.

Conflict of interest: The author has declared that no conflict of interest exists.
Citation for this article: *J. Clin. Invest.* **118**:1222–1223 (2008). doi:10.1172/JCI34654.