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SHORT COMMUNICATION

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

Lorenzo Lolli, Alan M Batterham and Greg Atkinson

Health and Social Care Institute, Teesside University, Middlesbrough, UK

Address for Correspondence:

Greg Atkinson

Health and Social Care Institute

School of Health and Social Care

Rm 1.16, Constantine Building

Teesside University

MIDDLESBROUGH

Tees Valley TS1 3BA

UK

greg.atkinson@tees.ac.uk

Twitter: [@Greg_at_TeesUni](https://twitter.com/Greg_at_TeesUni)

Tel: +44 (0) 1642 342758

26 **Summary**

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

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37 **Key words**

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

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51 **Introduction**

52 Atkinson and colleagues (Atkinson, *et al.* 2013) described an allometric approach for scaling
53 the flow-mediated change in arterial diameter to the baseline diameter measured at rest
54 (Dbase), which can vary substantially between and within individuals. Although this
55 approach was referred to as “Atkinson scaling” by McLay and colleagues (McLay, *et al.*
56 2017), the fundamental rationale and procedures of this approach are based on standard
57 allometric principles that were laid down several decades ago by other physiologists
58 (Albrecht, *et al.* 1993). One important reason for allometric scaling is to address the well-
59 known problem that ratio statistics can still be dependent on their denominators. Such
60 inconsistent size-scaling of a ratio over the measurement range can ultimately lead to biased
61 inferences in research and on individual patients.

62

63 The percentage flow-mediated dilation index (FMD%) seems to be one of the above
64 problematic ratio indices. In fact, several of the “expected differences” that were reported in
65 McLay *et al.*’s introduction section may be detrimentally affected by the inadequate scaling
66 of the FMD% index. For example, it has been shown in three separate datasets that the
67 assumed age-related decline in brachial FMD% has been over-estimated by previous
68 researchers, because Dbase tends to be larger with increasing age (Atkinson & Batterham
69 2013a; Atkinson & Batterham 2013b; Atkinson & Batterham 2015; Atkinson, *et al.* 2013).
70 (Atkinson & Batterham 2015) also explained how the apparent moderate association between
71 brachial FMD% and coronary vasodilation may also be biased by the natural dependency of
72 FMD% on resting artery diameters.

73

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

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

82

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

84 A fundamental purpose of allometric scaling is to “normalise” the numerator variable for the
85 denominator variable in a consistent manner across the measurement range (Albrecht, *et al.*
86 1993). The approach forwarded by Atkinson and colleagues clearly accomplishes this
87 purpose because the correlation between Dbase-adjusted flow-mediated dilation and Dbase
88 itself has been consistently shown to be close to zero ((Atkinson & Batterham 2015).
89 Nevertheless, McLay et al. (2017) have managed to formulate a new scaling approach which
90 does not address this fundamental *raison d'être* of size scaling. The reason McLay et al's new
91 approach does not serve its designed purpose is because an allometric exponent for the log-
92 log association between peak diameter (Dpeak) and Dbase was calculated, but then was
93 applied to a new index for which the flow-mediated change in diameter (Dpeak-Dbase) was
94 the numerator. An allometric exponent “works” on the denominator of interest (Dbase) only
95 if the numerator with which it was calculated actually remains the same numerator. The
96 equation that McLay et al. (2017) reported was as:

97

$$98 \text{ “New scaling FMD\%”} = \frac{\text{Dpeak} - \text{Dbase}}{\text{Dbase}^b} \times 100$$

99

100

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

108

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

120

121 **Interpretation of the “Atkinson approach”**

122 As part of their focus on supplying individually-adjusted allometric indices, McLay et al.
123 (2017) calculated the percentage mean difference between group mean values of the metric,
124 $D_{\text{peak}}/D_{\text{base}}^b$, where b represents the exponent derived from the $\log D_{\text{peak}} - \log D_{\text{base}}$
125 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 D_{peak}/D_{base}^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 D_{peak} and D_{base} 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 D_{base} is also wide (-0.44 to 0.21). With sufficiently-large samples, we have found that the
147 upper confidence limit for the D_{peak}-D_{base} exponent is consistently lower than 1 and the
148 index-D_{base} 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

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

152

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

164

165 **Allometric-adjusted individual values of FMD**

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

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

176

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

186

187 Since the relationship between Dbase and Dpeak is, in the data analysed by McLay *et al.*
188 (2017) approximately directly proportional, there is general agreement between the group
189 mean difference in individually-adjusted flow-mediated dilation and the group mean
190 differences in FMD%. Figure 1 presents the Dbase-adjusted FMD% against the original
191 Dbase measures, for which there is no evidence of a dependency of the index on Dbase. Here
192 we have individual adjusted values of flow-mediated dilation which can be interpreted in a
193 similar way to FMD%.

194

195 Incidentally, we note that McLay *et al.* (2017) derived a common slope (0.97) by just
196 regressing log Dpeak on log Dbase for the whole sample. This approach was surprising given
197 the sensible advice one of the authors provided in an earlier publication (Johnson, *et al.*
198 2000), i.e. that an exponent should be derived from a model which includes the group factor

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

206

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

216

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220 **Conflict of interest**

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

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