SHORT COMMUNICATION

Correct allometric analysis is always helpful for scaling flow-mediated dilation in research and individual patient contexts

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Summary

McLay et al. (2017) recently examined whether the allometric scaling of flow-mediated dilation influenced the mean difference between samples of young and older adults compared with the traditional percentage change approach. They also explored whether a new scaling calculation improved the ability to obtain individually-scaled flow-mediated dilation. In our response to their study, we can demonstrate that McLay et al. (2017) have (i) managed to formulate a new scaling index which does nothing to remove the dependency of that index on baseline diameter, and (ii) suggested, incorrectly, that the original allometric approach cannot be used to derive individual-adjusted values of flow-mediated dilation, which can be interpreted in a similar way to a percentage change.

Key words

flow-mediated dilation, allometry, scaling, endothelial function, vascular

Introduction

Atkinson and colleagues (Atkinson*, et al.* 2013) described an allometric approach for scaling the flow-mediated change in arterial diameter to the baseline diameter measured at rest (Dbase), which can vary substantially between and within individuals. Although this approach was referred to as "Atkinson scaling" by McLay and colleagues (McLay*, et al.* 2017), the fundamental rationale and procedures of this approach are based on standard allometric principles that were laid down several decades ago by other physiologists (Albrecht*, et al.* 1993). One important reason for allometric scaling is to address the well-known problem that ratio statistics can still be dependent on their denominators. Such inconsistent size-scaling of a ratio over the measurement range can ultimately lead to biased inferences in research and on individual patients.

The percentage flow-mediated dilation index (FMD%) seems to be one of the above problematic ratio indices. In fact, several of the "expected differences" that were reported in McLay et al.'s introduction section may be detrimentally affected by the inadequate scaling of the FMD% index. For example, it has been shown in three separate datasets that the assumed age-related decline in brachial FMD% has been over-estimated by previous researchers, because Dbase tends to be larger with increasing age (Atkinson & Batterham 2013a; Atkinson & Batterham 2013b; Atkinson & Batterham 2015; Atkinson*, et al.* 2013). (Atkinson & Batterham 2015) also explained how the apparent moderate association between brachial FMD% and coronary vasodilation may also be biased by the natural dependency of FMD% on resting artery diameters.

McLay et al. (2017) compared three different approaches for size-scaling the flow-mediated change in arterial diameter. First, we can explain why the "new approach" forwarded by

McLay et al. (2017) does not accomplish the fundamental aim in allometry of removing Dbase-dependency. Second, we can explain how it is possible to obtain individual Dbase-adjusted values of flow-mediated dilation, which can be interpreted in a similar way as an individual percentage change. Using the graph digitizer software, DigitizeIt (Braunschweig, Germany), we were able to extract the data presented in McLay et al.'s Figures 1 and 2 in order to support some of our arguments.

McLay et al's new approach does not remove Dbase-dependency

A fundamental purpose of allometric scaling is to "normalise" the numerator variable for the denominator variable in a consistent manner across the measurement range (Albrecht*, et al.* 1993). The approach forwarded by Altkinson and colleagues clearly accomplishes this purpose because the correlation between Dbase-adjusted flow-mediated dilation and Dbase itself has been consistently shown to be close to zero ((Atkinson & Batterham 2015). Nevertheless, McLay et al. (2017) have managed to formulate a new scaling approach which does not address this fundamental *raison d'etre* of size scaling. The reason McLay et al's new approach does not serve its designed purpose is because an allometric exponent for the log-log association between peak diameter (Dpeak) and Dbase was calculated, but then was applied to a new index for which the flow-mediated change in diameter (Dpeak-Dbase) was the numerator. An allometric exponent "works" on the denominator of interest (Dbase) only if the numerator with which it was calculated actually remains the same numerator. The equation that McLay et al. (2017) reported was as:

$$\text{"New scaling FMD\%"} = \frac{\text{Dpeak} - \text{Dbase}}{\text{Dbase}^{b}} \times 100$$

It can be seen that the only difference between the above equation and the traditional equation for FMD% is that the Dbase denominator is raised to the power of the calculated allometric exponent, which McLay et al. (2017) report to be 0.97. McLay et al. (2017) do not seem to realise that the only reason their new scaling FMD% seems to provide similar group mean values to FMD% is because the exponent happens to be very close to unity for their particular dataset. This will not be the case at all for more typical datasets for which the exponent is considerably lower than unity.

Moreover, McLay et al. (2017) showed in their own Figure 2 that the correlation between their derived index and Dbase is still as negative as that pertaining to FMD% itself. We can confirm with the other datasets we have previously analysed that their approach does not eradicate Dbase-dependency. For example, when McLay et al.'s approach is applied to the data that were extracted by Atkinson and Batterham (2013b) from (Celermajer*, et al.* 1992), the correlation between their new scaling index and Dbase is -0.78 (95%CI: -0.87 to -0.64), which is similar to the correlation between FMD% and Dbase in these same data. McLay et al. (2017) seem satisfied with their approach mainly because it provides similar percentage differences to that of FMD%, using their particular dataset. This is not a sound criterion for comparing different allometric models, and will not help researchers adjust their estimates of flow-mediated response for the sometimes substantial influence of Dbase.

Interpretation of the "Atkinson approach"

As part of their focus on supplying individually-adjusted allometric indices, McLay et al. (2017) calculated the percentage mean difference between group mean values of the metric, $Dpeak/Dbase^{b}$, where *b* represents the exponent derived from the logDpeak – logDbase regression line. McLay et al. (2017) erroneously reported in their Table 2 that this metric has

126 units of “equivalent %”. Atkinson and colleagues have never reported that this power
127 function ratio provides “equivalent %” values, and we cannot understand how McLay et al.
128 (2017) have neglected to recognise that the units of this metric are in fact mm/mm^{b}, and bear
129 no resemblance to a percentage index. Rather, it was highlighted by Atkinson and colleagues
130 that using the index of $Dpeak/Dbase^{e}$ results in a completely different metric vs FMD%,
131 which explains why mean group differences in this metric are 2-3 times higher than FMD%
132 when expressed as a percentage difference. Consequently, it was explained by Atkinson and
133 Batterham (2013b) how to back-transform the group differences on the logged scale in a way
134 that enabled an interpretation of group differences in a way similar to FMD%, and this is the
135 approach we used to routinely report group differences in subsequent studies (Atkinson &
136 Batterham 2013a; Atkinson & Batterham 2013b; Atkinson & Batterham 2015; Atkinson*, et*
137 *al.* 2013). Some of the results of this approach are presented in McLay et al’s Table 1, but not
138 discussed in-depth. We can provide below perhaps a more comprehensive interpretation of
139 these data.
140
141 The first point to note about the popliteal artery data presented by McLay et al. (2017) is that
142 they are derived from small samples and, therefore, estimates of the various statistics are
143 quite imprecise. For example, the allometric exponent between Dpeak and Dbase is 0.97, but
144 we calculate that the 95% confidence interval for this exponent is 0.91 to 1.04. We also
145 calculate that the 95% confidence interval for the correlation between scaling index and
146 Dbase is also wide (-0.44 to 0.21). With sufficiently-large samples, we have found that the
147 upper confidence limit for the Dpeak-Dbase exponent is consistently lower than 1 and the
148 index-Dbase negative correlation is typically at least moderate in magnitude. Nevertheless, in
149 the sample studied by McLay et al. (2017), it can be said that the point estimate exponent is

close to 1, so very little difference in flow-mediation dilation estimates would be expected between the allomteric approach and FMD% in this case.

McLay et al. (2017) reported that the mean (SD) difference in FMD% between age groups was 2.1% (4.1%). In their Table 1, the back-transformed group means (calculated so that they can be interpreted in similar percentage change terms, are 6.0% and 4.0% (to 1 d.p.) giving a group mean (allometrically adjusted) difference of 2.0%. As expected, this is similar to the mean difference in FMD% of 2.1% because the allometric exponent is close to unity, indicating direct proportionality between Dbase and Dpeak. We highlight that 95% confidence limits for these allometrically-adjusted group means can be obtained and these can be informative. The back-transformed mean difference is also informative as representing the ratio of the allomterically-adjusted group geometric means (Bland & Altman 1996). Importantly, unlike for the new approach described by McLay et al. (2017), all these estimates are properly adjusted for the influence of Dbase on the data.

Allometric-adjusted individual values of FMD

McLay et al. (2017) believed that the "Atkinson approach", i.e. general allometry, does not enable a researcher to estimate individual Dbase-adjusted values of the flow-mediated response in similar percentage change terms to that of FMD%. Hence McLay et al. (2017) seemed to concentrate on the calculation of individual power function ratios. Nevertheless, Albrecht et al. (1993) suggested a more informative approach is possible based on individual residuals, and we have experimented with this, and a number of other approaches. In a recent study on another scaling index (digit ratio), we derived individual-adjusted indices of 2nd finger length normalised for 4th finger length working directly in the raw arithmetic data

space (Lolli*, et al.* 2017). These data could be directly interpreted as allometrically-adjusted ratios for each participant. The same approach can be applied to flow-mediated dilation.

Conceptually, the algebraic derivation of adjusted indices for flow-mediated dilation is grounded on the theoretical assumptions regarding the use of raw residuals, which represent the true biological variability of the measurement outcome free from the influence of the independent variable (Albrecht*, et al.* 1993). Accordingly, the individually-normalised FMD% estimates can be derived directly from the allometric model residuals in raw arithmetic space, with the FMD% as the dependent variable and Dbase as the independent predictor. Each participant's residual can be derived as observed FMD% - predicted FMD% and then added to the predicted mean FMD% at the mean Dbase in the whole sample, to obtain individually adjusted FMD% free from the influence of Dbase (Figure 1).

Since the relationship between Dbase and Dpeak is, in the data analysed by McLay et al. (2017) approximately directly proportional, there is general agreement between the group mean difference in individually-adjusted flow-mediated dilation and the group mean differences in FMD%. Figure 1 presents the Dbase-adjusted FMD% against the original Dbase measures, for which there is no evidence of a dependency of the index on Dbase. Here we have individual adjusted values of flow-mediated dilation which can be interpreted in a similar way to FMD%.

Incidentally, we note that McLay et al. (2017) derived a common slope (0.97) by just regressing log Dpeak on log Dbase for the whole sample. This approach was surprising given the sensible advice one of the authors provided in an earlier publication (Johnson*, et al.* 2000), i.e. that an exponent should be derived from a model which includes the group factor

after first checking that a common slope was appropriate with a group x denominator interaction term (Batterham*, et al.* 1997). To quote Johnson *et al.* (2000), which was co-authored by Koval and Paterson, "*To confirm that men and women* [the group factor in this case] *could be compared using the same body size scaling exponent, the homogeneity of the BM (or FFM) coefficient was first verified by including a sex x lnBM (or lnFFM) variable into the linearised model.*". So the exponent of 0.97 that McLay et al. (2017) reported is incorrect anyway.

In conclusion, our re-analysis and re-interpretation of McLay et al.'s data have revealed that their new approach to individual adjustment does not actually adjust for Dbase at all. It only appears to provide similar group differences to FMD% because the exponent they calculated happens to be very close to unity. They have also incorrectly interpreted a power function ratio as providing "equivalent %" values, and overlooked all the steps Atkinson and colleagues reported for arriving at an index that can be interpreted as an "equivalent %". Finally, the various allometric analyses reported by Atkinson and colleagues on flow-mediated dilation and other ratios *can* form the basis of a robust method for deriving Dbase-adjusted FMD% for each individual in a sample.

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Conflict of interest

The authors have no conflicts of interest relevant to the content of this article.

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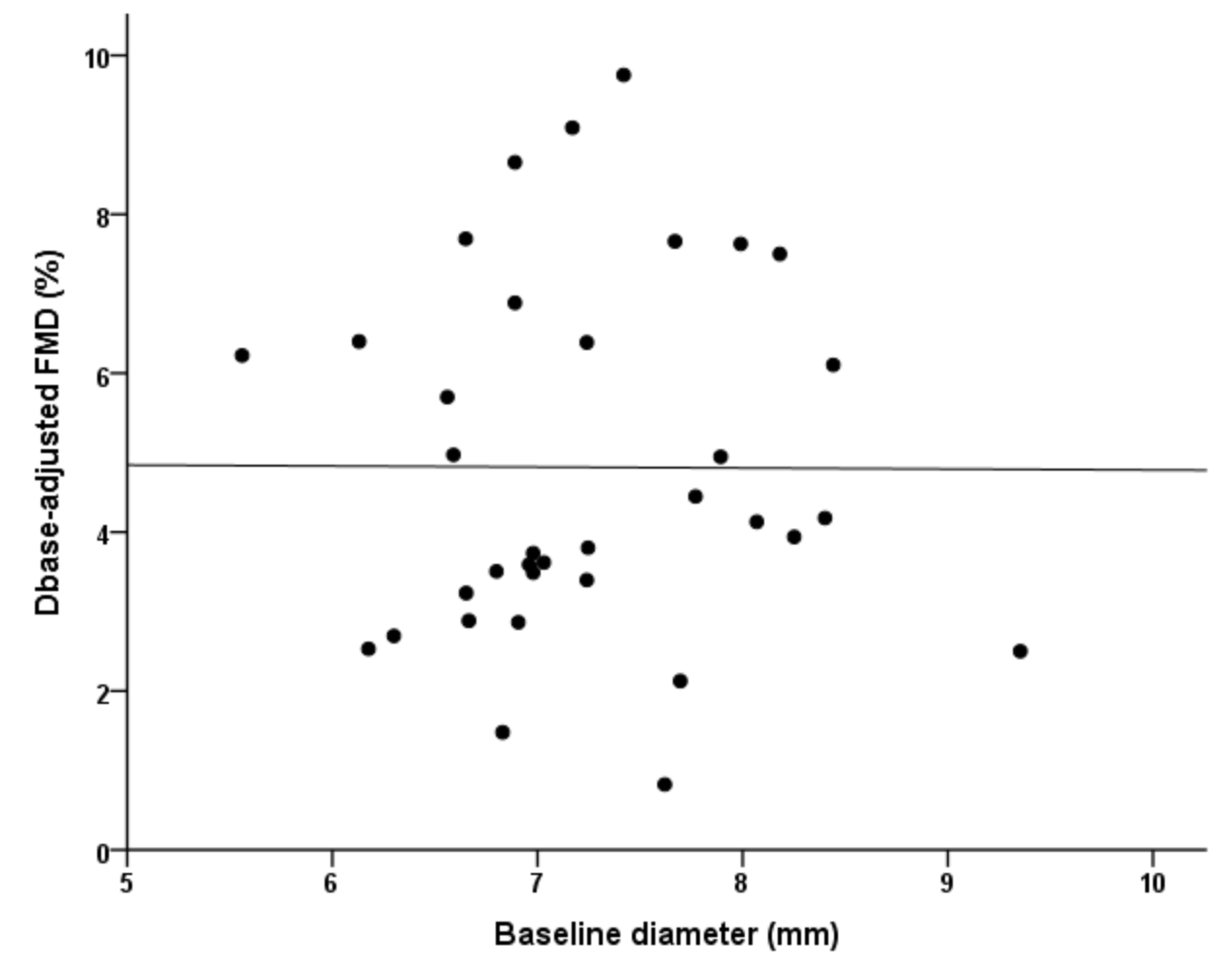
Dbase-adjusted FMD (%)
10
8
6
4
2
0
5
6
7
8
9
10
Baseline diameter (mm)