Brachial artery diameter, but not flow-mediated dilation, is associated with sleep apnoea in the Multi-Ethnic Study of Atherosclerosis

Greg Atkinson, Gerard Danjoux, Louisa Ells, Sophie Suri and Alan M Batterham

Health and Social Care Institute, School of Health and Social Care, Teesside University, UK

Running title: *Sleep apnoea and FMD%*

There was no financial support for any author of this study and no relationships with industry

G. Atkinson and AM Batterham gained access to the data and completed the statistical analysis. S. Suri assisted with literature searches and the writing of paper drafts. G. Danjoux and L. Ells commented on earlier drafts of the paper.

Total number of words: 2562

1 Figure

1 Table

Address for Correspondence:

Prof. Greg Atkinson

Health and Social Care Institute

School of Health and Social Care

Constantine Building

Teesside University

MIDDLESBROUGH, TS1 3BA, UK

greg.atkinson@tees.ac.uk

Tel: +44 (0) 1642 342758

Abstract

The percentage flow-mediated dilation of the brachial artery (FMD%) is purported to be an early indicator of atherosclerosis and has been reported to be reduced in people with obstructive sleep apnoea. Nevertheless, FMD% scales poorly for, and is concomitantly dependent on, initial artery diameter, which may, itself, be higher in obstructive sleep apnoea patients. Therefore, for the first time, we aimed to quantify the differences in initial diameter and properly-scaled flow-mediated dilation between people with, and without, sleep apnoea. The prevalence of physician-diagnosed sleep apnoea, as well as initial and peak diameters of the brachial artery were recorded for 3354 participants in the Multi-Ethnic Study of Atherosclerosis (MESA). Arterial data were analysed using FMD% and an allometric approach, which scales the flow-mediated response properly for initial diameter. In the sex, race and age-adjusted model, initial diameter was 0.19 mm larger in sleep apnoea patients (95%CI: 0.07 to 0.32 mm, *P=0.003*) and correlated negatively with FMD% (r= -0.43, 95%CI: -0.57 to -0.26, *P<0.0005*). Using this same adjusted model, FMD% was 3.8±2.7% for sleep apnoea patients (n=104) vs 4.4±2.7% for undiagnosed people (95%CI for difference: -1.12 to -0.07%, *P=0.028*). Allometric scaling halved this FMD%-indicated sample difference in flow-mediated dilation (95%CI: -0.7% to 0.1%, *P=0.19*). In conclusion, the initial diameter of the brachial artery is larger in MESA participants diagnosed with sleep apnoea compared with undiagnosed people. However, the difference in flow-mediated dilation between these two cohorts is trivial when the flow-mediated response is scaled properly for resting diameter.

Keywords: Sleep apnoea; Endothelial function; Biostatistics; Allometry

Introduction

Analyses of the large dataset from the Multi-Ethnic Study of Atherosclerosis (MESA) indicate that physician-diagnosed sleep apnoea is associated with an increased risk of cardiovascular events [1]. The causal pathway between sleep apnoea and cardiovascular disease has been proposed to involve the intermediary variables of elevated sympathetic activity, insulin resistance and obesity [2]. Other abnormalities in coagulation factors, platelet activation, inflammatory processes and/or endothelial function could also play a role in the pathogenesis of cardiovascular disease in sleep apnoea [2].

Ali *et al*. [3] recently reviewed the utility of various early indicators of cardiovascular events in obstructive sleep apnoea. One of these indicators was the percentage flow-mediated dilation of the brachial artery (FMD%), which is an indicator of endothelial function in humans [4]. Ali *et al*. [3] reported that, in most studies, mean FMD% is lower for patients with obstructive sleep apnoea *vs* healthy participants; an observation corroborated by Hoyos et al. [5] in a recent review specifically on sleep apnoea and endothelial dysfunction.

From the earliest studies onwards, FMD% has been reported to be negatively correlated, sometimes strongly, to initial artery diameter [4, 6]. Despite some attempts to explain this observation physiologically, there is evidence to suggest that it is due to the poor size-scaling of the FMD% ratio index itself [7]. This confounding of FMD% is important because initial artery diameter has been reported to predict the progression of subclinical atherosclerosis [8] and cardiovascular events [9], as well as being substantially higher in obstructive sleep apnoea patients [10]. This obfuscation of arterial structure and function by the FMD% index is seldom resolved in the literature. For example, less than half of the thirteen studies on FMD% reviewed by Ali *et al*. [3] actually presented data for initial diameter. In only one of these studies [10] was there an attempt to adjust FMD% for initial diameter. Consequently, it has been questioned to what extent the reported lower FMD% in

obstructive sleep apnoea is explained by the potentially higher initial artery diameter in these patients [11].

Therefore, we aimed to quantify any differences in initial brachial artery diameter and flow-mediated dilation (adjusted for artery diameter) between people who did and did not report physician-diagnosed sleep apnoea in the dataset from the Multi-Ethnic Study of Atherosclerosis (MESA). This dataset has also recently been analysed to explore other questions related to sleep apnoea, predominantly because the dataset is very large, population-based and involved comprehensive and standardised data collection methods [1, 12-14].

Methods

The MESA participants

The full study design for MESA has been detailed by Bild *et al*. [15]. In brief, MESA is a prospective cohort study on subclinical cardiovascular disease. The overall MESA sample comprises 6814 women and men, aged 45–84 years, recruited from six regions in the USA. The MESA was approved by the local Institutional Review Boards of each participatory study site.

The sleep apnoea question in MESA

During the second MESA examination, a self-administered sleep history questionnaire was administered [1, 12-14]. A question was "*Have you ever been told by a doctor that you had sleep apnoea (a condition in which breathing stops briefly during sleep)?"* Participants responses were either "*yes*", "*no*" or "*don't know*". Among the 6814 MESA participants, 678 either did not participate in the sleep history study or reported "*don't know*" to the sleep apnoea question and were therefore excluded from analysis.

The FMD% protocol in MESA

Full details the FMD% protocol in MESA are described by Yeboah *et al*. [9]. Of the 6136 participants who recorded a yes/no answer for the sleep apnoea question, 3354 completed the FMD% protocol (1692 women and 1662 men). Of these participants, 104 (23 women and 81 men) reported that a physician had diagnosed them with sleep apnoea, giving an overall prevalence of 3.1% (1.4% in women and 4.9% in men).

Data analysis

Data were analysed using FMD%, and an allometric approach [6,16,17]. In this approach, initial and peak diameters are logarithmically transformed (natural logarithm) and the differences between these values are calculated. These differences in diameter on the log scale are entered as the outcome in a general linear model with sleep apnoea diagnosis as the fixed factor and logarithmically-transformed initial diameter as a covariate. The resulting adjusted estimates of the flow-mediated response and associated 95% confidence intervals (CI) are obtained after back-transformation. The allometric scaling exponent of 'b' is derived from the log-linear transformation of the simple allometric model based on the equation;

$$\text{Peak diameter} = a \times \text{initial diameter}^{b}$$

The FMD% and allometric approaches were compared using unadjusted models and models adjusted for sex, race and age. It is extremely important not to covariate-adjust statistical models for variables that are on the causal pathway between exposure and outcome [18]. This issue has been highlighted by Levitzsky and Redline [19] specifically in the context of obstructive sleep apnoea and cardiovascular disease. These authors thought it crucial not to adjust for variables such as body mass, diabetes and hypertension when the association between obstructive sleep apnoea and cardiovascular outcomes is being investigated because these variables are on the proposed causal pathway [2]. Therefore, these variables were not entered as covariates in our statistical models.

Any ratio index like FMD% is naturally positively skewed even if the numerator and denominator are normally distributed [17]. Therefore, the FMD% index was also examined following natural logarithmic transformation. Descriptive sample statistics are mean ± standard deviation. The precision of inferential estimates is described by the 95% confidence limits.

Results

The correlation between FMD% and initial diameter was -0.43 (-0.57 to -0.26, *P<0.0005*) in the sleep apnoea patients (Figure 1) and -0.42 (-0.45 to -0.39, *P<0.0005*) in the undiagnosed participants. When FMD% was log-transformed, these correlations reduced slightly to -0.36 (-0.52 to -0.18, *P<0.0005*) and -0.40 (-0.43 to -0.37, *P=0.0005*) respectively. The regression slope for the FMD%-initial diameter relationship was -1.2 %/mm (95%CI: -0.7 to -1.7, *P<0.0005*) for sleep apnoea patients and -1.5 %/mm (95%CI: -1.4 to -1.6, *P<0.0005*) for undiagnosed participants.

The value of 'b' in the allometric model was 0.946 (0.924 to 0.969, *P<0.0005*) in the sleep apnoea patients and 0.942 (0.937 to 0.946, *P<0.0005*) in the undiagnosed cohort. Only when 'b' = 1.000 is a percentage index accurate for scaling a change in size across the full measurement range [16].

In the unadjusted model, the sample mean±SD estimate of FMD% was 3.8±2.6% for sleep apnoea patients vs 4.4±2.9% for undiagnosed participants (95%CI for difference: 0.01 to 1.14%, *P=0.045*, Table 1). In the model adjusted for sex, ethnicity and age, mean±SD estimates of FMD% remained unchanged (3.8±2.7 vs 4.4±2.7%) and the estimate of the difference between samples became more precise (95%CI: 0.07 to 1.12%, *P=0.028*).

In the unadjusted model, mean initial diameter was 0.45 mm larger in the sleep apnoea patients (95%CI: 0.28 to 0.61 mm, *P<0.0005*). This mean difference was 0.19 mm (95%CI: 0.07 to 0.32, *P=0.003*) in the model adjusted for sex, age and race. Use of the allometric approach to account for the confounding of initial diameter generally reduced the mean difference in flow-mediated response between sleep apnoea patients and healthy people. In the model adjusted only for initial artery diameter, the sample difference in adjusted flow-mediated dilation was 0.02% (-0.49 to 0.50, *P=0.92*). In the adjusted model, the difference between samples was 0.3% (95%CI: -0.1 to 0.7, *P=0.19*), which is approximately half the mean difference quantified with the FMD% index.

Discussion

It is vital that a ratio index scales consistently over the full range of measurements. In agreement with previous studies [6,16,17], the moderate-to-strong negative correlation between FMD% and initial diameter indicates that this assumption is also violated for sleep apnoea patients in the MESA (Figure 1). Our analyses suggest that the inappropriate scaling associated with FMD% leads to an exaggeration of the difference in flow-mediated response between people diagnosed with sleep apnoea and undiagnosed people in the MESA. Nevertheless, in agreement with previous studies [10], we found a clear mean difference in brachial artery diameter between people with and without sleep apnoea in the MESA.

Most of the past researchers on this topic have administered overnight sleep studies to their participants. Consequently, previous studies have tended to be relatively small and homogeneous in terms of participant sample [2]. A meta-analysis of the pooled mean difference in FMD% between people with and without sleep apnoea has yet to be undertaken on these past studies. However, Ali et al. [3] reported that this mean difference in FMD% tends to be 0.3-3.0%. The sample difference in FMD% in the present study of 0.6% lies within this range. A pertinent point is that statistical analyses have been covariate-adjusted for initial diameter in only one of these previous studies [10]. This research group

reported a substantial influence of initial artery diameter on the FMD% index. When the researchers adjusted for this confounding, the influence of a one-unit change in the Apnoea-Hypopnoea Index on FMD% was reported to be -0.09 % [10]. Therefore, the difference in FMD% (adjusted for initial diameter) between an apnoea-hypopnoea index of zero and twenty events/h can be estimated from this regression slope to be only 1.8%, which agrees with the clinically unimportant association found in our large population-based study.

The strengths of the present study were that it was large, population-based, multi-ethnic and involved comprehensive and standardized collection of data. Nevertheless, there are some limitations. First, our study was based on an observational cross-sectional design, which precludes the elucidation of the temporal relationships between variables in the causal pathway. Although it is thought most likely that sleep apnoea leads to endothelial dysfunction [2], the reverse could also be true. Interventions designed to improve the symptoms of sleep apnoea such as continuous passive airway pressure (CPAP) have been reported to improve FMD% in some studies [5], but none of these previous researchers has, again, covariate-adjusted the CPAP-mediated change in the flow-mediated response for any CPAP-mediated change in initial arterial diameter.

A second limitation is that the diagnosis of sleep apnoea was based on self-reported information, which may be influenced by recall bias. In MESA, it was questioned at the 2nd examination whether participants had ever been physician-diagnosed with sleep apnoea. The word “obstructive” was not included in the question. However, the prevalence of the other apnoeas (central and “mixed”) is known to be much lower than that of obstructive sleep apnoea [20]. Therefore, it is likely that the vast majority of the sleep apnoea patients in MESA had obstructive sleep apnoea. It is also likely that polysomnography was used to diagnose sleep apnoea in the MESA participants who reported diagnosis, since this method has been a part of the sleep apnoea clinical pathway for several decades in North America [21].

In conclusion, the sex, race and age-adjusted mean FMD% of the MESA participants who reported physician-diagnosed sleep apnoea was 0.6% lower than those who participants who did not report such a diagnosis. This mean difference was 0.3% and not statistically significant when the confounding influence of initial artery diameter was allometrically-adjusted for. Therefore, the MESA participants who reported physician-diagnosed sleep apnoea do not demonstrate a clinically important reduction in flow-mediated dilation.

Acknowledgement

This Manuscript was prepared using the Multi-Ethnic Study of Atherosclerosis (MESA) research materials obtained from the NHLBI Biologic Specimen and Data Repository Information Coordinating Center and does not necessarily reflect the opinions or views of the MESA or the NHLBI. MESA was supported by contracts N01-HC-95159 through N01-HC-95169 from the National Heart, Lung, and Blood Institute and by grants UL1-RR-024156 and UL1-RR-025005 from NCRR. The authors thank the other investigators, the staff, and the participants of the MESA (http://www.mesa-nhlbi.org).

References

1. Yeboah J, Redline S, Johnson C, Tracy R, Ouyange P, Blumenthalf RS, Burke GL, Herrington DM. Association between sleep apnea, snoring, incident cardiovascular events and all-cause mortality in an adult population: MESA. *Atherosclerosis,* 2011, 219: 963-968.
2. Jean-Louis G; Zizi F; Clark LT; Brown CD; McFarlane SI. Obstructive sleep apnea and cardiovascular disease: role of the metabolic syndrome and its components. J Clin Sleep Med 2008, 4: 261-272
3. Ali, S.S., Oni, E.T., Warraich, H.J., Blaha, M.J., Blumenthal, R.S., Karim, A., Shaharyar, S., Jamal, O., Fialkow, J., Cury, R., Budoff, M.J., Agatston, A.S., Nasir, K. Systematic

review on noninvasive assessment of subclinical cardiovascular disease in obstructive sleep apnea: new kid on the block! *Sleep Med Rev*, 2014, Article in Press.

4. Celermajer DS, Sorensen KE, Gooch VM, et al. Non-invasive detection of endothelial dysfunction in children and adults at risk of atherosclerosis. Lancet 1992, 340:1111–1115
5. Hoyos CM, Melehan KL, Liu PY, Grunstein RR, Philips CL. Does obstructive sleep apnea cause endothelial dysfunction? A critical review of the literature. Sleep Medicine Reviews 20 (2015) 15e26.
6. Atkinson G, Batterham AM. Allometric scaling of diameter change in the original flow-mediated dilation protocol. *Atherosclerosis*, 2013a, 226: 425-427
7. Atkinson G, Batterham AM. The clinical relevance of the percentage flow-mediated dilation index. *Curr Hypertens Rep*, 2015, 17: 1-9.
8. Halcox JPJ, Donald AE, Ellins E, *et al*. Endothelial Function Predicts Progression of Carotid Intima-Media Thickness. *Circulation,* 2009, 119: 1005-1012.
9. Yeboah J, Folsom AR, Burke GL, *et al*. Predictive value of brachial flow-mediated dilation for incident cardiovascular events in a population-based study: the Multi-Ethnic Study of Atherosclerosis. *Circulation,* 2009, 120: 502–509.
10. Namtvedt SK, Hisdal J, Randby A, *et al*. Impaired endothelial function in persons with obstructive sleep apnoea: impact of obesity. *Heart*, 2013, 99: 30–4
11. Atkinson G. Correspondence: Impaired endothelial function in obstructive sleep apnoea: Allometric scaling can help estimate the true difference in flow-mediated response. *Heart,* 2013, 99: 968-969.
12. Chew M, Xie J, Klein R, Klein B, Cotch MF, Redline S, Wong TY, Cheung N. Sleep apnea and retinal signs in cardiovascular disease: the Multi-Ethnic Study of Atherosclerosis. Sleep Breath 2015, DOI 10.1007/s11325-015-1177-z
13. Teodorescu, M., Barnet, J.H., Hagen, E.W., Palta, M., Young, T.B., Peppard, P.E. Association between asthma and risk of developing obstructive sleep apnea. J Am Med Assoc, 2015, 313: 156-164.

14. Lin, G.-M., Colangelo, L.A., Lloyd-Jones, D.M., Redline, S., Yeboah, J., Heckbert, S.R., Nazarian, S., Alonso, A., Bluemke, D.A., Punjabi, N.M., Szklo, M., Liu, K. Association of Sleep Apnea and Snoring with Incident Atrial Fibrillation in the Multi-Ethnic Study of Atherosclerosis. Am J Epidemiol, 2015, 182: 49-57.

15. Bild DE, Bluemke DA, Burke GL, *et al*. Multi-ethnic study of atherosclerosis: objectives and design. *Am J Epidemiol*, 2002, 156: 871–81.

16. Atkinson G, Batterham AM, Thijssen DHJ, Green DJ. A new approach to improve the specificity of flow-mediated dilation for indicating endothelial function in cardiovascular research. *J Hypertens,* 2013, 31: 287-291.

17. Atkinson G, Batterham AM. The percentage flow-mediated dilation index: A large-sample investigation of its appropriateness, potential for bias and causal nexus in vascular medicine. *Vasc Med*, 2013b, 18: 354-365

18. Schisterman EF, Cole SR, Platt RW. Overadjustment bias and unnecessary adjustment in epidemiologic studies. Epidemiology 2009; 20: 488-495.

19. Levitzky YS and Redline S. Epidemiological evidence for an association between sleep apnea, hypertension and cardiovascular disease. In: Sleep Apnea, Implications in Cardiovascular and Cerebrovascular Disease. (edited by TD Bradley and JS. Floras) CRC Press, 2009: 163–179

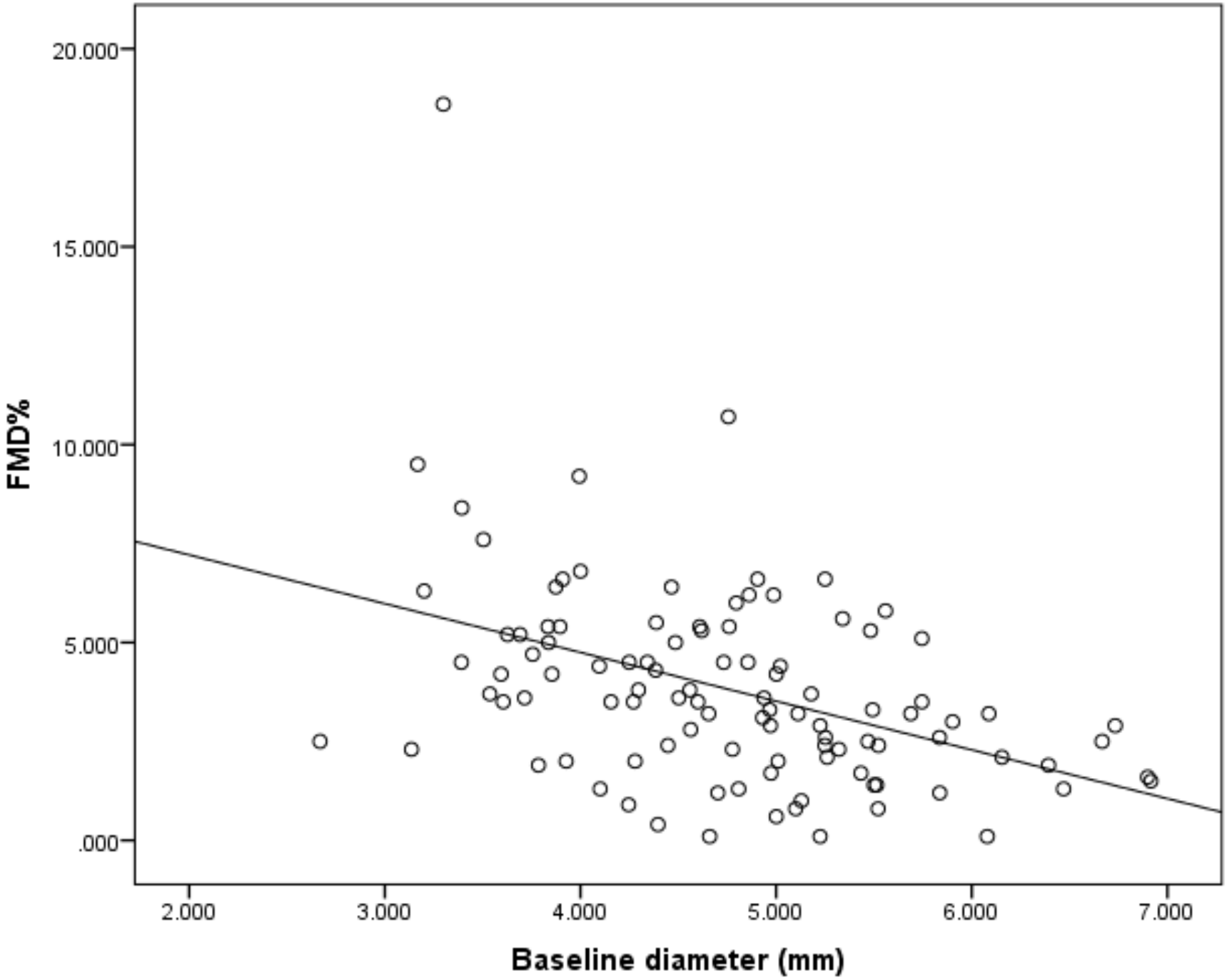
20. Morgenthaler TI, Kagramanov V, Hanak V *et al*. Complex sleep apnea syndrome: is it a unique clinical syndrome? *Sleep*, 2006, 29: 1203-1209.

21. Qaseem A, Dallas P, Owens DK, Starkey M, Holty JC, Shekelle P. Diagnosis of obstructive sleep apnea in adults: A clinical practice guideline from the American College of Physicians. *Ann Intern Med,* 2014, 161: 210-220

Table 1. Variables measured during the flow-mediated dilation protocol for people in MESA who did, and did not, have physician-diagnosed sleep apnoea (Estimates not adjusted for race, sex and age).

Variable	Sleep apnoea (n=104) Mean ± SD	Undiagnosed (n=3250) Mean ±SD	95%CI for difference between samples
Initial diameter (mm)	4.76 ± 0.89	4.31 ± 0.83	0.28 to 0.61
Peak diameter (mm)	4.93 ± 0.88	4.49 ± 0.82	0.28 to 0.60
Absolute diameter change (mm)	0.17 ± 0.10	0.18 ± 0.11	-0.01 to 0.01
FMD% (%)	3.8 ± 2.6	4.4 ± 2.9	0.01 to 1.14
D_{base}-adjusted FMD (%)	4.4 ± 2.4	4.3 ± 2.4	-0.5 to 0.5

Figure 1. The negative moderate correlation between initial artery diameter and FMD% for the sleep apnoea patients in MESA.


20.000
15.000
10.000
5.000
.000
FMD%
2.000
3.000
4.000
5.000
6.000
7.000
Baseline diameter (mm)