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Review

# The use of percentages and size-specific indices to normalize physiological data for variation in body size: wasted time, wasted effort?

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**Abstract**

Researchers commonly compute percentages or size-specific indices in an attempt to remove effects of body size from physiological data. Unfortunately, such ratios seldom eliminate the influence of body size on a physiological response and the ratios introduce major (but often unrecognized) problems with respect to statistical analysis and interpretation of the data. Indeed, these shortcomings of ratios frequently lead investigators to arrive at incorrect conclusions in otherwise flawless experiments. A superior alternative to using ratios combines graphical analysis and the analysis of covariance, which is a widely available statistical routine that uses least-squares regression to remove effects of body size from physiological data. Accordingly, we counsel researchers to discontinue forming ratios in an attempt to normalize physiological data for variation in body size and to adopt a reliable alternative. We also advise readers of scientific research not to place great confidence in results of studies that use ratios for scaling. © 1999 Elsevier Science Inc. All rights reserved.

*Keywords:* ANCOVA; Body size; Percentages; Ratios; Scaling; Size-adjustments

## 1. Introduction

The rate or intensity of a physiological process is usually higher in large individuals of a species than in small ones, so part of the variation in most physiological data results from variation in size of the animals being studied. This size-induced variation in physiological response contributes to 'background noise' in planned experiments with groups of animals that are matched for size and is a major confounding factor in descriptive studies focusing on groups of animals that differ in size [9,17,18]. Many physiologists consequently try to adjust their data to remove the influence of size—often by computing a percentage or size-specific index, that is, by computing some kind of a ratio that uses a measure of body size as the denominator (Table 1). Recent issues of Comparative Biochemistry and Physiology A contain numerous examples of ratios that were computed by well-intentioned workers who had the aforementioned goal in mind.

Forming a ratio, however, seldom removes the effects of body size on the variable of interest. Moreover, statistical analysis of data expressed as a ratio is commonly biased and unreliable and a ratio is subject to multiple (often opposing) interpretations. The insidious effects of ratios were first noted more than a century ago by the famous statistician Karl Pearson [19], whose concerns have been repeated and elaborated on numerous occasions (Table 2). Nevertheless, many investiga-

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tors still are unaware of the general problem that ratios engender and the practice of forming ratios to scale data continues to be widespread, despite the fact that conclusions from the affected studies are not well founded. We address these several points and then offer a set of recommendations for dealing with the problem of ratios. Our remarks are directed primarily toward younger scientists and other newcomers to the field, because these colleagues are more likely than others to be unaware of the pitfalls awaiting those who use ratios injudiciously.

## 2. Problems with ratios

### 2.1. Ratios seldom remove effects of body size

The practice of computing ratios to normalize data for variation in body size is based on the fundamental (but often unrecognized) assumption that the physiological response of interest varies as a fixed proportion of the measure of body size [2,10,11,17,18,23,25–27]. This condition (which is known to some workers as 'isometry', [14,17]) is satisfied when a plot of data on a graph with linear coordinates can be described by a straight line passing through the origin (Fig. 1A). Thus, the $y$-intercept for the line is zero and the equation for the line takes the general form of $y = bx$. Dividing both sides of the equation by the measure of body size yields $y/x = b$. The term to the right of the equal sign (i.e. the term $b$) is a constant of proportionality that is independent of body size.

A percentage or size-specific index will yield an appropriate adjustment for body size in cases where the preceding assumption is satisfied (Fig. 1B), but the assumption is satisfied only rarely in nature. In most instances the physiological response does not vary as a fixed proportion of the measure of body size (which condition is known to some workers as 'allometry', [14,17]). A plot of such data on a graph with linear coordinates yields a curve or, more commonly, a straight line that fails to pass through the origin (Fig. 1A). The equation for the latter takes the general form of $y = a + bx$. Dividing both sides of the equation by the measure of body size yields $y/x = a/x + b$. No constant of proportionality exists to the right of the equal sign. Forming a ratio in such cases does not remove effects of body size [5,6,11,17,23,24,26] and a plot of the ratio against the measure of body size usually yields a line with a significant correlation (Fig. 1B). The slope of the line depends on whether the $y$-intercept is positive or negative in the original bivariate plot and on how much the $y$-intercept differs from zero (Fig. 1). Even a small, 'non-significant' departure of the intercept from zero can introduce an important bias into the data [2].

Table 1
Examples of ratios that are commonly used by physiologists to correct, or normalize, data for variation in size of the animals under investigation

| Index | Method of computation |
|---|---|
| Percentage | (Organ mass/body mass) * 100 |
| Gonosomatic index | (Gonad mass/body mass) * 100 |
| Relative clutch mass | Mass of clutch/mass of postparturient female |
| Mass-specific metabolism | Rate of oxygen consumption/body mass |
| Area-specific water loss | Rate of water loss/surface area |
| Relative locomotion rate | Speed/body length |
| Relative consumption rate | Rate of food consumption/body mass |
| Relative growth rate | Rate of change in size/initial size |

Table 2
A partial, chronological list of biologists who have tried in recent years to alert their colleagues to the manifold dangers in using ratios to scale data for variation in body size

| Year | Worker | Reference |
|---|---|---|
| 1949 | Tanner | [25] |
| 1962 | Weil | [27] |
| 1965 | Dinkel | [11] |
| 1976 | Atchley et al. | [6] |
| 1977 | Anderson and Lydic | [4] |
| 1982 | deVlaming et al. | [10] |
| 1985 | Reist | [24] |
| 1986 | Gould | [14] |
| 1986 | Prothero | [21] |
| 1987 | Packard and Boardman | [17] |
| 1988 | Packard and Boardman | [18] |
| 1991 | Jackson and Somers | [15] |
| 1992 | Raubenheimer and Simpson | [23] |
| 1993 | Kronmal | [16] |
| 1993 | Albrecht et al. | [1] |
| 1995 | Allison et al. | [2] |
| 1995 | Raubenheimer | [22] |

### 2.2. Ratios introduce biases into statistical analyses

Workers who form ratios in an attempt to normalize physiological data for variation in body size unwittingly introduce important biases into their data and statistical analyses. We illustrate this point by examining two sets of hypothetical data—one to illustrate the problems that ratios introduce into planned experiments making use of groups of animals that are matched for size and the other to demonstrate the potential for ratios to mislead investigators conducting descriptive studies on groups of animals that differ substantially in

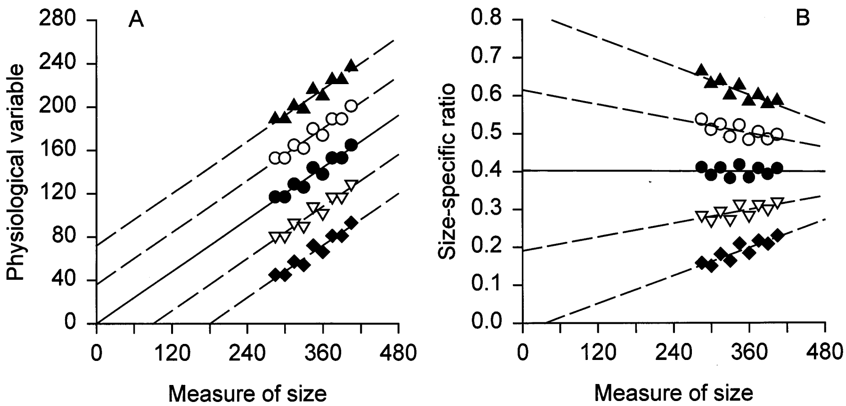


Fig. 1. Hypothetical data illustrate the importance of the assumption that is fundamental to using size-specific indices to scale data for variation in body size—namely, the assumption that the rate or intensity of the physiological response varies as a fixed proportion of the measure of body size. (A) Values for a physiological variable are plotted against corresponding values for body size. The data are displaced to the right of the $y$-axis because this is commonly the case with real data. Lines fit to the five data sets have identical slopes but different $y$-intercepts. The data set depicted by closed circles satisfies the aforementioned assumption, because the best-fit line passes through the origin of the graph. The other data sets do not satisfy the assumption, because intercepts are other than zero. (B) Data for the physiological variable in the preceding examples have been 'normalized' by dividing each value by the corresponding measure of size. Effects of size have been removed only from data that satisfy the aforementioned assumption (closed circles): the line that has been fit to these data is the only line having a slope of zero.

size. Although the numbers have been chosen for convenience and to make a point, they are representative of real problems in the analysis of real data [5,7,8,11,13,18,20,25–27]. We also illustrate an alternative method of analysis—a method that combines graphical and computational analysis. The alternative method is not constrained in practice by limiting assumptions because conclusions to which an investigator is led by the computational analysis must agree with those to which s/he is led by visual examination of the graphical display.

### 2.2.1. Increasing precision in planned experiments

Two samples of nine are drawn from the same population and submitted to study. One sample becomes the treatment group and the other becomes the control. Animals in both groups are measured for size and measurements are taken soon thereafter for the physiological variable of interest.

We first prepare a bivariate plot of the physiological variable against the measure of body size, so that we can examine the data visually for patterns and trends (Fig. 2A). This graph reveals that the groups were matched for size; that the physiological responses were quite variable for animals in both groups; and that the rate or intensity of the physiological variable was generally higher in large animals than in small ones. The envelope enclosing data for animals in Group 1 is somewhat higher than that for animals in Group 2, but statistical analysis (by ANOVA) of unadjusted values for the physiological variable does not distinguish between the responses of animals in the two groups (Fig. 2B).

Perhaps the problem with the preceding statistical test is that background noise is too great to enable us to identify treatment effects. Accordingly, we might increase the sensitivity of the test if we were to adjust the physiological variable to remove the contribution of body size to overall variation in the data (Fig. 2A). We therefore form size-specific indices by dividing values for the physiological variable by corresponding values for the measure of body size and then present the scaled data in a dot plot for examination (Fig. 2C).

An analysis of variance (ANOVA) is performed next on the ratios to test for treatment effects. The $F$-ratio from this analysis is too small (and the associated probability too high) for us to conclude that the treatment had any effect whatsoever on the physiological response of interest (Fig. 2C). Thus, we would probably conclude just the opposite, that is, that responses by animals in the two groups were, in fact, identical. Such a conclusion would be incorrect, however. The problem with the preceding analysis is that forming ratios did not fully remove effects of body size on the data (see Fig. 1) and this, in turn, prevented the ANOVA from being as sensitive as it might have been [3,4].

We can illustrate this point by submitting the data to an analysis of covariance (ANCOVA), which is not nearly as intimidating as its name might imply. ANCOVA is a blend of two statistical procedures with which virtually all physiologists are familiar: regression and the ANOVA [9,12]. Regression removes effects of body size from the variable of interest and ANOVA then provides a sensitive test of the adjusted data [9,12,17,18,23,26].

In the present example, the physiological variable is a function of body size for animals in both groups (Fig.

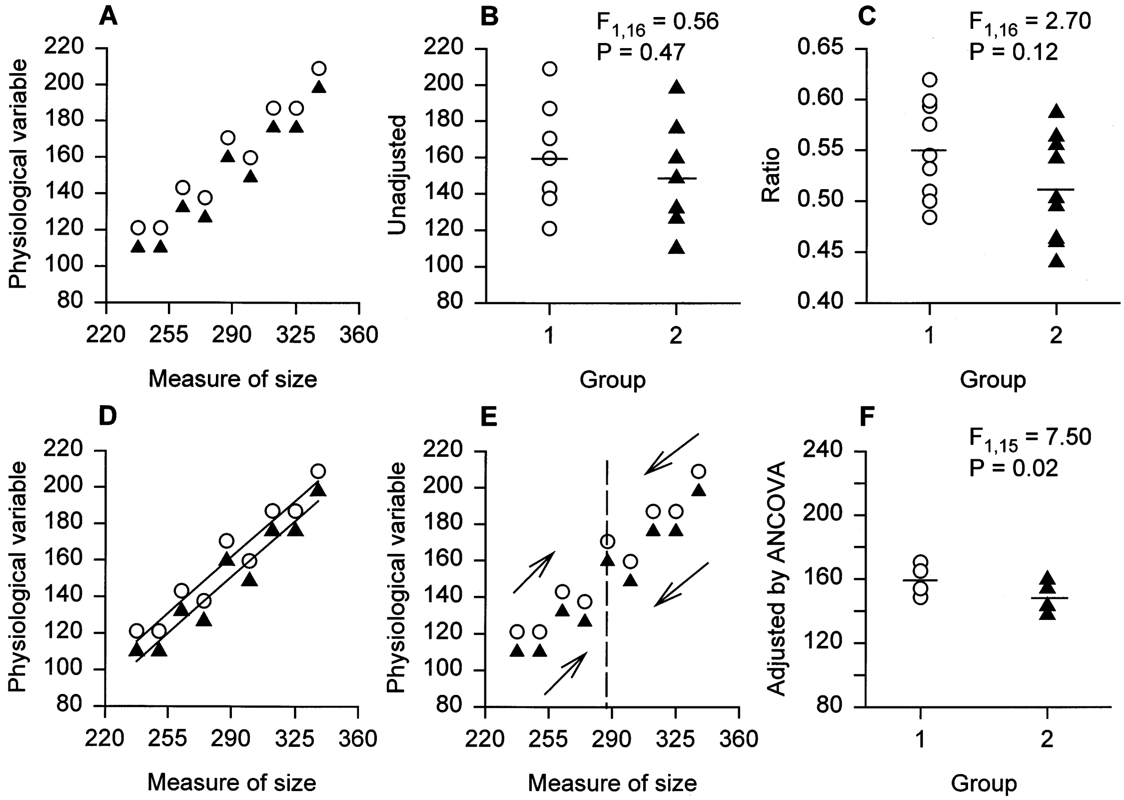


Fig. 2. (A) Hypothetical values for a physiological variable are plotted against measures of body size for animals in matched treatment and control groups. Open circles, Group 1; closed triangles, Group 2. (B) Unadjusted values for the physiological variable are projected onto the vertical axis and then presented in a dot plot to allow visual comparison of the two groups. The difference between the means (horizontal lines) is small relative to variation within groups, so a statistical analysis (ANOVA) cannot distinguish between the groups. (C) Values for the physiological variable have been divided by corresponding values for body size in an attempt to remove effects of body size. The variation in the data is smaller here than in unadjusted values and an ANOVA yields a slightly larger $F$-ratio. The $F$-ratio still is too small, however, for us to conclude that a treatment effect has been detected. (D) Lines have been fit to each of the data sets by the method of least squares. The slopes for the lines are identical. (E) An 'average' slope is used to adjust values for the physiological variable upward for small individuals in each group and downward for large individuals. Adjustments are made to the grand mean (dashed vertical line) for body size of the 18 animals in the study. (F) Adjusted values show a much tighter clustering than unadjusted values because all variation resulting from differences in body size has been removed from the data. The $F$-ratio from an ANOVA is large enough to reveal a treatment effect, with responses of animals in Group 1 being higher than those for animals in Group 2.

2A). Accordingly, we first fit least-squares regression lines to each of the data sets and then compare the slopes (Fig. 2D). When the slopes turn out not to differ—they are, in fact, identical in this example—we take a weighted average. This average slope is then used to adjust data upwards for small animals and downwards for large animals (Fig. 2E), so that all individuals are treated like they were of the same, average size for the 18 animals in the study [17]. The adjusted values for the physiological variable are more tightly clustered than the unadjusted values were (Fig. 2F), because none of the size-induced variation remains in the data set.

ANOVA performed on the adjusted values reveals a treatment effect (Fig. 2F). The $F$-ratio is too high (and the associated probability too low) for the differences in adjusted values to have resulted from chance, so we are led to believe that the treatment caused a change in the rate or intensity of the physiological variable. Although this conclusion is consistent with subjective impressions gained from our preliminary examination of data in the bivariate plot (Fig. 2A), it is not the conclusion to which we were led by the analysis of ratios (Fig. 2C). Indeed, the limited sensitivity of statistical tests performed on ratios is a major drawback to the use of percentages and size-specific indices to scale physiological data [17,18,23].

### *2.2.2. Removing confounding effects of body size from descriptive studies*

Two samples of nine animals again are obtained for study, but this time they are drawn from different species, populations, ages or sexes. The animals are measured for body size and the physiological variable of interest is then assessed for each individual. The data for the two groups are plotted against the measure of body size (Fig. 3A), thereby revealing that the physiological variable varies with body size and that animals

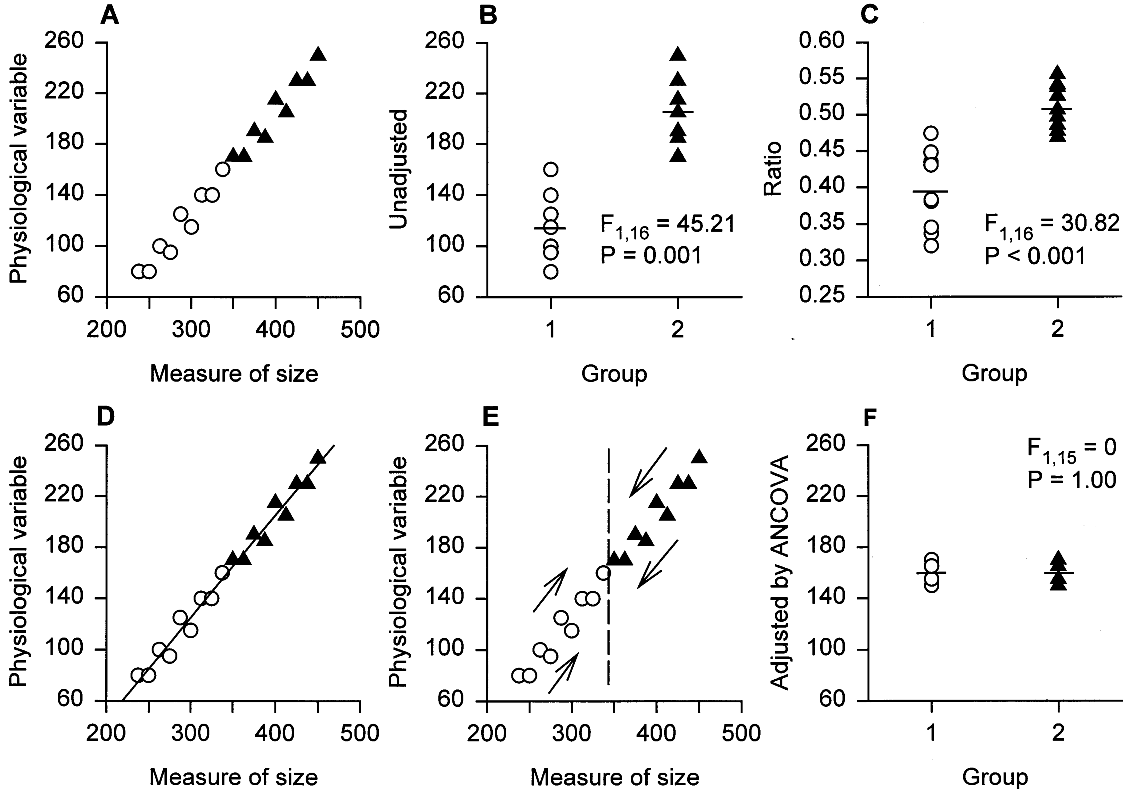


Fig. 3. (A) Hypothetical values for a physiological variable are plotted against measures of body size for two groups of animals that differ in average size. Open circles, Group 1; closed triangles, Group 2. (B) Values for the physiological variable are projected onto the vertical axis and then presented in a dot plot to allow visual comparison of the two groups. The mean (horizontal line) for Group 2 is significantly larger than the mean for Group 1, as is indicated by the large *F*-ratio from an ANOVA. (C) Values for the physiological variable have been divided by corresponding measures of body size in an attempt to remove confounding effects of body size. ANOVA indicates that means differ significantly, so physiological responses by animals in the two groups appear to differ even after the data have been normalized for effects of body size. (D) Lines have been fit to each of the data sets by the method of least squares. The slopes for the lines are identical. (E) An 'average' slope is used to adjust values for the physiological variable upward for small individuals in each group and downward for large individuals. Adjustments are made to the grand mean for body size of the 18 animals in the study (dashed vertical line). (F) Analysis of adjusted values reveals that differences between the two groups were a manifestation of differences in size.

in Group 2 are larger overall than those in Group 1. Indeed, we have the impression that data for animals in Group 2 are little more than an extension of data for animals in Group 1.

If data for the physiological variable are simply presented as a dot plot, without any regard for the effects of body size, we see that values for Group 2 are, indeed, higher than those for Group 1 (Fig. 3B). ANOVA confirms that the means can be distinguished statistically (Fig. 3B). However, the difference between the groups may result simply from the substantial difference in body size.

Suppose that we normalize the physiological data by expressing them as size-specific indices (i.e. by dividing by the measure of body size) and that we then display the ratios in a dot plot (Fig. 3C). The distribution of data for Group 2 is a bit 'tighter' than that of data for Group 1, but as physiologists, we are not overly concerned (albeit we probably should be!). ANOVA on the ratios indicates that values for animals in Group 1 differ significantly from those for animals in Group 2. The plot of data (Fig. 3C) indicates that scaled values are lower for Group 1 than for Group 2, that is, that a group effect remains even after effects of body size have seemingly been removed. However, this conclusion again is not the one to which we were led by our subjective examination of the bivariate plots (Fig. 3A).

We can also examine the data by ANCOVA. Regression lines are fit to each of the data sets (Fig. 3D) and the slopes are evaluated. The slopes do not differ significantly (indeed, they are identical in this case), so we compute a weighted mean. This average slope is used to adjust values for the physiological variable to a common value for body size (Fig. 3E) and an ANOVA is then performed on the adjusted values (Fig. 3F). A plot of adjusted data reveals that values for the physiological variable are the same for animals in Group 1 as for those in Group 2 once confounding effects of body size have been removed and this impression is supported by outcome of the ANOVA. Interestingly, this finding accords well with our subjective impressions of the bivariate plots and regressions.

The conclusion from the examination of ratios differs from that emerging from examination of a bivariate plot plus ANCOVA. The reason for this is simple: the ratios did not correct fully for variation in body size (see Fig. 1) and this introduced a bias into the data. The bias was manifested in a statistical test leading to a conclusion opposite to the correct one.

### *2.3. Ratios are subject to multiple interpretations*

Suppose that some physiological variable is measured on animals in each of two groups and suppose also that the data are 'normalized' by expressing them as a ratio of body size. If values for the normalized data are higher for Group 1 than for Group 2, the implicit assumption is that the physiological variable was of greater intensity for animals in Group 1 than for those in Group 2. This need not be the case, however [2,17]. The difference might result from variation only in the denominator variable. We can illustrate this point by using measurements for some physiological variable (like hematocrit) that we know in advance does not vary with body size.

We first plot values for the physiological variable against corresponding measurements of body size for animals in both groups (Fig. 4A). Inspection of the data reveals that animals in Group 2 were substantially larger than those in Group 1, but little else. The two sets of measurements for the physiological variable overlap considerably and we detect no apparent relationship between the value for the physiological response and body size in either group.

If we had automatically computed ratios to scale the data for body size, however, we would have discovered that the 'normalized' data differ significantly between the two groups (Fig. 4B). Values for Group 2 are substantially lower than those for Group 1, so we might have concluded that the level of response was greater in the latter group than in the former once an 'appropriate' adjustment had been made for body size. This conclusion is incorrect, as the initial inspection of plotted data clearly revealed.

Examination of the data by ANCOVA lends support to subjective impressions gained from inspecting the original graph (Fig. 4A). The potential covariate (body size) is unimportant and the ensuing ANOVA on unadjusted data yields no evidence whatsoever of any difference between the two groups (Fig. 4C). This is not the same conclusion that we would have reached had we used ratios.

## 3. Burden of proof

Forming a ratio to scale physiological data for variation in body size will not always lead investigators to arrive at an incorrect conclusion [8,18]. Treatment effects in planned experiments, and differences among groups in descriptive studies, sometimes are so pronounced that the correct conclusion is reached regardless of the method that is used to scale and analyze the data. But how is a reader of a scientific paper to know whether a conclusion based on the analysis of a ratio is correct or not? Have the authors confirmed that the physiological variable of interest varies as a fixed proportion of the measure of

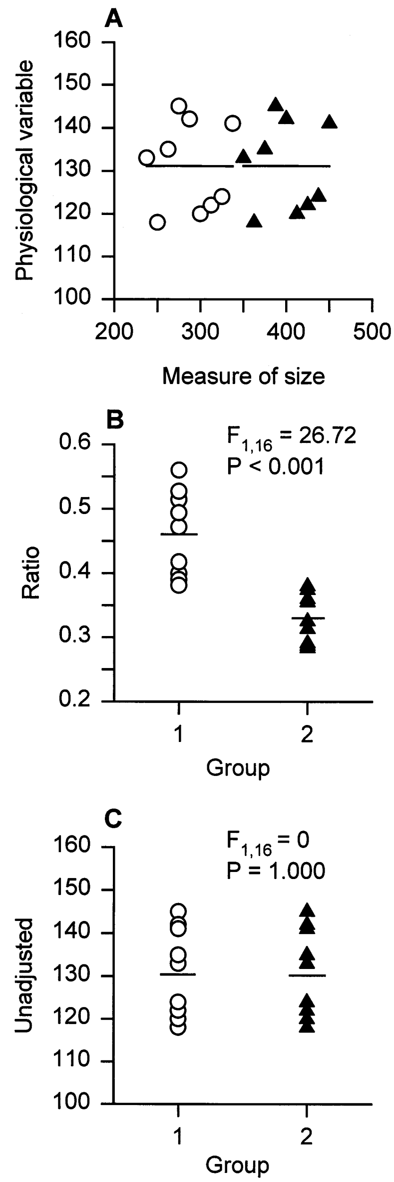


Fig. 4. (A) Hypothetical values for a physiological variable are plotted against measures of body size for two groups of animals that differ in average size. Open circles, Group 1; closed triangles, Group 2. Lines fit to each of the data sets have slopes of zero. (B) Values for the physiological variable were divided by corresponding values for the measure of body size. The two groups differ in mean (horizontal lines) for the 'normalized' data. (C) The data do not require any adjustment for variation in body size, so ANOVA is performed on unadjusted values. The means for the two groups are identical. The aforementioned difference between groups in the mean for size-specific measurements is attributable entirely to the difference is size.

body size in the animals under investigation? Have they shown that a difference in mean ratio between groups actually reflects a difference in the numerator variable and not the denominator variable? Have they demonstrated that an analysis of data scaled by regression leads to the same conclusion as the analysis of ratios?

If the answers to these questions are 'no', we suggest that the investigators in question have failed to accept a reasonable burden of proof in reporting their research to the scientific community, that is, they have not provided enough information to enable readers independently to evaluate the methods and results of the study. Without the aforementioned validation for the use of a ratio, readers may have no recourse but to dismiss conclusions from the study, no matter how carefully the work might have been done and how important the work might otherwise have been.

## 4. Recommendations

We believe that the aforementioned problems with ratios render them unsuitable for use in normalizing data for variation in body size and therefore tender the following recommendations to authors and readers of physiological research:

1. We counsel authors to discontinue using percentages and size-specific indices in an attempt to scale data for variation in body size within and among groups. Although ratios are simple to form and seemingly simple to interpret, they actually are exceedingly deceptive and potentially misleading [2,11,17,18,25]. Indeed, the value of research that relies on ratios is severely undermined, to the detriment of both the author and the journal. Effects of body size can be removed from data sets by using regression in the form of ANCOVA (or multiple regression). This procedure is widely available in statistical software for MacIntosh and Windows. We illustrate the simplicity of the procedure by reproducing in the Appendix A the output of the ANCOVA performed on data summarized in Fig. 2. We urge workers not to apply ANCOVA without also performing a graphical analysis, however.

2. We also advise readers not to take too seriously the conclusions from any report that relies on percentages or size-specific indices to control for effects of body size on the variable of interest. Such conclusions are unfounded at best and often incorrect [5,7,8,11,13,18,20,25,27]. We realize that we are advising colleagues to disregard numerous studies, which may have been properly designed and carefully executed. Unfortunately, the use of ratios to scale data may invalidate what is otherwise good research. Readers cannot determine what is valid and what is not without a great deal more information than most authors provide.

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## Appendix A

Data for group membership, size and the (physiological) variable of interest.

| Group | Size | Variable |
|---|---|---|
| 1 | 237.5 | 121 |
| 1 | 250 | 121 |
| 1 | 262.5 | 143 |
| 1 | 275 | 137.5 |
| 1 | 287.5 | 170.5 |
| 1 | 300 | 159.5 |
| 1 | 312.5 | 187 |
| 1 | 325 | 187 |
| 1 | 337.5 | 209 |
| 2 | 237.5 | 110 |
| 2 | 250 | 110 |
| 2 | 262.5 | 132 |
| 2 | 275 | 126.5 |
| 2 | 287.5 | 159.5 |
| 2 | 300 | 148.5 |
| 2 | 312.5 | 176 |
| 2 | 325 | 176 |
| 2 | 337.5 | 198 |

Control information for performing an ANCOVA using SAS version 6.12

```
data temp; infile 'ratio.dat';
input group size variable;
proc glm;
class group;
model variable = group size;
lsmeans group/ stderr;
run;
```

Summary of output from SAS

| Source of variation | df | Mean square | *F*-ratio | *P* |
|---|---|---|---|---|
| Group | 1 | 544.5 | 7.50 | 0.015 |
| Size | 1 | 14 520.0 | 200.00 | $<0.001$ |
| Error | 15 | 72.6 | – | – |

Least squares means

| Group | Variable | S.E. |
|---|---|---|
| 1 | 159.5 | 2.84 |
| 2 | 148.5 | 2.84 |

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