APPENDIX-I (Eye)

## "Snapshot" Epidemiology: Why Lipid Levels Only APPEARED Less Important at Older Ages



### • Part 1. Emergence of a Clinical Disappointment and a Scientific Puzzle

In the early 1950's, the Donner team advanced evidence --- initially from case-control studies --- that all members of the Std Sf 0-400 serum lipoproteins (LDL, IDL, and VLDL) are significantly and independently elevated in Ischemic Heart Disease (Appendix-E). We proposed that arterial exposure, over time, to elevated levels of these serum lipoproteins causes acceleration of and greater amounts of coronary artery atherosclerosis. During that same period, prospective studies were launched, including one at the Lawrence Livermore Laboratory --- a study which included all members of the entire lipoprotein spectrum, low and high density, except for the Std Sf 400+ lipoproteins.

#### 1a. Confirmations --- Including a Perplexing Observation

By 1965, the prospective studies provided some major and extensive confirmation of the case-control findings, but there were some major unresolved questions, too (Gofman 1966). The powerful picture which had emerged by 1965, from two independent prospective studies --- Livermore and Framingham, both of which were based upon ultracentrifugal analysis --- was of an extremely strong relationship of Std Sf 0-400 lipoproteins with Ischemic Heart Disease (IHD) for men entering the study at 30-39 years of age. Moreover, the same lipoprotein classes, Std Sf 0-20 and Std Sf 20-400, which had been proven to be independently associated with IHD risk in the case-control studies, were shown prospectively to be strongly associated with IHD risk in men (more details in Appendix-E, Part 12).

Both prospective studies also confirmed a perplexing observation from the early case-control studies --- namely, the IHD-lipoprotein association appeared to be weaker in the older groups than in the younger groups of such studies.

#### 1b. What Made This Observation "Perplexing" for Many Years?

The observed decline, in the IHD-lipoprotein relationship with advancing age, represented both a scientific puzzle and a clinical disappointment. It hardly made sense that a group of circulating lipoproteins, measured at relatively young ages, could be causally related to development of coronary atherosclerosis --- and NOT be causally related if they were measured, instead, at ages beyond 55 years. The clinically disappointing aspect was that it appeared no medical benefit could be achieved by attention to lipoprotein levels beyond 55 years of age, since IHD risk appeared so weakly associated with Std Sf 0-400 lipoprotein level.

Further consideration of the puzzle identified a reason for the apparent age-related decline in association of Std Sf 0-400 lipoprotein levels with IHD risk. The word "apparent" must be stressed, since our subsequent analysis (Gofman 1978) led us to conclude that, most probably, the Std Sf 0-400 lipoproteins are causally important for coronary atherogenesis and risk of clinical IHD at all ages, including advanced ages. It is the purpose of Appendix-I to summarize that analysis, and to reflect on the limits of "snapshot epidemiology" in general.

### • Part 2. "Snapshot Epidemiology": Nature Is Not Obliged to Be Convenient

Whether one studies how a disease relates to some aspect of the blood in a population, or to

some other parameters, the easy parameters to evaluate are those which are essentially constant, both acutely and over decades. In such a simplified situation, individuals can be ranked according to the parameter at one point in life, and be relied upon to remain in that rank order throughout the decades during which a process such as atherosclerosis develops.

The usual prospective study takes a "snapshot" (a single measurement) of a parameter at one point in time, and neglects its past history as well as its future behavior. If such studies deal with a stable parameter, one "snapshot" can suffice.

### 2a. A Severe Inconvenience in Medical Research

But Nature is not compelled to be kind to medical investigators, and only a very few parameters are fixed diurnally and over decades. Diurnal variation deserves special mention. The vague medical notion, that stable parameters are more important for disease development than are those with substantial diurnal variation, has no basis. Nature is under no obligation to prevent a highly variable parameter from being the key element in a disease process, even at the loss of convenience for the medical investigator.

If the parameter under study is not fixed, the investigators in a "snapshot" study can hope that the non-measured exposures do not change the relative RANKING (established by the "snapshot" measurement) of a study's participants upon that parameter. The investigators start their study with an appreciable gradient in measurements, from low to high. But without their knowing it, 10 years after the "snapshot" or 10 years before the "snapshot," many participants may have OTHER places in the ranking (due to changes in the parameter which were never measured). The "snapshot" rank-order may be a poor (even misleading) indicator of differences in lifetime exposure to the parameter, if the parameter is quite unstable over time.

It appears that a proper prospective study would start early in childhood, measure crucial parameters at least annually (and with a check on diurnal variation), and would follow this procedure for the full lifespans of the participants.

### 2b. The Serum Lipoprotein Levels: Not a Stable Parameter

Ischemic Heart Disease (Coronary Artery Disease) has two phases in time: (1) Coronary atherogenesis, which is a silent pathological process occurring over decades, and (2) Clinical Ischemic Heart Disease, which is a relatively or extremely abrupt announcement of the pathological process --- a process which may well continue, if its announcement was not fatal.

The Lipid Hypothesis means that risk of a fatal outcome is higher in persons with higher average exposure to atherogenic lipoproteins over TIME (all other co-actors being equal). Therefore, variation of exposure-level matters, daily and over the lifespan.

Nonetheless, virtually all reported prospective studies, on the relationship of serum lipoprotein levels and Ischemic Heart Disease, have failed to find out how serum concentrations of the Std Sf 0-12, 12-20, 20-100, and 100-400 lipoproteins VARIED in each participant, acutely over daily cycles and chronically over decades of life. We must fault the magnificent Framingham Study and our own Livermore Study on both counts.

It turns out that the Livermore Study contains evidence which WARNS that a remarkably large share of American adults change their lipoprotein measurements even during a brief period of 18-months. Part 3 presents that evidence.

## • Part 3. Remarkably Frequent Changes in Weight and Lipoprotein-Levels: Livermore Data

By 1952, it was already evident that obesity was associated with unfavorable serum elevation of certain atherogenic lipoproteins (Gofman 1952-b, p.514). The fact, that serum lipoprotein levels rise with weight-gain and fall with weight-loss, was illustrated in Appendix-F, Part 12, by some specific evidence.

Here, we add to that evidence with some interesting measurements from the Livermore

Lipoprotein Study. Of the 2,297 persons in the Livermore database, 374 individuals were examined twice, with the average interval between examinations being 1.5 years. The findings were as follows for this interval of 1.5 years:

Number whose weight changed by less than 5 pounds: 164 persons.

Number who lost five pounds or MORE: 73 persons.
Average loss of weight = 10.7 pounds.
Avg change in Std Sf 0-400 lipoprotein level = decline by 5.0 mg/dl for each pound of weight lost.

Number who gained five pounds or MORE: 103 persons.
Average gain of weight = 9.6 pounds.
Avg change in Std Sf 0-400 lipoprotein level = rise by 5.0 mg/dl for each pound of weight gained.

Number who did not change weight: 34 persons.
Stable weight was associated with stable Std Sf 0-400 lipoprotein level.

There were 176 persons (73+103) who either gained an average of about ten pounds (4.54 kilograms) in the 1.5 year interval or lost about ten pounds in that interval. None of the individuals had been advised, requested, or instructed by the Livermore Laboratory to change weight. Since there was a total of 374 individuals who were studied twice, it follows that (176 / 374), or 47 percent of the Livermore population sample showed large changes in weight and Std Sf 0-400 lipoprotein levels in the brief span of 1.5 years. This remarkable fact is ordinarily obscured by the AVERAGE weight-gain trend during the decades of adulthood in the United States.

How does this information relate to "snapshot" epidemiology? One illustration can suffice for endless variations.

Suppose that two individuals, A and B, have the same age, and have equal levels of Std Sf 0-400 serum lipoproteins up to a given time. Both have followed the mean trend of rising serum lipoprotein levels with advancing age (shown in Appendix-F, Box 5). Now, suppose that during the 1.5 years which follow the "given time," person A gains 10 pounds of weight (as did 27.5% of the Livermore employes) and person B loses 10 pounds of weight (as did 19.5% of the Livermore employes).

Then we come along and enroll them in our "snapshot" prospective study about the relationship of serum lipoprotein levels with Ischemic Heart Disease. At this point, A has appreciably higher levels than B, although the levels had been the same until 1.5 years earlier. In such a study, having no prior lipoprotein measurements of A and B, we in effect assume (but mistakenly) that A and B have differed in their lifetime exposure to the atherogenic lipoproteins. And we assume that they will continue to differ. In truth, however, A and B have had nearly equal INTEGRATED exposure, prior to our "snapshot." Moreover, the data above certainly shatter the assumption that their "snapshot" measurements will remain constant during the follow-up years.

Part 4 combines the information In Part 3 with the information from Appendix-F, Box 5, to address the APPARENT weakening of the male IHD-lipoprotein relationship at older ages.

## • Part 4. Why Lipoprotein Levels Only APPEAR Less Important at Older Ages

The data and graphs in Appendix-F, Box 5, show that average serum levels of the Std Sf 0-20 and 20-400 lipoproteins rise steeply in males from about age 18 to age 42. Then the increase flattens out or is already declining, on the average. Our interpretation of the curving-over of these plots is that it results from an increase in the number of men who shift, from positive caloric balance or from caloric equilibrium, into negative caloric balance as they age. It can be anticipated that in culturally different populations, these curvatures might be different, if customary weight-behavior is different.

The same Box 5 (Appendix-F) shows that levels for females during adulthood are lower than levels for males --- until the women catch up with the men at about age 50 for the Std Sf 0-20 lipoproteins and at about age 59 for the Std Sf 20-100. After these ages, the female levels exceed the male levels. Box 5 makes it evident that the average female has a lower INTEGRATED exposure to

the atherogenic lipoproteins than the average male. Their age-adjusted MortRates from Ischemic Heart Disease have always been much lower than male rates, too (Table 41-B).

## 4a. Age at "Snapshot": Males 30-39, and Females

Despite the rising, non-constant lipoprotein levels so obvious in Box 5, investigators necessarily hope in "snapshot" prospective studies that individual participants do, at least, retain their relative RANK ORDER with respect to Std Sf 0-400 lipoprotein levels. For men in the age-band of about the 20-35 years, we are lucky, simply because relatively few individuals (relative to older age-bands) "fall out of order" during the steep upward trend in average weight (positive caloric balance being the rule).

Thus, at the end of a 12-year follow-up, we observed a very strong positive association of Std Sf 0-400 lipoprotein level with IHD risk, for those men who entered the "snapshot" prospective study between 30 and 39 years of age (Gofman 1966, p.683, Table 3). We suggest that the association would have been stronger yet, if not for perturbations in lipoprotein levels caused by relatively acute fluctuations in weight (Part 3). Moreover, at 12 years after the "snapshot," the surviving men had an average age of about 48 years, which means they had spent little time beyond the age when peak-exposure to Std Sf 0-400 lipoproteins changes to its downward direction.

What about the women of ages 30-49 years at their "snapshot" measurement? Their average age was 44.4 years then, and about 56 years at follow-up. Because of the much lower rates of IHD in females than males, there were still too few cases of de novo IHD (16 cases in 1,635 women) to obtain much statistical power (Gofman 1966, p.683, Table 7). The statistically strongest IHD-lipoprotein associations occurred in the female cohort of ages 50-55 (average age of about 65 at follow-up). In this group, there were 39 de novo cases among 468 women (Gofman 1966, p.683, Table 8). But for the oldest female cohort (having ages 56-69 at "snapshot" and a mean age of about 71 years at follow-up), the statistically significant IHD-lipoprotein associations appear to have vanished (Gofman 1966, p.684, Table 9). So, we do not know to what extent --- if any --- the discussion here is applicable to females.

## 4b. The Mis-Match between True Rank-Order and Ostensible Rank-Order

By contrast with the men ages 30-39 at "snapshot," men who were ages 40-49, or 50-59, or 60-69 at the time of their "snapshot" measurement, spent most or all of their follow-up time at ages when the AVERAGE level of Std Sf 0-400 lipoproteins was declining (Appendix-F, Box 5).

The observed change, from average rise in lipoprotein level to average decline, can have two possible explanations. Either all the men are changing in this manner, or some are still rising in lipoprotein level (and in weight-level) while an increasing proportion are leveling out or declining in lipoprotein level (and in weight-level) with increasing age. We suggest that experience favors the second interpretation.

Under the latter interpretation, the change from an average rise in lipoprotein level to an average decline, offers extra opportunities for individual participants to experience major, unrecorded changes in their lipoprotein RANK ORDER within their age-band.

The men who were ages 40-49, at their "snapshot" measurement, have experienced not only more years of acute lipoprotein fluctuations (Part 3) than the 30-39 year cohort, but a growing share of men in the 40-49 age-group shift from increasing levels to decreasing levels of serum lipoproteins. In the 40-49 age-group, these circumstances cause the rank order of Std Sf 0-400 lipoprotein levels to become materially perturbed (if not massively so), relative to the rank order assigned by the "snapshot" measurements.

A mis-match is developing, between the true rank order and the ostensible rank order.

The result: The association of Std Sf 0-400 level with IHD risk in males appears to be markedly weakened. The IHD-lipoprotein association necessarily appears to weaken even more, as age at the "snapshot" measurement increases. When 58 is the average age at "snapshot" (age 70 at follow-up), all of the IHD-lipoprotein association appears to be lost for males in a prospective study (Gofman 1966, p.683, Table 6).

The REASON is that the "snapshot" Std Sf 0-400 level tells us less and less about the integrated Std Sf 0-400 lipoprotein exposure, as age-at-entry to such a study becomes higher. It is INTEGRATED exposure which relates to risk of IHD (all other co-actors being equal).

## 4c. The Need to Find Out the True Rank Order

This problem in "snapshot" epidemiology is not going to go away, and the problem is not peculiar to the study of the IHD-lipoprotein relationship. It applies to many etiologic co-actors in Ischemic Heart Disease and in other disorders which build up over decades. What is needed is an appreciable increase in the frequency of measuring the key variables, if one hopes to reduce the frequency of FALSE conclusions.

Parts 4a and 4b suggest that "snapshot" epidemiology came close to concealing the now well-confirmed IHD-lipoprotein relationship --- and might have done so, were it not for the inclusion of the men ages 30-39 at their "snapshot" measurement. Parts 3 and 4 help to explain why this group was able to avoid the pitfall.

The nature of the pitfall indicates that the APPARENT loss of the IHD-lipoprotein relationship, in men measured only at advanced ages, was probably not a REAL absence of the relationship. Recognition of this pitfall moves us to warn that serum lipoproteins which are proven atherogens at one age almost certainly remain atherogenic at even the most advanced ages.

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