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Estimation of the maximum flow-mediated brachial artery response using local regression methods

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Abstract

We consider methods for estimating the maximum from a sequence of measurements of flow-mediated diameter of the brachial artery. Flow-mediated vasodilation (FMD) is represented using the maximum change from a baseline diameter measurement after the release of a blood pressure cuff that has been inflated to reduce flow in the brachial artery. The influence of the measurement error on the maximum diameter from raw data can lead to overestimation of the average maximum change from the baseline for a sample of individuals. Nonparametric regression models provide a potential means for dealing with this problem. When using this approach, it is necessary to make a judicious choice of regression methods and smoothing parameters to avoid overestimation or underestimation of FMD. This study presents results from simulation studies using kernel-based local linear regression methods that characterize the relationship between the measurement error, smoothing and bias in estimates of FMD. Comparisons between fixed or constant smoothing and automated smoothing parameter selection using the generalized cross validation (GCV) statistic are made, and it is shown that GCV-optimized smoothing may over-smooth or under-smooth depending on the heart rate, measurement error and measurement frequency. We also present an example using measured data from the Buffalo Cardio-Metabolic Occupational Police Stress (BCOPS) pilot study. In this example, smoothing resulted in lower estimates of FMD and there was no clear evidence of an optimal smoothing level. The choice to use smoothing and the appropriate smoothing level to use may depend on the application.

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1. Introduction

Brachial artery ultrasound measurements of artery diameter taken before and during a transient increase in blood flow, caused by the inflation and subsequent release of a blood pressure cuff on the forearm, provide a noninvasive evaluation of brachial artery flow-mediated vasodilation (FMD) (Corretti *et al* 2002). Under appropriate experimental conditions, the FMD response is a marker for the endothelial response to flow-induced shear stress which includes the release of nitric oxide (NO) leading to vasodilation (Pyke and Tschakovsky 2005). Methods for measurement and image analysis have varied from study to study, and efforts to standardize methods are ongoing (Corretti *et al* 2002, Pyke and Tschakovsky 2005). Sources of the measurement error for brachial diameter measurements include intra- and inter-observer variabilities. This study will focus on the effects of intra-observer variability and its potential influence on estimates of maximum artery diameter after cuff deflation. While intra-observer variability for diameter measurements has been reported to be small, with the coefficient of variation (CV) less than 4%, when measurements are converted to a diameter change from the baseline or a per cent change from the baseline the CVs become larger than 20% meaning that intra-observer variability represents a significant source of variation in the parameters of interest (Herrington *et al* 2001, Faulx *et al* 2003). Sources of intra-observer variability can include subtle variations in choices of placement of boundary lines during reading and calculation of vessel diameter along with variations in image quality from the vessel or participant movement during scanning. Intra-observer variability can lead to statistical bias in the analysis of diameters after reading. As will be demonstrated in this study, choosing the maximum value of the FMD response without some smoothing to reduce the influence of the measurement error leads to positively biased estimates of the subject-specific peak diameters. This bias is then averaged over a sample during estimation of the mean FMD response parameters, leading to biased estimates of the mean absolute difference from the baseline and mean per cent difference. Smoothing or regression modeling of subject-specific brachial artery responses provides one approach to dealing with this bias. The purpose of this study is to examine and quantify the effects of an additive measurement error, smoothing, heart rate and different measurement rates (e.g., reading diameter measurements with different numbers of cardiac cycles between measurements) on the presence of bias in simulated and measured FMD response data.

2. Methods

2.1. Measurement

Brachial diameter measurements are typically taken in a temperature-controlled room, while the study participant is in a relaxed (resting for 15 min before examination) state since mental stress can affect vasodilator responses (Harris *et al* 2000). Measurements are taken before (baseline), during (pre-cuff) and after cuff inflation using continuous ultrasound measurements. Image analysis is then performed by trained readers using standard methods including gating measurements of brachial diameter at a specific part of the cardiac cycle (e.g., onset of the *R*-wave marking end diastole) (Corretti *et al* 2002). After image analysis, the diameter data are transformed from pixels to millimeters and the maximum response is determined.

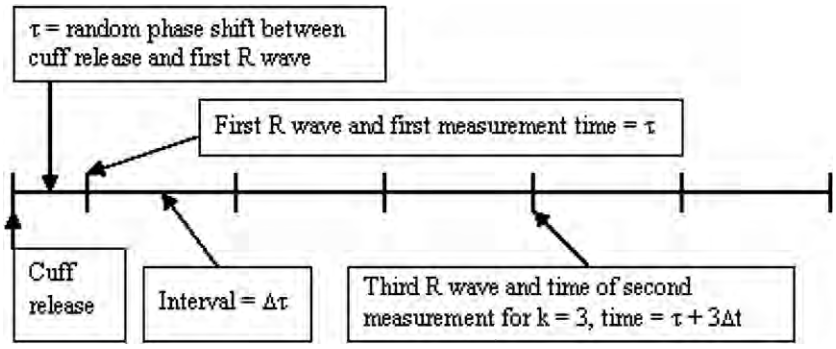

Figure 1. Time sequence diagram for the brachial diameter measurement model given in equation (1).

2.2. Theoretical model

The following model provides a useful approximation to the measurement process used to gather the FMD diameter data presented in a later section of this study. This model is developed here as a means of studying the results of varying parameters in the measurement and analysis process.

Let the measured diameter function during the flow-mediated dilation of the brachial artery be denoted by

$$DM(t) = D(t)C(t; \tau, \Delta t) + \varepsilon. \tag{1}$$

$D(t)$ is a continuous theoretical diameter function with a shape similar to that given by figure 2, and $C(t; \tau, \Delta t)$ is a measurement function that produces values at approximately regular intervals $k\Delta t$, where Δt is the time increment between cardiac cycles and k is an integer representing the number of cardiac cycles between successive diameter measurements. Although the quantity Δt is a function of the heart rate, which is not constant over time, it will be assumed to be constant for purposes of model simplicity and modeled using the average heart rate for an individual. The term ε represents an additive measurement error assumed to be normally distributed with mean zero and variance σ^2. The rationale for using an additive normal measurement model is discussed in section 2.4.

A refinement of the measurement function includes the addition of a random phase shift to account for the phase difference of up to one cardiac cycle between the time of cuff release and the first full QRS interval. For m measurements, as $h = 0, \ldots, m-1$, we have

$$\begin{aligned} C(t; \tau, \Delta t) &= 1 \qquad \text{if} \quad t = \tau + hk\Delta t, \\ C(t; \tau, \Delta t) &= 0 \qquad \text{if} \quad t \neq \tau + hk\Delta t, \end{aligned} \tag{2}$$

where τ is distributed uniform $(0, \Delta t)$ and C is a function with values of one at distance $k\Delta t$ apart and values of zero elsewhere as defined above. Figure 1 provides a time diagram illustrating the time sequence of measurements for the model in equation (1).

For the purposes of this study, we will assume that the function $D(t)$ is a smooth function with unique global maximum. Our general goal is then to estimate

$$D\max = \max(D(t)), \tag{3}$$

using m diameter measurements given by $DM(t)$ as $t = \tau, \ldots, \tau + (m-1)k\Delta t$.

2.3. FMD diameter function estimation

The data resulting from the measurements of brachial artery diameter during the FMD response take on values that are not readily modeled using a known functional form, which is needed to perform nonlinear regression. Nonparametric regression methods provide an approach to modeling the FMD response without assuming a given functional model. These methods also have the advantage of being local in nature, meaning that the estimated functional value at a given point of interest is influenced by the data points that are relatively close to that point. The extent to which functional estimates are local is commonly controlled by a smoothing parameter which defines the proportion of data points to be included in each predicted functional value. A simple example of this approach would be a three-point moving average with weights $(1/4, 1/2, 1/4)$ where each estimate is influenced by the immediate surrounding data points in addition to the middle point of the moving average, with the middle data point having more influence. Assuming that we have ten measurements over time, choosing a smoothing parameter of 0.3 would be equivalent to choosing a three-point moving average. The weights would be analogous to the kernel function used in local regression (sometimes called loess regression). There are various methods available for smoothing data or fitting nonparametric regression models, including local regression methods, smoothing splines, and semiparametric regression models based on maximum likelihood estimation (Cleveland 1979, Cleveland and Grosse 1991, Hastie *et al* 2001, Ruppert *et al* 2003). Choosing among these methods is not a simple matter, but for the measured brachial data in this study smoothing splines and local regression gave similar results. We chose local linear regression (loess) for these analyses because it is a natural extension of a weighted moving average and has the capacity to automatically select the optimal smoothing parameter based on the optimization of a specified fit parameter. The smoothing parameter, usually denoted by the symbol λ, defines the width of the smoothing window. This is similar to the example given above for moving averages where $\lambda = 0.3$, indicating that 30% of the data are included in the smoothing window. The loess method also makes use of a kernel smoothing function that weights points in the middle of the smoothing window more heavily than the surrounding points. For automated smoothing parameter selection, we used the generalized cross-validation (GCV) parameter for choosing a subject-specific smoothing parameter value (Hastie *et al* 2001). This method approximates the leave one out cross-validation approach to model selection.

The mean square error (MSE) provides a measure of the prediction error which can further be expressed in terms of the bias–variance decomposition (Hastie *et al* 2001). The D max estimate bias and variance are presented in this study to assess the smoothing method's ability to produce quality estimates of D max. Bias is of particular interest because in estimating a maximum for a function with an additive measurement error, simply choosing the largest data value produces a positive bias in the final estimate of mean difference from the baseline diameter to the maximum diameter (D max). This bias arises from choosing the maximum for each function in a sample of functions in the presence of the measurement error. When choosing the global maximum for each function, we are more likely to choose a point with a positive measurement error than with a negative measurement error and this bias is then averaged over a sample of differences from the baseline diameter to the maximum diameter leading to a positive bias in the estimation of the mean FMD response.

2.4. Simulation method

The model given by equation (1) can be simulated to provide samples for studying properties of different ways of estimating maximum FMD. The first step in this process provides a

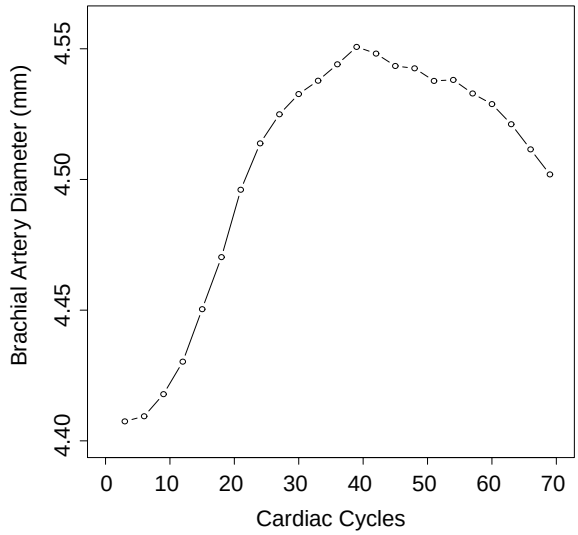

Figure 2. Mean brachial diameter function from measured data.

continuous diameter function by fitting a third-order smoothing spline to the mean diameter function from a data set. This smoothing spline is imposed to provide continuity for the realistic functional shape taken from measured data, as is given in figure 2. This provides a starting point for simulation experiments with varying measurement rates ($k\Delta t$), varying levels of measurement error and different levels of smoothing. The simulation then becomes a simple matter of generating a normal random variable given by ε with mean zero and variance σ^2, then generating a uniform random variable given by τ and forming the function of equation (1) by evaluating $D(t)$ for each $t = \tau + hk\Delta t$ for $h = 0, \ldots, m-1$, for a given value of Δt, and adding the random error term.

All simulations for this study were performed using the *R* language. The cubic smooth spline used to model the theoretical diameter function $D(t)$ was fitted to the data using the *R* procedure *smooth.spline*. The maximum diameter was found through the optimization procedure *optimize*, which is based on golden section and successive parabolic interpolation. The optimize procedure gives the global maximum of the diameter function similar to a simple data search, but is computationally faster and convenient since it is already implemented in the *R* language. This maximum is considered as the true value. Simulated measurements were calculated using the predicted values from the smooth spline at each measurement point $(\tau, \tau + k\Delta t, \tau + 2k\Delta t, \ldots, \tau + (m-1)k\Delta t)$ and adding random measurement errors from $N(0, \sigma^2)$. In order to estimate D max for each simulated diameter function, a local linear regression was fitted for each generated sample function using the *loess* procedure, and then the maximum diameter on the loess curve was searched by the optimization procedure. The simulation was repeated a large number of times (10 000). The mean square error and bias were obtained from these simulations. Automatic smoothing parameter selection was also implemented by minimizing the GCV value for loess fits, i.e. through the optimization procedure, the minimum GCV was searched with the smoothing parameter varying between 0 and 1. The GCV was computed as defined in Hastie *et al* (2001). The maximum diameter was picked from the loess fit with minimum GCV.

Two separate simulation studies were performed in order to examine the effect of smoothing on estimation of the maximum diameter (D max). For both sets of simulations,

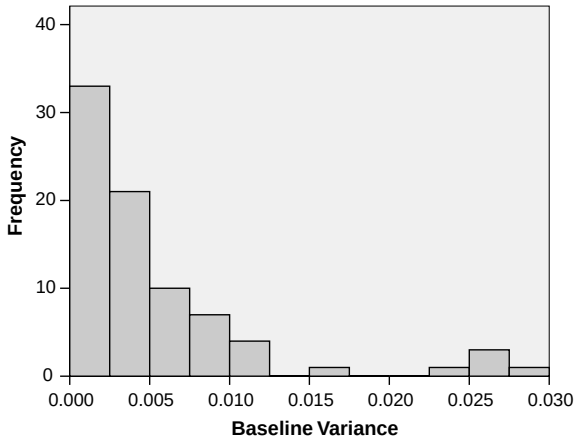

Figure 3. Distribution of within-person baseline diameter measurement variance σ^2 (mm^2) from BCOPS data ($n = 81$).

we varied input parameters as follows: $\sigma^2 = 0.002$, 0.003 and 0.006 mm^2; $k = 3$ and 4 cardiac cycles between diameter measurements; and heart rate = 40, 60 and 80 beats per minute. There were $n = 10\,000$ simulations for each combination of these parameters. The three levels of the measurement error ($\sigma^2 = 0.002$, 0.003 and 0.006 mm^2) were selected to be representative of the distribution of values obtained from analyzing the variance from repeated baseline measurements, as is given by figure 3. The variance at the baseline for repeated measurements was used as an approximation of the measurement error for input to the simulations. This seems a reasonable choice for studying methods for estimating the peak diameter within individuals since it includes intra-observer variability and any other sources of variability between diameter measurements (for an individual) that are relatively local in time. Before using the baseline variance as an approximation to the measurement error, for repeated measurements within an individual, we confirmed in a sample of 81 measured diameter responses that there was no trend or difference in diameters across the five baseline measurements. We checked the validity of assuming an additive normal measurement error by calculating the residual or difference between each individual measurement and the mean over five baseline measurements for each of the 81 participants. We then plotted each residual on a normal scale confirming a good approximate fit to the normal distribution (data not shown). We also confirmed the validity of an additive error model by plotting each residual against the baseline mean diameter to confirm a basic pattern with slope zero centered at the origin of the horizontal axis and no nonlinear features. Tests for linear correlation between the residuals and baseline diameter confirmed our visual observation of no relationship between the diameter and measurement variability. This means that an additive error model is adequate since there is no evidence of a multiplicative error relationship with diameter. Details of the study design and analysis for the 81 measured responses will be presented in the following section of this paper.

The first simulation study examines the relationship between minimum bias in the estimation of D max and the smoothing parameter magnitude. Simulations were performed across a range of smoothing parameters, for a linear loess smoothing regression, varying from 0.2 up to 1.0 by steps of 0.05 (e.g., 20%–100% of the data points included in the local linear smoothing). The smoothing parameter associated with the smallest bias in estimating D max was saved along with the D max estimate variance.

The second simulation study was performed to examine the properties of D max estimates taken from regressions with the smoothing parameter selected using the GCV method. This method optimizes the overall fit of the regression model to the data, but is not specifically targeted at providing unbiased estimates of D max. In contrast to the first set of simulations, we obtained a distribution of smoothing parameters within each iteration of $n = 10\,000$ simulations and calculated D max estimate bias and variance within each sample of 10 000 simulated FMD responses and GCV selected smoothing regressions.

2.5. Observed data methods

The observed data for this study were from a random sample of 100 officers from the Buffalo, New York, Police Department. The study design and brachial response measurement protocol are described in detail elsewhere (Joseph *et al* 2005, Violanti *et al* 2006). These measurements were taken during November 2001 through April 2003. A total of 99 participants agreed to complete the brachial ultrasound examination. There were 42 women and 57 men in the sample with ages ranging from 29 to 64 years. However, seven participants with the following conditions were excluded from brachial ultrasound measures: Raynaud's syndrome ($n = 2$), prior myocardial infarction ($n = 1$), cardiac pacemaker ($n = 1$), hypertension ($n = 1$), sprained wrist ($n = 1$) and patient discomfort ($n = 1$). Among the remaining 93 participants, 12 additional brachial scans were not of sufficient quality to be read due to participant movement or inadequate tape recordings. In total, 81 participants had acceptable brachial scans for analysis. These data were used for generating the mean brachial response curve for input to the simulation studies described above and to develop the measurement error distribution given by figure 3.

3. Results

3.1. Simulation results

Figure 4 provides an illustration of the relationship between bias and smoothing using the simulation methods described above. Note that the bias is positive with a magnitude of about 0.048 mm diameter where the smoothing parameter is $\lambda = 0.2$ and reaches minimum bias where the smoothing parameter value is $\lambda = 0.6$. The bias is negative for smoothing above $\lambda = 0.6$, meaning that the smoothing is trimming the top of the diameter function.

Table 1 shows the summary results for the first set of simulations with optimal (i.e. minimum bias in the estimate of D max) smoothing parameter values varying from $\lambda = 0.35$ to $\lambda = 0.90$ depending on the measurement error, measurement parameter (k) and heart rate. As expected when the measurement error increases, the amount of smoothing needed to have minimal bias also increases. As the number of cardiac cycles (k) between diameter measurements increases, the optimal smoothing level decreases highlighting the fact that taking diameter measurements less frequently increases the possibility of over-smoothing for the same smoothing parameter value. This results from the fact that with larger k, there are fewer measurements around the true maximum of the diameter function for a given time interval, so the same level of smoothing is covering a longer time window. Similarly, as the heart rate decreases the optimal smoothing level decreases because there will be fewer measured diameters around the true maximum of the diameter function to use in estimating the maximum. The bias and variance estimates in table 1 represent the values of these parameters for the estimation of D max where the bias was minimized. Since the solution is numerical,

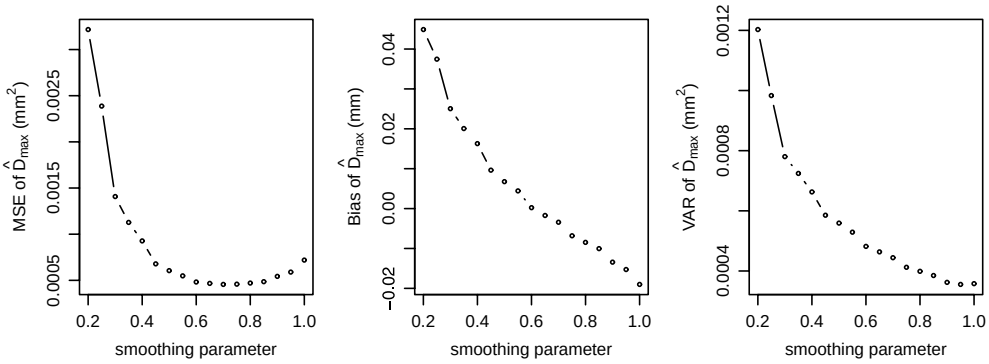

Figure 4. Maximum diameter estimate mean square error (MSE), bias and variance as functions of the smoothing parameter magnitude. Results are from 10 000 simulations with the heart rate = 60 beats min^{-1}, measurement error variance = 0.006 mm^2 and measurements at $k = 3$ cardiac cycles apart.

Table 1. Minimum bias smoothing parameters using an additive error model with the mean diameter curve and linear loess smoothing model to estimate the peak diameter *D* max. Simulation $n = 10\,000$ per combination of the measurement error, measurement parameter (k) and heart rate.

Simulation input parameters				*D* max estimate	
Measurement error variance, σ^2 (mm^2)	k[a]	Heart rate	Smoothing parameter	Bias (mm)	Variance (mm^2)
0.002	3	40	0.35	0.0008	0.0003
0.002	3	60	0.45	0.0007	0.0002
0.002	3	80	0.70	−0.0003	0.0002
0.002	4	40	0.30	−0.0008	0.0003
0.002	4	60	0.40	0.0004	0.0002
0.002	4	80	0.45	0.0005	0.0002
0.003	3	40	0.40	0.0008	0.0004
0.003	3	60	0.55	−0.0008	0.0003
0.003	3	80	0.90	−0.0002	0.0004
0.003	4	40	0.30	0.0013	0.0005
0.003	4	60	0.45	−0.0022	0.0003
0.003	4	80	0.55	−0.0007	0.0003
0.006	3	40	0.45	−0.0004	0.0006
0.006	3	60	0.60	0.0002	0.0005
0.006	3	80	0.90	0.0039	0.0007
0.006	4	40	0.40	−0.0015	0.0008
0.006	4	60	0.50	0.0003	0.0006
0.006	4	80	0.60	0.0002	0.0005

[a] Cardiac cycles between diameter measurements.

the bias values differ from zero by a small amount related to the discrete increment in the smoothing parameter value used in minimizing the bias.

The results of the second simulation study are presented in table 2. This method produces estimates of the individual level *D* max that, for the most part, tend to have a negative bias from over-smoothing as indicated by the bias estimates in table 2. Along with these results is the distribution of smoothing parameters for given levels of the measurement error, heart rate and measurement parameter *k*. The variation in smoothing parameter values, for a given level of simulation input parameters, is fairly large with values for the interquartile range that

Table 2. Generalized cross validation (GCV) estimate of an optimal smoothing parameter using an additive error model with the mean diameter curve and linear loess smoothing model to estimate the peak diameter D max. Simulation $n = 10\,000$ per combination of the measurement error, measurement parameter (k) and heart rate.

Simulation input parameters			Smoothing parameter distribution				D max estimate	
Measurement error variance, σ^2 (mm^2)	k[a]	Heart rate	First quartile	Median	Mean	Third quartile	Bias (mm)	Variance (mm^2)
0.002	3	40	0.39	0.53	0.50	0.61	−0.0068	0.0004
0.002	3	60	0.42	0.62	0.59	0.73	−0.0020	0.0003
0.002	3	80	0.43	0.68	0.64	0.81	0.0049	0.0003
0.002	4	40	0.36	0.43	0.43	0.51	−0.0088	0.0005
0.002	4	60	0.42	0.57	0.53	0.62	−0.0056	0.0004
0.002	4	80	0.42	0.62	0.59	0.73	−0.0021	0.0003
0.003	3	40	0.43	0.57	0.54	0.64	−0.0074	0.0006
0.003	3	60	0.47	0.67	0.62	0.76	−0.0012	0.0005
0.003	3	80	0.48	0.73	0.67	0.87	0.0072	0.0005
0.003	4	40	0.39	0.47	0.47	0.57	−0.0105	0.0007
0.003	4	60	0.46	0.61	0.57	0.69	−0.0055	0.0005
0.003	4	80	0.46	0.67	0.62	0.76	−0.0014	0.0005
0.006	3	40	0.49	0.62	0.61	0.75	−0.0077	0.0010
0.006	3	60	0.51	0.74	0.68	0.86	0.0022	0.0009
0.006	3	80	0.53	0.76	0.71	0.91	0.0126	0.0009
0.006	4	40	0.40	0.55	0.54	0.64	−0.0127	0.0013
0.006	4	60	0.50	0.66	0.63	0.76	−0.0049	0.0010
0.006	4	80	0.52	0.75	0.68	0.86	0.0021	0.0009

[a] Cardiac cycles between diameter measurements.

Table 3. Mean per cent change in brachial artery diameter by tertiles of waking AUCI and by smoothing parameter for women only.

		Smoothing parameter											
		0[a]		0.2		0.4		0.6		0.8		GCV[b]	
Tertile	n	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Low	7	7.45	1.8	6.45	2.1	5.46	1.9	4.93	2.0	4.64	1.9	5.32	2.0
Medium	8	8.56	2.8	7.26	2.8	6.12	2.8	5.63	2.8	5.51	2.8	6.19	3.0
High	7	4.15	1.7	3.08	1.9	1.96	1.9	1.49	1.8	1.33	1.7	2.30	2.5
p-linear[c]		0.012		0.014		0.010		0.012		0.013		0.041	
p-quadratic[d]		0.011		0.026		0.028		0.028		0.020		0.050	

[a] Smoothing parameter of zero means no smoothing (i.e. per cent change calculated from raw data).
[b] Automatic smoothing parameter selection through the GCV (generalized cross-validation) criterion.
[c] p-linear: tests linear trend in mean per cent change in brachial artery diameter across the tertiles.
[d] p-quadratic: tests curvilinear trend in mean per cent change in brachial artery diameter across the tertiles.

approach 0.4. This level of variation in the GCV-automated smoothing parameters was larger than expected.

3.2. Results of analyses with observed data

Results from the analysis of an association between FMD, characterized by a per cent change from the baseline diameter, and waking cortisol response (measured by the area under the curve above the first waking cortisol for four salivary cortisol measurements taken over the first hour of waking, AUCI) are presented in table 3. Note that the sample size for this analysis

is smaller than that of the overall data set because the association reported here only applies to women (it was not significant in men) and because of further exclusions from missing waking cortisol measurements. This analysis was done for a range of fixed smoothing parameter values and the GCV-automated parameter selection method. As would be predicted, more smoothing leads to lower mean response values. The standard deviations do not seem to be very different across the fixed smoothing parameter values, but the GCV method seems to have higher standard deviation values and produces less significant results. We also checked the relationship between smoothing parameter values and sample standard deviation over the whole sample and found that the GCV method does tend to lead to higher sample standard deviation (data not shown). This can be explained by the fact that the data-driven choice of the smoothing parameter results in different smoothing levels for each participant. This may inflate the sample variance of D max estimates, in comparison to the D max estimates from a fixed smoothing parameter, because of the added variability in the smoothing parameter.

4. Discussion

We have shown that estimation of the FMD response using raw diameter data can lead to bias in the estimation of the average response. This bias is an artifact of choosing the maximum value of a function with a measurement error. This process leads to a positive bias because when we choose the peak value of a measured function, we are more likely to choose a point with a positive measurement error than with a negative measurement error. Nonparametric regression analysis provides a useful way to explore this issue. This method is implemented by fitting a nonparametric regression to the raw data and then estimating the peak response for predicted values from the regression. Nonparametric regression is typically based on some sort of a smoothing approach, which requires the choice of a smoothing parameter to govern how much the data are smoothed to produce the functional fit. We have demonstrated, for simulated data, that the optimal (e.g., leading to minimum bias estimates of the mean response) choice of this smoothing parameter depends on how often the brachial ultrasound data are measured (e.g., number of cardiac cycles between image analyses), heart rate and measurement error levels. The results of the simulation studies show that bias is minimized by increasing the smoothing parameter for higher levels of the measurement error and heart rate, while less smoothing is optimal when the number of cardiac cycles between diameter measurements increases. One of the limitations of these results is the fact that the numerical estimation of a smoothing parameter leading to minimum bias estimation of the maximum diameter requires that the true maximum of the FMD diameter function be known. This issue was simplified in the simulation studies by removing inter-individual variation in the brachial response and using a mean curve with one value for the maximum diameter. While the simulation results are helpful in describing the way that estimate bias and smoothing interact with the measurement error, heart rate and measurement spacing, they do not provide a general way to obtain minimum bias estimates of peak diameter. In addition to this, minimum bias smoothing is not available as a general theoretical development or as a feature of existing nonparametric regression applications. Methods for optimizing the general fit of a nonparametric regression model to data are readily available. We tested one such method, the automated GCV method, with respect to its ability to produce quality estimates of the individual level D max for simulated data, and we examined the resulting distribution of smoothing parameters for varying levels of the measurement parameter k, measurement error and heart rate. This method produces estimates of the individual level D max that tend to have a negative bias from over-smoothing. This over-smoothing phenomenon is referred to as *trimming the hills* and is a known property of local regression, particularly using the linear fit. One way to address this bias from

over-smoothing is to use a quadratic local regression (Hastie *et al* 2001). However, our experience has been that this approach is not as useful for the observed brachial data because the quadratic local regression tends to be too sensitive to points at the beginning and end of the measured function and leads to incorrect specification of D max. Simply using a fixed smoothing parameter for all FMD curves provides an alternative to the GCV smoothing. We found that this approach may be a good choice for analyses where associations between D max and other variables are of interest since the GCV method appears to inflate the sample variance above the level found with minimal smoothing (e.g., $\lambda = 0.2$). For analyses of associations, it is clear that the optimal strategy may include computing the association over a range of smoothing parameters from no smoothing up to $\lambda = 0.8$ and possibly the GCV, and use of the minimum smoothing level that produces the maximum estimate of association. This approach could also be adapted to work for experimental studies testing for differences in FMD between treatment groups. The implications of using nonparametric regression for obtaining FMD estimates for clinical purposes are less clear than for research purposes. Clinical use of FMD necessarily involves comparing measurements for individual FMD against reference values. Since selecting the appropriate smoothing level for this purpose is not straightforward (e.g., measurement error level for an individual patient is unknown), then these results may be less applicable in a clinical than in a research setting.

Variation in mean FMD has been found between study populations with similar characteristics. Various reasons for these differences have been proposed and tested in a meta analysis by Bots *et al* (2005). In the discussion of this meta analysis, it is pointed out that variations in the technical aspects for measuring FMD and in risk factor distributions do not completely explain these differences in mean FMD. While a few studies mention using smoothing to obtain FMD estimates (Herrington *et al* 2001), many do not. Because of this, it is difficult to say whether different data smoothing methods can explain some of the variation in FMD estimates between similar study populations. We have shown that higher levels of the measurement error can lead to higher estimates of FMD using raw data and that smoothing the raw data can reduce this bias but that care must be taken to avoid over-smoothing, which leads to underestimates of FMD.

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References

Bots M L, Westerink J, Rabelink T J and de Koning E J P 2005 Assessment of flow-mediated vasodilation (FMD) of the brachial artery: effects of technical aspects of the FMD measurement on FMD response *Eur. Heart J.* **26** 363–8

Cleveland W S 1979 Robust locally weighted regression and smoothing scatterplots *J. Am. Stat. Assoc.* **74** 829–36

Cleveland W S and Grosse E 1991 Computational methods for local regression *Stat. Comput.* **1** 47–62

Corretti M C *et al* 2002 Guidelines for the ultrasound assessment of endothelial-dependent flow-mediated vasodilation of the brachial artery: a report of the International Brachial Artery Reactivity Task Force *J. Am. Coll. Cardiol.* **39** 257–65

Faulx M D, Wright A T and Hoit B D 2003 Detection of endothelial dysfunction with brachial artery ultrasound scanning *Am. Heart J.* **145** 943–51

Harris C W, Edwards J L, Baruch A, Riley W A, Pusser B E, Rejeski W J and Herrington D M 2000 Effects of mental stress on brachial artery flow-mediated vasodilation in healthy normal individuals *Am. Heart J.* **139** 405–11

Hastie T, Tibshirani R and Freidman J 2001 *The Elements of Statistical Learning* (New York: Springer)

Herrington D M, Fan L, Drum M, Riley W A, Pusser B E, Crouse J R, Burke G L, McBurnie M A, Morgan T M and Espeland M A 2001 Brachial flow-mediated vasodilator responses in population-based research: methods, reproducibility and effects of age, gender and baseline diameter *J. Cardiovasc. Risk* **8** 319–28

Joseph P N, Dorn J, Violanti J M, Andrew M E and Riley W A 2005 Examining the brachial artery reactivity curve measured by b-mode ultrasound in the buffalo cardio-metabolic occupational police stress (BCOPS) pilot study *J. Vasc. Ultrasound* **29** 199–206

Pyke K E and Tschakovsky M E 2005 The relationship between shear stress and flow-mediated dilatation: implications for the assessment of endothelial function *J. Physiol.* **568** 357–69

Ruppert D, Wand M P and Carrol R J 2003 *Semiparametric Regression* (Cambridge: Cambridge University Press)

Violanti J M *et al* 2006 The Buffalo Cardio-Metabolic Occupational Police Stress (BCOPS) pilot study: methods and participant characteristics *Ann. Epidemiol.* **16** 148–56