Letter to the Editor

The Claim of "Lack of Effect of High Fat Intake on Serum Lipid Levels"

Dear Sir:

The paper by G. V. Mann under the above title in the issue of May-June 1955 of this JOURNAL[1] provides an interesting case study in statistical fallacy applied to inadequate evidence. The resulting conclusions will not impress anyone who examines the data, but busy readers often forego critical analysis of the papers they scan. It may be useful, therefore, to underscore the failure of this paper to justify its title and to use the example to point out some elementary requirements in experimental design and analysis.

The evidence in this paper is obtained from two "control" blood samples from each of two young men, followed by five samples during the succeeding 15 days on a special kind of high fat diet. The possibilities and limitations of valid conclusions can be predicted in advance. Aside from noting that any conclusions must be restricted to this situation of healthy young men changing from the control diet used to the "pemmican" diet for 15 days, the limitations are great.

Obviously the hypothesis under test is the question: "Does the high fat diet result in a elevation of one or more of the items of measurement in the blood serum?" The simplest and most impressive confirmation conceivable would be the situation where all of the experimental values are higher than any of the preceding controls. Such high consistency can rarely be expected in experiments when the characteristics measured have much spontaneous variation such as the case with these serum lipid fractions.

But the data show, surprisingly, that for cholesterol this perfect consistency with the hypothesis obtains for both subjects. The same is true for the measurements of S_f 12–20 for subject "G" but not for subject "B." The recorded values for S_f 21–35 and S_f 35–100 are inconsistent.

With no more ado, then, we conclude that the cholesterol data are in conformity with the hypothesis and that no greater consistency than found could, in fact, emerge from two such experiments. In regard to S_f 12–20, the data for subject "G" show the same perfect consistency but this is not true for subject "B," so for S_f 12–20 the general hypothesis about all young men is not supported, though the results are consistent with the hypothesis that in *some* young men this high fat diet results in an increase in the S_f 12–20 in the serum. The results with S_f 21–35 and S_f 35–100 admit of no useful conclusion.

The use of "t" tests or other elaborations cannot change these conclusions but will clarify the problem. The key, of course, lies in the consideration of variability, particularly the question of intra-individual variability. Where there are large numbers of subjects and observations the familiar procedure would be followed, with computation of standard deviations and errors after getting assurance that the measures are normally distributed within both control and experimental periods. This is not possible, of course, with only 2 control observations and it is highly hazardous to attempt with so few as 5 experimental observations. But suppose we assume that these young men are like other American men who have been studied so we may attribute to them the same kind of intra-individual variability found in more extensive studies? Let us, in effect, examine the quantitative possibilities before undertaking the experiments.

In "control" studies on healthy men in the United States we have made hundreds of repeated blood samplings on the same individuals and they yield intra-individual standard deviations of ±18 to ±20 mg per 100 ml about the individual cholesterol means and ±7 to ±9 mg per 100 ml about the individual means for S_f 12–20. Others have had the same experience and even within a single day the intra-individual

variability is large. Mann himself has reported[2] average intra-individual standard deviations for repeated blood samples taken within 24 hours to be, for cholesterol, ±15.8 mg per 100 ml in 8 patients and ±10.8 mg per 100 ml in 9 "controls." The corresponding values for S_f 12–20 were ±5.8 and ±4.0 mg per 100 ml.[2]

We can, therefore, estimate roughly the least size of an effect on the high fat diet that would be "proved" at the 5 per cent level of probability from 2 control and 5 experimental observations, assuming that the variability of the serum values on the high fat diet is no greater than on the control diet.

If we take the true intra-individual standard deviation of the individual means to be ±18 mg per 100 ml for cholesterol and ±7 mg per 100 ml for S_f 12–20, we can compute the standard error of the difference (S. E. diff.) between the control and experimental means:

$$\text{S. E. diff.} = \sqrt{(N_1 + N_2)\, V_p/(N_1N_2)}$$

where V_p is the pooled variance, computed as

$$V_p = \frac{(N_1 - 1)\, V_1 + (N_2 - 1)\, V_2}{(N_1 - 1) + (N_2 - 1)}$$

V_1 and V_2, of course, are the variances, or standard deviations squared, for the control and experimental means, respectively.

In the present problem, where $N_1 = 2$, $N_2 = 5$, $V_1 = 18 = V_2$, for cholesterol, the standard error of the difference of the means proves to be ±15.1 mg per 100 ml. And for S_f 12–20, where $V_1 = 7 = V_2$, S. E. diff. = 5.8 mg per 100 ml. In order to reach the 5 per cent level of probability, we must insist that the "t" value, i.e., the ratio of the difference between the means divided by the standard error of that difference, be 2.57 for 5 degrees of freedom. Let Δ = the difference between the means. Then $\Delta/15.1 = 2.57$ and $\Delta = 38.8$ mg per 100 ml, the difference between the cholesterol means required to show statistical significance. For S_f 12–20 we have $\Delta/5.8 = 2.57$ and $\Delta = 14.9$ mg per 100 ml. In other words, from 2 control and 5 experimental observations no effect could be demonstrated at $P = 0.05$ unless the true mean effect of the high fat diet in many observations were to raise the cholesterol level by about 39 mg per 100 ml.

But we have *two* subjects and sets of observations. We should, in other words, combine the observed probabilities. In the simplest and most favorable case, where both experiments yield results having identical probabilities, this would mean that, since (0.23) (0.23) = 0.05, we could accept two minimal values for "t" corresponding with the 0.23 level of probability or, with five degrees of freedom, "t" = 1.36. Hence we might hope, in the most favorable case, to establish the hypothesis of an effect of high fat with two experiments, each having 2 control and 5 experimental observations, with a true mean fat effect as small as 20.54 mg per 100 ml of increase in cholesterol in each of two subjects.

With proper experimental planning, all of this should have been clear before the experiments were made, in which case, no doubt, a larger and more suitable experiment plan would be devised. In any case, it is indefensible to attempt to disprove the hypothesis by the device of using "t" tests separately on each of these very small series of observations.

Let us follow the procedure of Mann in the analysis who says, "the mean levels of the five observations during the Pemmican treatment were compared with the mean control (two observations) by the application of "t" tests" (pp. 230–231). The cholesterol data are readily reconstructed from his Figs. 1 and 2. They are given here in Table I.

TABLE I

Cholesterol Values, in mg per 100 ml, Read Off from Figs. 1 and 2 in the Paper by Mann[2]

Subject	Control	Experiment	Post-experiment
"G"	248, 243	301, 271, 264, 273, 280	266
"B"	298, 267	327, 304, 301, 316, 321	305

The "t" values for cholesterol prove to be, with 5 degrees of freedom in each case, "t" = 2.97 for "G," and "t" = 2.68 for "B." Standard statistical tables show that for 5 degrees of freedom the 5 per cent level of probability is reached at "t" = 2.57 and the 0.01 per cent level at "t" = 4.03. The "t" analysis says that, given the two observations on cholesterol to start with, the subsequent five observations

on "G" during Pemmican feeding would arise by chance in less than 4 per cent of trials and those on "B" in less than 5 per cent of trials. The combined probabilities of the two experiments indicate that the higher values on fat feeding would arise by chance in something fewer than 2 out of 1,000 trials. The best estimate from these experiments is that the change to a high fat diet results in mean rises in serum cholesterol of 32.1 ± 10.8, and 31.3 ± 11.7 mg per 100 ml, for "G" and "B," respectively.

But Mann says that his use of the "t" test "revealed no evidence that subject B had influenced his serum lipid levels by this high fat intake. The cholesterol and S_f 12–20 levels of subject G indicated only suggestive evidence ($0.10 > P > 0.05$) that they had been increased by the treatment." How is this possible from these data? Perhaps in his computation Mann included the post-experiment observations as part of his "control," though he explicitly indicated he used *two* observations and not three.

But if the post-experiment observations are used we unwittingly introduce an additional hypothesis: "The post-experiment observations are not influenced by the previous high fat diet and are proper samples of the control universe." Can we prove this? On the contrary, computation indicates that these post-experiment observations are not homogeneous with the controls. Let us combine the observations on "G" and "B" by expressing the control and post-experiment observations on each subject as percentage of the mean control value for that subject. We have then, 4 control values, 101.0, 99.0, 105.5, 94.5, and 2 post-experiment values, 108.4, 108.0, the means of these being 100.0 and 108.2, respectively. But the standard errors of these means prove to be ±2.63 and ±0.16, standard error of the difference between means is ±3.42 and "t" = (108.2 − 100.0)/3.42 = 2.40. At 4 degrees of freedom this means that the odds are about 14 to one that the post-experiment values do not belong in the same universe as the control values.

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REFERENCES

1. MANN, G. V.: Lack of effect of a high fat intake on serum lipid levels. AM. J. CLIN. NUTRITION 3: 230, 1955.
2. CHANDLER, H. L., LAWRY, E. Y., POTEE, K. G., and MANN, G. V.: Spontaneous and induced variations in serum lipoproteins. *Circulation* 8: 723, 1953.

COMMENT BY DR. MANN

Dear Sir:

I believe that Keys is correct when he accuses me of bias in the interpretation of these data. These observations do indicate that the dietary treatment increased the serum cholesterol levels. It now appears to me that it would be more appropriate to compare the observed *differences* between control and treatment levels at the several observations rather than to compare the measured levels as I first did and as Keys so elegantly elaborated. The method with differences will show a probability of about 0.01 that these might be attributed to chance.

It is of interest to compare this limited experience—and these experiments were the extent of my facilities—with the variability we have observed in adult men in their usual environment both with and without dietary alterations. Since we have published these findings it is not necessary to use the plentiful but still undocumented estimates of Keys.

In the studies with Walker[1] we found that a short period of positive calorie balance accomplished with a very low fat diet increased the serum cholesterol levels of two young men 38 and 70 mg per 100 ml, respectively. In another experiment[2] in which three young men gained weight rapidly for 28 days while con-