Shear rate normalisation is not essential for removing the dependency of flow-mediated dilation on baseline artery diameter: Past research revisited

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Abstract

Normalisation of percentage flow-mediated dilation (FMD%) by shear rate (SR) was originally proposed to account for variability in the FMD% stimulus and in resting artery diameter (D_{base}). It was not known at that time that FMD%-D_{base} dependency is caused by the poor allometric properties of FMD% itself. Therefore, data from a seminal study on FMD%/SR normalisation were extracted and re-analysed. The absolute change in arterial diameter was found to be strongly inversely proportional to D_{base} (r= -0.7, P<0.0005) and the allometric exponent was 0.82 (95%CI: 0.78-0.86), rendering use of FMD% inappropriate. Allometric scaling eliminated the originally-reported D_{base-} dependency without any need for SR normalisation (r=0.0, P=0.96). The SR-FMD% correlation reduced from 0.69 to 0.37, following D_{base}-adjustment. This reanalysis indicates that allometric scaling (i) renders FMD%/SR normalisation redundant for removing D_{base}-dependency, and (ii) allows the influence of SR on the flow-mediated response to be quantified without the confounding influence of D_{base}.

Key words: Allometric scaling, Normalisation, Endothelial function, Spurious correlation

Introduction

The two primary variables of interest that are measured during the flow-mediated dilation protocol are resting (D_{base}) and peak artery diameter (D_{peak}). Typically, the change in diameter (D_{peak} - D_{base}) is expressed as a percentage of D_{base}, to give the percentage flow-mediated dilation index (FMD%). Because this final calculation of the FMD% scaling index is grounded fully in statistics and not physiology, it follows that any potential problems with the properties of FMD% *per se* can only be solved satisfactory by applying more appropriate statistics. Therefore, protocol factors like cuff placement, timing of data capture or duration of cuff occlusion do not alter the fact that the ubiquitously-calculated "output" summary index for the purpose of data analysis is FMD%.

It is clear that FMD% is calculated to "normalise", statistically, the change in diameter (D_{diff}) to D_{base}. Nevertheless, up to two-thirds of the variability in FMD% can still be explained by variability in D_{base} (Celermajer et al., 1992; Pyke et al., 2004). A negative slope is almost always found between FMD% and D_{base}. Therefore, FMD% is *not* normalising for D_{base} consistently across the measurement range (Atkinson and Batteram, 2013a). To remove the D_{base}–dependency problem, Pyke *et al*. (2004) proposed that FMD% should be, in turn, normalised by measurements of shear rate. When Pyke et al. (2004) divided FMD% by shear rate, the correlation between FMD%/SR and D_{base} was found to be -0.43 (P=0.002). Although this correlation is "moderate" in magnitude, and statistically significant, Pyke *et al*. (2004) inferred from it that shear rate normalisation "virtually eliminated" D_{base}-dependency, and is, therefore, "*essential for examining endothelial-dependent flow-mediated dilation between groups differing in baseline arteries*".

In light of new information on the allometric relationship between D_{base} and D_{peak}, and the statistical inadequacy of the FMD% index to represent this relationship properly (Atkinson *et al*., 2013a; Atkinson and Batterham, 2013a; 2013b; 2013c), two new fundamental questions have emerged about using shear rate normalisation to remove D_{base}-dependency;

(i) Why should FMD% be normalised for shear rate (in order to remove the dependency of FMD% on D_{base}) when it is the FMD% index itself which causes D_{base}-dependency in the first place?

(ii) How can the influence of shear rate *per se* on the flow-mediated response *per se* be isolated and quantified accurately when the influence of D_{base} on both these variables is so substantial? This question is grounded in the issue of spurious correlations whereby the correlation between two variables, shear rate and FMD% is biased by the influence of a "third variable", D_{base}.

These questions have yet to be answered using real data relating to shear rate and FMD%. It is, therefore, pertinent to re-examine such data published in a seminal study on shear rate normalisation in order to answer these questions.

Methods

Using dedicated data extraction software (DigitizeIt, Koln, Germany), the data presented in a past study on the value of normalising FMD% for shear rate were re-analysed (Pyke *et al*., 2004). DigitizeIt (http://www.digitizeit.de/index.html#Features) allows the extraction and digitization, into x-y data, of graphical information that has been presented in previous publications. Specifically, all the data from the Figure 7A in a past publication were extracted (Pyke *et al*., 2004), and this Figure is reproduced (with permission) as Figure 1A in the present paper. The extracted values of D_{base} and FMD% can be found in a supplementary SPSS file named "Extracted Data.sav". From these data, values of D_{peak} and D_{diff} were calculated using the same approach as presented in a previous study (Atkinson and Batterham, 2013b). These data can also be found in the supplementary SPSS file.

The digitisation process entails scrutiny of an enlarged version of the Figure. The minimum and maximum points of the x and y axes are first digitised. Then the exact centre of each x-y data-point is digitised. Values of D_{peak} were calculated from D_{base} + (D_{base} x FMD% / 100). D_{diff} was then calculated from D_{peak} – D_{base}. The D_{base} and FMD% data were extracted three times by the same person and the coefficient of variation for intra-individual error was less than 0.001%. The digitised example shown in Figure 1A corresponds to the minimum values of D_{base} and FMD% reported by Pyke *et al*. (2004).

Statistical Analysis

Using the allometric approach presented in previous studies (Atkinson *et al*., 2013a; Atkinson and Batterham, 2013a; 2013b), the natural logarithms of D_{base} and D_{peak} were obtained. The D_{diff} data were also analysed on this natural log scale by subtracting log-tranformed D_{base} from log-transformed D_{peak}. The allometric relationship between D_{base} and D_{peak} was then described using a General Linear Model in the Statistical Package for the Social Sciences (SPSS). Log-transformed D_{peak} was the outcome and log-transformed D_{base} was the covariate. From this model, the allometric exponent of the relationship between D_{peak} and D_{base} can be obtained (Albrecht, 1992). This allometric exponent (a) was then used to calculate a more accurate size scaling index of the form D_{peak}/D_{base}^{a}. This method of calculating the allometric exponent was cross-validated with a non-linear regression approach (Packard and Boardman, 1999). Data are presented in the results as mean (standard deviation) and 95% confidence intervals.

Results

Forty-four pairs of D_{base}-FMD% data were extracted and these are presented in Figure 1B - in the same manner as the original Figure in the previous publication (Figure 1A). These two

Figures of D_{base} vs FMD% are almost identical. The mean (SD) values of the extracted data are also very similar to those reported in the published paper. For the extracted data, the mean (SD) values of D_{base}, FMD% and D_{peak} were 4.44 mm (0.54), 6.3% (3.0) and 4.71 mm (0.48) respectively. The same mean (SD) values that were reported in the original publication were 4.45 mm (0.52), 6.3% (3.1) and 4.72 mm (0.46).

From the previously reported coefficient of determination, $r^2 = 0.639$, the FMD%-D_{base} correlation can be calculated to be -0.80. The same correlation for the extracted data was -0.82. Previously, the least squares regression slope for this relationship was reported to be -4.75 %/mm (Pyke *et al*., 2004). The slope for the extracted data was a similar -4.52 %/mm. The correlation between the absolute change in diameter and D_{base} was -0.70 for the extracted data, indicating that smaller (not larger) absolute changes in arterial diameter are associated with relatively larger diameters of arteries (Figure 2A).

The allometric exponent for the D_{base}-D_{peak} relationship was 0.82 (95%CI = 0.78-0.86) from the log-log model and 0.82 (95%CI: 0.77-0.86) with the non-linear regression approach. This correct allometric exponent was then used to calculate an index of flow-mediated dilation that is free of the influence of D_{base}, by dividing D_{peak} by $D_{base}^{0.82}$. This index was still moderately correlated to FMD% (r=0.57, P<0.0005), but completely free from the influence of D_{base} (r=0.00, P=0.96, Figure 2B). The partial correlation (adjusting for the influence of D_{base}) between shear rate and FMD% was found to be 0.37.

Discussion

One of the proposed benefits of normalising FMD% for arterial shear rate is to eradicate the influence of initial artery diameter, thereby allowing FMD%, to be interpreted between arteries of different size (Pyke *et al*., 2004). Nevertheless, in this, and other (Atkinson and Batterham, 2013a; 2013b), datasets, D_{diff} is actually found to be inversely proportional to D_{base}, which completely compromises the use of FMD%. The present re-analysis of a seminal study clearly demonstrates that shear rate normalisation is not needed to eradicate any dependency on D_{base}, because such dependency is completely absent when allometric scaling is applied. Importantly, this allometric approach also allows the true influence of shear rate on the flow-mediated response *per se* to be quantified accurately without the confounding influence of D_{base}. It is likely that at least some of the association between shear rate and FMD% is spurious, i.e., explained by the “third variable” of D_{base}

The allometric exponent was found to be 0.82 for the past data, and the upper confidence limit of this estimate was less than the value of 1 needed for appropriate use of FMD%. This exponent is even lower than the estimates (0.90-0.94) that have been derived from other samples (Atkinson *et al*., 2013a; Atkinson and Batterham, 2013a; 2013b). This difference in the estimates of exponents may be explained by bias associated with pseudoreplication of data (detailed below), and/or it may be due to sampling error. Importantly, because this and other exponents are less than unity, FMD%, is not appropriate for accurately quantifying the relative change in flow-mediated dilation.

Although not as parsimonious as the allometric approach (Atkinson and Batterham, 2013a), the partial correlation between shear rate and FMD%, adjusting for the influence of D_{base}, can be calculated. This partial correlation was almost half the magnitude of the originally reported correlation between shear rate and FMD%. Shear rate is proposed as the stimulus for the flow-mediated response, but proper quantification of the effects of this stimulus requires proper adjustment for the influence of D_{base}, that is measured at the beginning of the protocol. The FMD% index clearly does not remove this influence and therefore generates spuriousness between itself and shear rate.

Forty-four pairs of FMD%-D_{base} data were extracted from the previous study, whereas the number of participants (n=8) recruited and the number of data collection times that were administered (n= 2 x 3) suggests that forty-eight pairs of data should have been present in

Figure 1A (the original Figure) and, therefore, the extracted data presented in Figure 1B. Assuming that there was no loss of data in the original study, this disparity could be due to some of the x-y data-points in Figure 1A being precisely superimposed on top of each other, i.e. four pairs of observations could have shown exactly the same values of D_{base} and FMD%. Nevertheless, the impact of this difference on the primary finding is likely to be negligible, given the close similarity between the scatterplots of Figures 1A and 1B, as well as the close similarity between the published sample statistics and those derived from the present re-analyses.

It is noted that “pseudoreplication” was present in the original data analyses completed by Pyke *et al*. (2004). Pseudoreplication refers to the pooling together of data from repeated measurements made on the same participants, and analysing the data in this pooled form, rather than taking into account the repeated measurements in the statistical model (Lazic, 2010). It was important for us to follow exactly the same analysis procedures as in the original publication in order to compare the results accurately. Because pseudoreplication was present in both this and the past data analyses, it is unlikely to influence the primary finding that shear rate normalisation was not needed to remove the D_{base}-dependency presented in the previous study.

Conclusions

Although normalisation of FMD% for the shear rate stimulus has intuitive appeal, it is fraught with problems because FMD% is retained as the index of change, and so the D_{base}-dependency problem is not solved properly. Essentially, the extent to which shear rate *per se* influences the flow-mediated response *per se* is difficult to quantify accurately as long as FMD% is the outcome of interest because it does not adjust for the influence of D_{base} properly.

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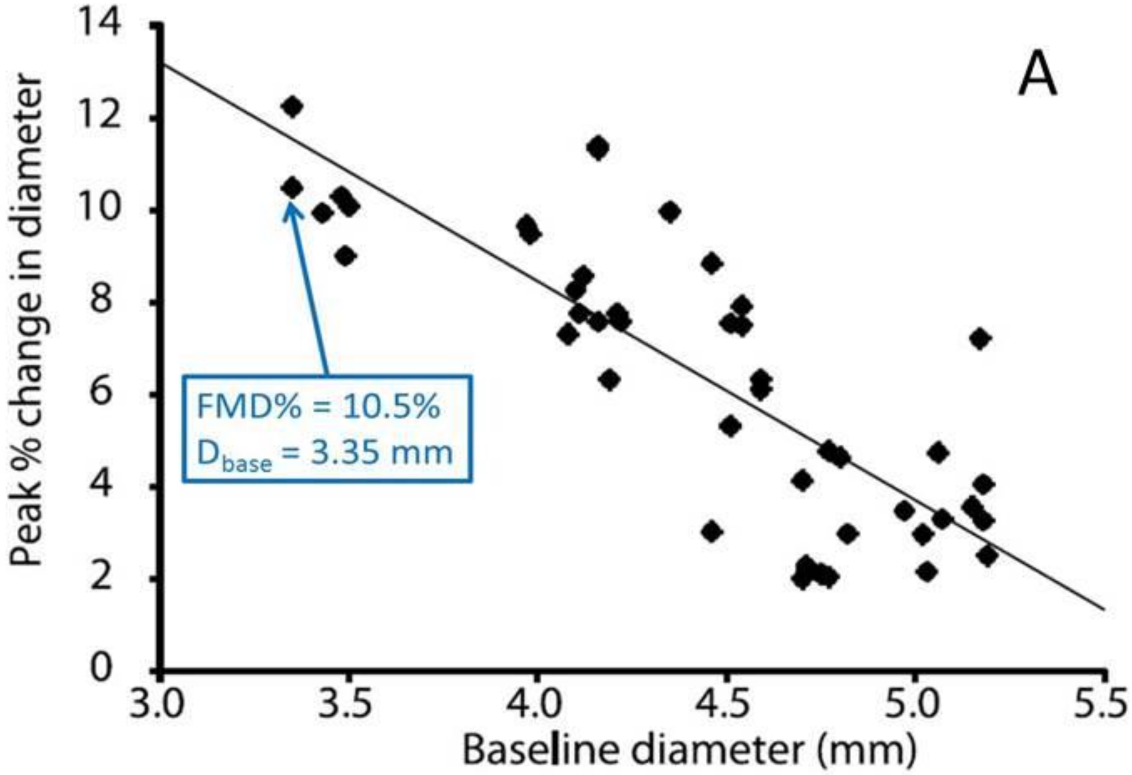
List of Figures


A
Peak % change in diameter
Baseline diameter (mm)
FMD% = 10.5%
D base = 3.35 mm
14
12
10
8
6
4
2
0
3.0
3.5
4.0
4.5
5.0
5.5

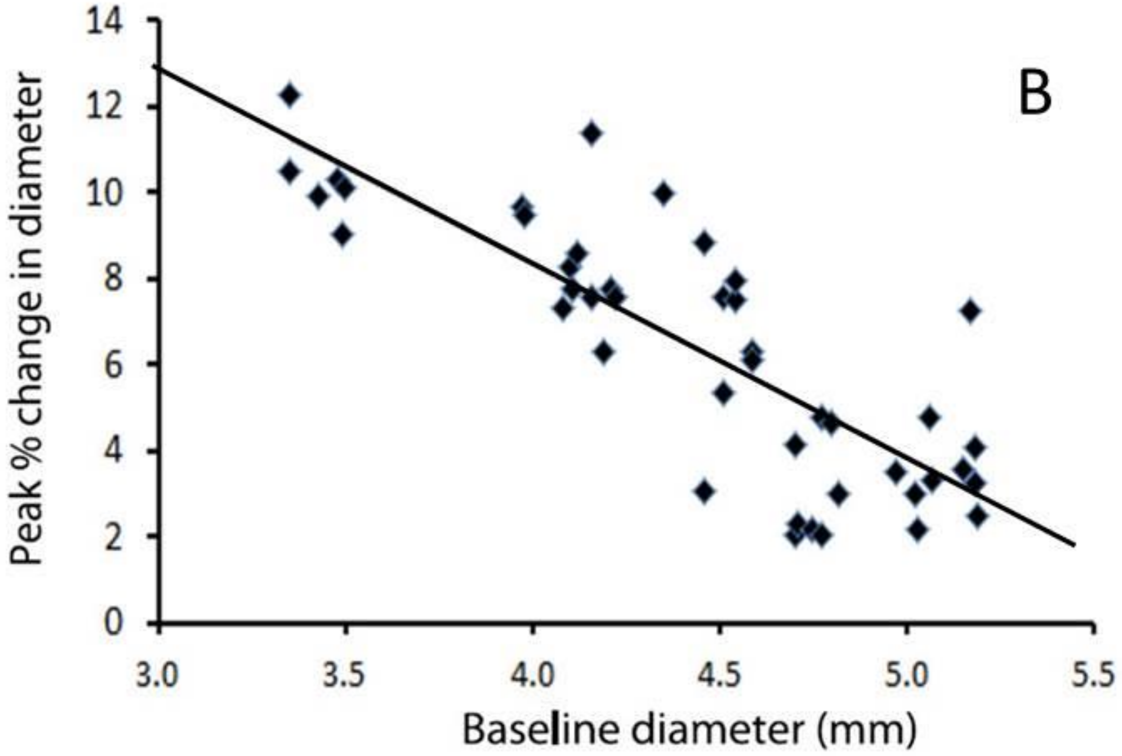

B
Peak % change in diameter
Baseline diameter (mm)
14
12
10
8
6
4
2
0
3.0
3.5
4.0
4.5
5.0
5.5

Fig.1

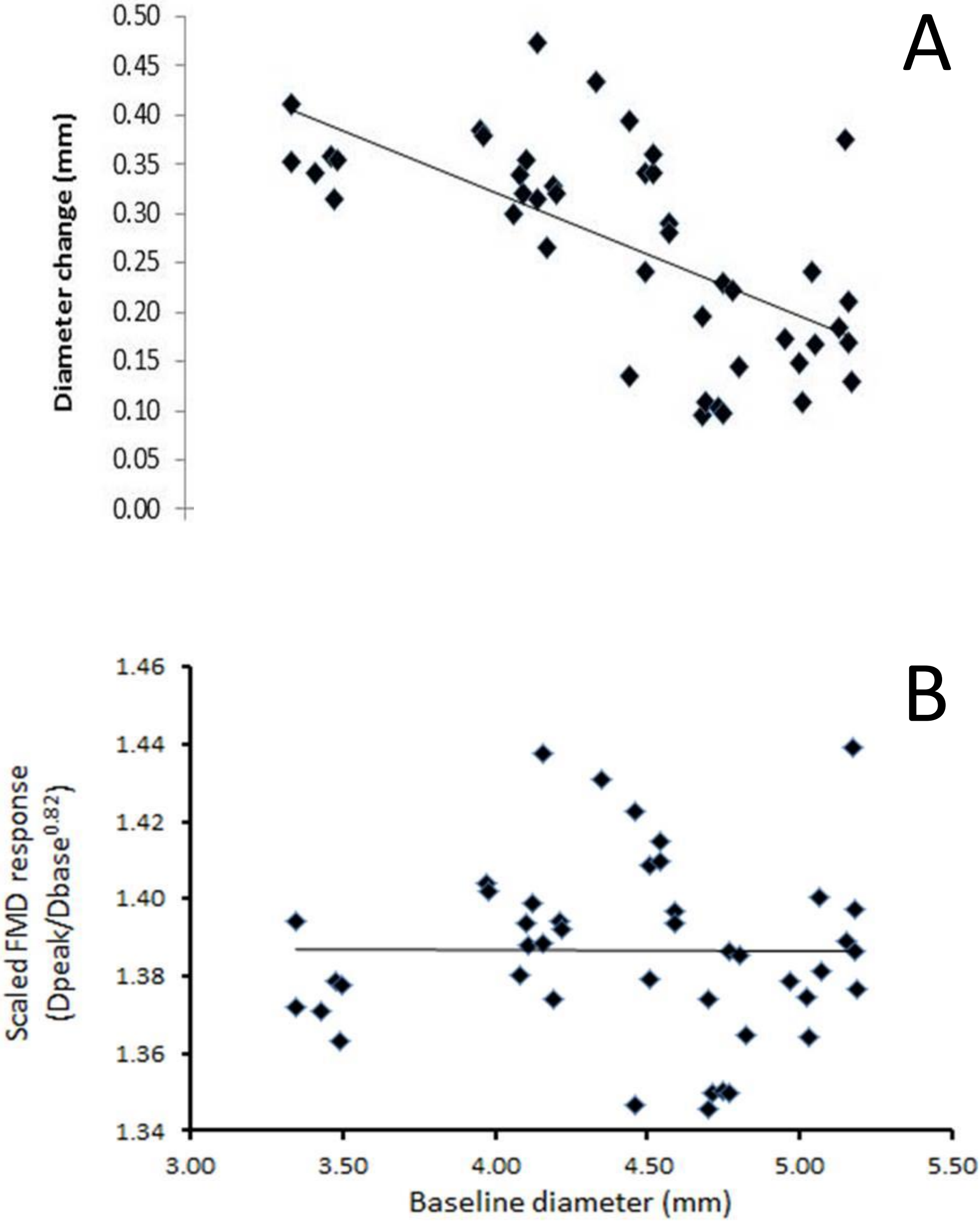

A
Diameter change (mm)
0.50
0.45
0.40
0.35
0.30
0.25
0.20
0.15
0.10
0.05
0.00
B
Scaled FMD response
(Dpeak/Dbase$^{0.82}$)
1.46
1.44
1.42
1.40
1.38
1.36
1.34
3.00
3.50
4.00
4.50
5.00
5.50
Baseline diameter (mm)

Fig 2.