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Commentaries

Does weight gain lead to weight loss?

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Can we lose body weight permanently merely by carrying extra weight around with us for a time? That is the startling implication of a study by Adams et al. (2001). They reported that weight surgically implanted in mice resulted in weight loss that was proportionate to the weight of the implant and that persisted after weight removal. Adams et al. hypothesized that "*sensory perception of body mass is a critical regulator of metabolic tissue mass and is capable of resetting a biological set point of body mass*."

This is a remarkable claim with important theoretical and practical implications. Indeed, the finding was cited by Shanas et al. (2002) as a reason for not implanting transmitters in spiny mice. Its importance did not escape the notice of *Nature Science Update*, which featured the research under the headline 'Weight gain causes weight loss' (Whitfield, 2001). Unfortunately, there is reason to question whether the phenomenon is real.

Mice were assigned to groups by matching on body weight rather than by randomization, the 'gold standard' of experimentation. While this had the desired effect of eliminating body weight variability, it also created groups with significant pre-operative differences in food intake, a critical variable in this experiment. These pre-experimental differences compromise their post-experimental comparisons.

The authors' statistical analysis is a second cause for concern. They used the Newman–Keuls procedure, a controversial test that does not control the family-wise error rate (Curran-Everett, 2000). Inspection of the standard error bars on their figures suggests that many of their comparisons were not significant as claimed. I confirmed this by measuring the error bars and using the data to calculate two-tailed *t*-tests. Most were not significant.

The data presented in their fig. 2 on changes in body weight after implantation are critical to their conclusions. First, I note that the data on the body weights of the mice plus their implant weights duplicate the data on true body weights, which alone provide meaningful data. Their fig. 2C presents this information as a function of implant weights. My analysis indicates that none of these comparisons is significant. For fig. 2D, which reports decreases in true body weight from pre-operative body weight, my analysis confirms that the decrease in body weight for the 2 g implanted group was significantly greater than for either of the two control groups. However, the 3 g implanted group did not differ significantly from any group. Consequently, these data do not support the claim that the weight loss was "*proportional to the mass of the implant*" (p. 1729).

Changes in food intake were also investigated, and "*a clear trend for a reduction in food intake with increased implant mass*" (p. 1732) was reported. Yet this claim is unsupported by statistical evidence. Moreover, while I confirm that the intake of the 3 g group was significantly lower than that of the sham control group, it did not differ significantly from the implant control group. Thus, the evidence for a proportionate decline in food intake as a function of increasing implant weight is unconvincing.

Following the experimental phase, the implants were removed from half the mice in each group and all groups were monitored for an additional seven weeks. These data, reported separately for each implant weight condition, are also problematic. The data are presented as changes in body weight from pre-operative body weight. Only the data that give the true body weights of these mice minus their implant weights are meaningful. Except for a brief period, the true body weights of those still carrying implants did not differ from those in which the implants had been removed, both maintaining true body weights approximately 1 g below their pre-operative weights. There was no proportionate effect of implant weight. However, because data for the two control groups were not provided, the significance of this small decrease in weight compared with pre-operative weight five or more weeks earlier cannot be evaluated. Thus, it cannot be concluded that the decrease in weight was caused by the prior experience of carrying an implant weight.

There is also an anomalous result. The weight of the groups with implants removed showed a transient increase that peaked on day 3 after implant removal. This represented a return to pre-operative body weight for the 1 g and 2 g groups. More remarkably, for the 3 g group, it represented an increase of almost 10% *above* pre-operative weight. This is an unusual response that the authors fail to directly note. Instead, they discuss the transient increase only in relation to the body weights of the mice including their implant weights, which obscures this puzzling result.

Overall, I commend the authors for an original and intriguing hypothesis. Unfortunately, their study failed to

randomize subjects to groups, used questionable statistical analysis, failed to provide data for control groups for comparisons after the implants were removed, and reported an anomalous result. Consequently, the only reasonable answer to the question 'does weight gain lead to weight loss?' is that we cannot yet claim that it does.

I thank Ken Steele and Helen Black for their helpful comments.

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Response to 'Does weight gain lead to weight loss?'

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We were glad to learn that our study 'A novel mechanism of body weight regulation' (Adams et al., 2001) was of interest to Dr Black and we thank him for his creativity in re-interpreting our data (Black, 2003). The methods he used for this re-interpretation, while imaginative, are incorrect *inasmuch as they are inappropriate to our experimental system*. Black states four subjects of concern that invalidate our claims. These are: (1) failure to randomize; (2) questionable statistical analysis; (3) failure to provide controls for fig. 4; and (4) an 'anomalous result'.

1. Failure to randomize

The first question that Black addresses is the division of animals into one of five groups. He suggests randomization to be the 'gold standard'. We, of course, considered randomization, but were forced to create initially five groups with similar body weights. Had we randomized by body weight, then a justifiable criticism would be that significant differences in both pre-operative body weight and food intake would "*compromise [our] post-experimental comparisons*." Furthermore, as comparisons among animals were made on the basis of their change from the pre-operative mean, any concerns about pre-operative difference are moot, as each individual variable is, in fact, normalized by its own initial value.

2. Questionable statistical analysis

Black's second concern (and, in reality, the primary basis for his criticism) is the statistical analysis that we employed. Black cites Curran-Everett (2000) as describing the Student–Newman–Keuls *post-hoc* test that we used as "*controversial*". However, Curran-Everett points out in his excellent paper that the Student–Newman–Keuls test may be a flawed 'multiple comparison test'. We emphasize the term 'multiple comparison', because nowhere in his paper does Curran-Everett suggest that a *t*-test (a test that is appropriate only for comparing the differences of two means) would be an appropriate test for multiple comparisons. With his reference to Curran-Everett, Black is aware that to repeat our statistical analysis, a multiple comparison test is essential. We clearly state in our original paper (Adams et al., 2001) that we use a two-way analysis of variance (ANOVA) test using both 'implant weight' and 'time from implantation' as covariates. The bar graphs included in the paper were representative of the mean change with each experiment; they were not representative of the raw data. Therefore, we believe that Black's reanalysis of our data and the conclusions drawn from that reanalysis are incorrect.

To return to Curran-Everett and his criticism of the Student–Newman–Keuls procedure, underestimation of error caused by repeated measurements is a serious concern. In addition to the Student–Newman–Keuls test, the least significant difference (LSD) test also fails to control the probability of making a type I error. Furthermore, another multiple comparison procedure, the Bonferroni inequality test, is flawed in the other direction in that it may, in fact, fail to detect actual differences. As reported by Curran-Everett (2000), these three test are the three most commonly used multiple comparison tests in journals published by the American Physiological Society. To confirm that the results we presented in the original paper are significant, we have returned to our original data and reanalyzed it using a more conservative Tukey multiple comparison procedure in place of the Student–Newman–Keuls test. We found only two small differences in what the tests determined were significant. These are both shown in Fig. 1. Unlike the Student–Newman–Keuls test, the Tukey test did not determine significant differences between the sham control or the implant control groups and the

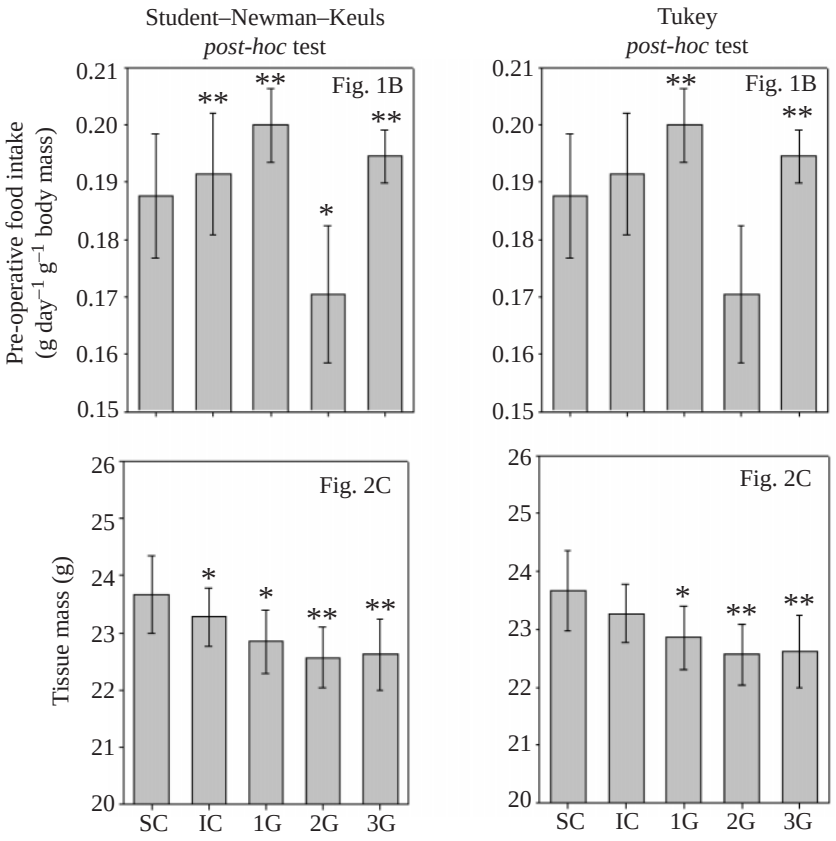

Fig. 1. Difference in results of two *post-hoc* tests. SC, sham control; IC, implant control; 1G, 1 g implanted group; 2G, 2 g implanted group; 3G, 3 g implanted group. Fig. 1B: **P*<0.05 in comparison to sham controls; ***P*<0.05 in comparison to 2G group. Fig. 2C: **P*<0.05 in comparison to sham control; ***P*<0.05 in comparison to sham and implant controls and 1G group.

2 g implant group for food intake (fig. 1B in our original paper). Therefore, despite Black's concerns regarding pre-operative food intake, a more conservative *post-hoc* test actually shows *fewer* pre-operative differences. The second change was seen in fig. 2C of the original paper. The Tukey test did not recognize a significant difference between the sham control and the implant control. There were no other differences between the results of the two tests. The differences that we did see do not alter our interpretation of the data or the conclusions that are presented in the paper.

3. Failure to provide data for control groups for fig. 4

Black suggests, "*because data for the two control groups were not provided, the significance of this small decrease in weight…cannot be evaluated*." The controls for each of these experiments are animals with similar implanted weight, not the sham and implant controls of the previous experiment. While Dr Black's concern with the types of controls could be further debated, the findings are clear: when the implant was removed from animals there was no compensatory increase in body weight.

4. An 'anomalous result'

Black ends his critique by decrying "*an anomalous result*." We really have no response to this statement, because all we have presented is an experimental paradigm, outcome data and a possible explanation of our findings. In fact, the results are provocative and in our opinion they deserve further investigation.

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