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Assessment for the possibility of a 'first night effect' for wrist actigraphy in adolescents

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Abstract

Objectives: Evidence of a 'first night effect' has been documented for polysomnography. The possibility of this has not been previously assessed in wrist actigraphy, yet may have important implications for the study design of future sleep research. We sought to examine potential evidence of a 'first night effect' for wrist actigraphy in adolescents across weekdays and weekend nights for multiple sleep outcomes.

Design: Three-year prospective cohort study (Midlands Adolescent Schools Sleep Education Study).

Setting: Eight secondary schools in the Midlands region of the United Kingdom (UK).

Participants: Adolescents (aged 11-13 years at baseline) were recruited to the study and requested to wear wrist actigraphy for seven consecutive days/nights at each annual assessment for three years.

Primary outcome measures: We compared multiple sleep outcomes (total sleep time, wake after sleep onset, sleep efficiency, sleep onset latency, number of awakenings, length of awakenings, sleep onset time) when the device was administered on a weekday and weekend and compared these to other nights to identify possible evidence of a 'first night effect' for wrist actigraphy.

Results: No significant differences were found between any sleep outcomes when the first night of wrist actigraphy was on a weekday compared to other weekdays. When the first night was measured on a weekend (Friday), average total sleep time was significantly greater (486±5 minutes) compared to the second night (Saturday; 496±6 minutes), p=0.01.

Conclusions: We found no evidence to support a 'first night effect' for wrist actigraphy in our adolescent sample. The first night of actigraphy data should not be disregarded in future studies that deploy this technique to measure sleep over prolonged time periods.

Article summary

Strengths and limitations of this study

- This is the first time the possibility of a 'first night effect' for wrist actigraphy used to measure sleep has been investigated;
- A large sample of adolescents were instructed to wear wrist actigraphy for seven consecutive days, annually for three years;
- We compared multiple sleep parameters from the first night when the device was issued to all subsequent nights on both weekdays and weekends separately;
- The study findings are limited to healthy adolescents and may not be generalizable to other age groups, countries or specific patient populations.

Introduction

Polysomnography (PSG) is the gold standard method for the accurate determination sleep and its quality. Laboratory PSG involves recordings via multiple electrodes and sensors that may disturb sleep and the recording my not reflect usual sleep. A key criticism of PSG is the "first night effect"[1]. Agnew and colleagues observed a reduction in total sleep time, alterations to sleep architecture as well as poorer sleep quality on the first night of PSG assessment compared to the second[1]. It is purported that multiple factors are responsible for these observations, including both the equipment and the environment[2]. Furthermore, more recent evidence suggests these effects may extend beyond the first night[2].

The importance of sleep pertaining to health is increasingly recognised. Thus, establishing methods to accurately measure sleep, in the absence of a sleep laboratory, has been a recent focus. Advances in technology have made it possible to monitor sleep over prolonged periods in the absence of a laboratory. Sleep researchers are frequently utilising wrist actigraphy to monitor sleep-wake activity across multiple days/nights. It has been recommended that five nights of actigraphy data be required to enable an accurate representation of sleep[3]. It is possible, although currently unknown, if there is a 'first night effect' for wrist actigraphy when used to monitor sleep, as observed in PSG. If this effect does exist for wrist actigraphy, this has important implications for the design of future studies and data analysis. The psychosocial aspects of recruitment to a study involving wrist actigraphy for sleep monitoring may subsequently influence sleep behaviour on the first night and beyond, before adaptation is achieved. We therefore sought to examine potential differences between multiple sleep parameters between the first weekday night the wrist

actigraphy was worn compared to subsequent weekday nights in a large sample of adolescents.

Methods

Nine secondary schools, from the Midlands region of the UK, were approached to participate in the Midlands Adolescents Schools Sleep Education Study (MASSES). Eight schools agreed to take part and were recruited to the study. Details of the MASSES have been previously described[4,5]. In brief, parents of students registered in year 7 or year 8 of participating schools were sent a letter regarding study participation during the first term of the 2011/12 academic year. There was an 80% parental response rate and 892 adolescents were eligible for study participation. Adolescent participants were included in the study if they had parental consent, provided personal assent, did not have a diagnosed sleep disorder, were not taking sleep medication, or had not travelled to a different time zone four weeks prior to data collection. All participants were aged between 11-13 years and registered in UK education at baseline. Study assessments were performed at baseline and annually thereafter for two years. Ethical approval was granted from the University of Birmingham Research Ethics Committee (ERN_08-437).

Of the total sample, students were randomly selected to wear wrist actigraphy (GT3X+, The ActiGraph, FL, USA) at each annual assessment (n=332 at baseline, n= 324 at 1-year follow up and n=236 at 2-year follow up). Participants were instructed to wear the actigraph on their non-dominant wrist for seven consecutive days/nights. Data were downloaded using the manufacturers software (ActiLife, Version 6) and

sleep was automatically scored according to pre-defined algorithms[6]. Multiple sleep parameters were derived for each 24-hour period that the device collected data. These included 1) total sleep time (minutes); 2) wake after sleep onset (minutes); 3) sleep efficiency (%); 4) sleep onset latency (minutes); 5) number of awakenings; 6) length of awakenings (minutes); 7) time of sleep onset. We compared all of the mean sleep variables obtained from the wrist actigraphy, comparing those who were administered with the device on the first weekday (Monday), to all other subsequent weekdays (Tuesday, Wednesday, Thursday) that the device was worn. We further explored potential differences in sleep outcomes for when adolescents were administered the device on a Tuesday compared to subsequent consecutive nights (Wednesday and Thursday). We then examined possible differences between those issued with wrist actigraphy on a Wednesday and compared sleep outcomes on the next day (Thursday). Lastly, we assessed significant differences in sleep variables between when the device was issued on a Friday and compared these data to Saturday night to determine potential 'first night effect' for weekend nights.

Statistical analysis

Analysis was performed using Stata (StataCorp. 2013) on all available data. Data are summarized as means (standard error), unless otherwise stated. To compare sleep variables between 2 different nights, Paired t tests were used for normally distributed variables (total sleep time & number of awakenings) and Wilcoxon sign rank test for skewed variables (wake after sleep onset, sleep efficiency, sleep onset latency & length of awakenings). Normality was assessed using Kolmogorov–*Smirnov* test. P values <0.05 were considered to be significant.

Results

At baseline, the mean age of the total sample was 12.0±0.7years (42.4% boys). Table 1 highlights the mean (standard error) of all sleep features obtained from the wrist actigraphy across all weekdays. We show comparisons between sleep parameters when participants were administered wrist actigraphy on the first weekday (Monday) and compare these data to the subsequent weekday nights (Tuesday, Wednesday, Thursday). No significant differences were observed for any sleep measure between Monday (first night) with any other weekdays (Tuesday, Wednesday or Thursday), where $p \geq 0.05$.

Table 2 shows comparisons between sleep variables for the first night of issue (Tuesday) and subsequent weekdays (Wednesday and Thursday). There were no significant differences in any sleep variable between the first night and the two subsequent nights that the actigraphy was worn.

The potential of a 'first night effect' was also assessed when participants were issued the wrist actigraphy on a Wednesday and sleep variables were compared to the next night (Thursday). Comparisons between all sleep features and these days are depicted in Table 3, which highlights no significant differences for any sleep variable assessed, where $p \geq 0.05$ for all.

We also report on differences in sleep outcomes when the first night was Friday (weekend) and compared these to the other weekend night (Saturday), see Table 4. We observed a significant difference in average total sleep time on the first night

(Friday) compared to the second night (Saturday), 486±5 and 469±6 minutes, respectively (p=0.01).

Discussion

We present novel findings from the first study to examine the possibility of a 'first night effect' for wrist actigraphy used to monitor multiple sleep outcomes. We found no evidence of a 'first night effect' for any of the seven sleep features derived from actigraphy in a large sample of adolescents. The only significant difference observed was for total sleep time at the weekend when the first night (Friday) was, on average, 17 minutes longer compared to the second weekend night (Saturday).

Acebo and colleagues previously recommended at least five nights of valid actigraphy data is required for an accurate representation of sleep in paediatric populations[3]. Up until now, it has remained unclear if the first night of actigraphy data can be used for sleep analysis or disregarded due to the possibility of the 'first night effect'. Clearly, if wrist actigraphy demonstrates a 'first night effect' then this would have important implications for future study design development, which includes actigraphy to monitor sleep as well as data analysis plans. We found no evidence, however, to support this phenomenon for wrist actigraphy used to monitor sleep over prolonged periods of time in adolescents without a sleep disorder.

Multiple studies have highlighted evidence to support the 'first night effect' for PSG in patient populations[7-10], healthy individuals[2,10-12] and in pediatrics[13,14]. A variety of possible explanations for this phenomenon have been linked to the environment as

well as the equipment. PSG, unless portable, requires the individual to sleep in an unfamiliar environment (sleep laboratory), where different conditions are likely to be encountered. These include external noises, different light exposure and room temperature, bed comfort and more. Given that wrist actigraphy permits the individual to sleep in their natural environment, these external alterations that may influence sleep outcomes are automatically controlled for and are therefore overcome. Thus, the possibility of a 'first night effect' for wrist actigraphy to monitor sleep is not likely to occur due to the environment, but rather the equipment.

PSG is an intrusive technique, which has been shown to alter multiple sleep outcomes on the first night of sleep assessment as compared to subsequent nights[1,2,7-14]. Reasons for this include restriction of movement and awkwardness about the equipment, which may cause unrest and hyperarousal as a result of the PSG equipment. Despite wrist actigraphy being much less invasive than PSG, the presence of equipment to monitor sleep may alter sleep outcomes through physical and psychological effects. For example, the knowledge of being entered into a research study where sleep is monitored using wrist actigraphy may subsequently influence sleep behaviours via psychological mechanisms. Wearing the device may result in individuals purposely intending to alter sleep behaviours (until acclimatisation is achieved), to either distort the data or have more socially desirable sleep outcomes, knowing that sleep is being investigated. If these intentional sleep behaviour alterations occur then this will result in misrepresentation of the sleep outcomes assessed. The physical presence of wrist actigraphy may paradoxically contribute to distorted sleep alterations due to discomfort, particularly if the individual does not habitually sleep while wearing a wrist device. Furthermore, depending on the type of actigraphy and the initialisation

process, some can emit light to indicate that the device is collecting data, which is likely to cause sleep disruption. The aforementioned are arguments that support the potential 'first night effect' for wrist actigraphy for sleep assessment. Contrary to PSG findings, our wrist actigraphy data showed no evidence to support a 'first night effect'. Factors relating to the sleep environment and equipment, which may be responsible for differences between the first night and subsequent nights of PSG assessment were not shown to influence subsequent sleep behaviour in adolescents wearing wrist actigraphy.

The findings from our study indicated a significant difference for total sleep time at the weekend when the first night was a Friday, compared to the Saturday. The difference, although statistically significant, was an average of just 15 minutes more on the first night. This can be explained by the possibility of accumulated weekday sleep debt, which is repaid at the first opportunity (e.g. the first weekend night). Fifteen minutes of additional sleep is not, however, likely to compensate for the amount of sleep debt that a typical adolescent accrues across the school week. For example, Kim and colleagues found that adolescents sleep, on average, 2 hours and 42 minutes less on weekdays compared to weekend nights[15]. On closer inspection, there was no evidence of weekend catch-up sleep in our adolescent cohort where average sleep quantity and quality did not differ during the week compared to weekends.

Whilst our study is the first to investigate the possibility of a 'first night effect' for wrist actigraphy on sleep outcomes, we acknowledge several limitations. Firstly, we combined data from participants across three study time points. Repeated exposure to

wrist actigraphy may have resulted in participants becoming more acclimatised to wearing the device during the second and third assessment. However, wrist actigraphy was administered annually at three time points, diminishing possible acclimatisation to the device. Secondly, our findings may not be generalizable to other populations. For example, different age groups and patient populations, particularly those with sleep disorders, may exhibit different patterns and could be the focus of future studies. Finally, whilst we recognise that multiple types of wrist actigraphy have been validated against PSG for multiple sleep outcomes in many groups,[16-30] not all sleep parameters included in our study have been validated. It remains a possibility that some differences may be present between the first night and subsequent nights according to actigraphy type/manufacture, given that multiple devices are now available.

In conclusion, we found no evidence to support a 'first night effect' for wrist actigraphy to monitor sleep in a large cohort of adolescents. The first night of actigraphy data collected to measure sleep should be included and not disregarded at this stage. However, further efforts are required to determine if these findings are consistent in different age groups, patient populations and with the application of different types of wrist actigraphy that are currently available and utilised for sleep research.

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Author contributions

TA and ST made substantial contributions to the conception and design of the study. TA acquired the study data. OMO performed data analysis. All authors contributed to interpretation of the study results. TA wrote the first draft and the other two authors critically revised it for important intellectual content. All authors have approved the final version of the manuscript and agree to be accountable for all aspects of the work performed.

Competing Interests

No, there are no competing interests

Data Sharing

No additional data available.

Table 1: Weekday differences in sleep features between Monday (first night) compared to all other weekdays

Sleep measure	Monday (n=95)	Tuesday (n=94)	p value	Wednesday (n=94)	p value	Thursday (n=93)	p value
TST (minutes) [a]	440 (5)	437 (7)	0.65	443 (10)	0.77	449 (10)	0.32
WASO (minutes) [b]	69 (45)	71 (55)	0.88	67 (59)	0.72	67 (44)	0.98
SE (%) [b]	86.1 (9.3)	86.3 (10.5)	0.84	87.1 (11.6)	0.86	86.5 (9.6)	0.70
SOL (minutes) [b]	2 (4)	1 (4)	0.63	2 (4)	0.36	2 (4)	0.82
Number of awakenings [a]	23 (1)	22 (1)	0.29	21 (1)	0.07	21 (1)	0.05
Length of awakenings (minutes) [b]	3 (1)	3 (1)	0.12	3 (2)	0.34	3 (1)	0.11
Sleep onset time		S: 9.1% L: 46.6% E: 44.3%		S: 10.2% L: 54.6% E: 35.2%		S: 9.1% L: 52.3% E: 38.6%	

Data are presented as: [a] mean (standard error), paired t test; [b] median (interquartile range), Wilcoxon Signed-rank test.
TST=total sleep time; WASO=wake after sleep onset; SE=sleep efficiency; SOL=sleep onset latency;
S=Within 30 minutes of Monday; L=more than 30 minutes later than Monday; E=more than 30 minutes earlier than Monday.

Table 2: Weekday differences in sleep features between Tuesday (first night) and all other weekdays

Sleep measure	Tuesday (n=161)	Wednesday (n=159)	p value	Thursday (n=159)	p value
TST (minutes) [a]	452 (5)	462 (5)	0.06	446 (4)	0.28
WASO (minutes) [b]	64 (39)	60 (43)	0.20	58 (44)	0.26
SE (%) [b]	87.4 (7.1)	88.1 (6.9)	0.07	87.7 (8.6)	0.32

SOL (minutes) [b]	2 (4)	2 (4)	0.38	2 (4)	0.31
Number of awakenings [a]	22 (1)	21 (1)	0.33	22 (1)	0.83
Length of awakenings (minutes) [b]	3.0 (1.1)	2.8 (1.3)	0.16	2.8 (1.3)	0.06
Sleep onset time		S: 15.8% L: 47.5% E: 36.7%		S: 12.2% L: 54.0% E: 33.8%	

Data are presented as: [a] mean (standard error), paired t test; [b] median (interquartile range), Wilcoxon Signed-rank test.
TST=total sleep time; WASO=wake after sleep onset; SE=sleep efficiency; SOL=sleep onset latency;
S=Within 30 minutes of Tuesday; L=more than 30 minutes later than Tuesday; E=more than 30 minutes earlier than Tuesday.

Table 3: Weekday differences in sleep features between Wednesday (first night) compared to Thursday

Sleep measure	**Wednesday (n=185)**	**Thursday (n=188)**	**p value**
TST (minutes) [a]	442 (4)	437 (4)	0.20
WASO (minutes) [b]	68 (43)	65 (46)	0.40
SE (%) [b]	86.1 (9.3)	87.1 (7.5)	0.54
SOL (minutes) [b]	2 (4)	2 (4)	0.38
Number of awakenings [a]	24 (1)	22 (1)	0.05
Length of awakenings (minutes) [b]	3 (2)	3 (1)	0.96
Sleep onset time		S: 9.4% L: 52.4% E: 38.3%	

Data are presented as: [a] mean (standard error), paired t test; [b] median (interquartile range), Wilcoxon Signed-rank test.

TST=total sleep time; WASO=wake after sleep onset; SE=sleep efficiency; SOL=sleep onset latency;
S=Within 30 minutes of Wednesday; L=more than 30 minutes later than Wednesday; E=more than 30 minutes earlier than Wednesday.

Table 4: Weekend differences in sleep features between Friday (first night) and Saturday

Sleep measure	Friday (n=169)	Saturday (n=168)	p value
TST (minutes) [a]	486 (5)	469 (6)	*0.01*
WASO (minutes) [b]	73 (52)	72 (41)	0.50
SE (%) [b]	85.9 (7.7)	86.1 (8.1)	0.84
SOL (minutes) [b]	3 (4)	2 (4)	0.19
Number of awakenings [a]	25 (1)	25 (1)	0.86
Length of awakenings (minutes) [b]	3 (1)	3 (1)	0.39
Sleep onset time		S: 8.5% L: 61.5% E: 30.0%	

Data are presented as: [a] mean (standard error), paired t test; [b] median (interquartile range), Wilcoxon Signed-rank test.
TST=total sleep time; WASO=wake after sleep onset; SE=sleep efficiency; SOL=sleep onset latency;
S=Within 30 minutes of Friday; L=more than 30 minutes later than Friday; E=more than 30 minutes earlier than Friday.

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STROBE 2007 (v4) Statement—Checklist of items that should be included in reports of *cohort studies*

Section/Topic	Item #	Recommendation	Reported on page #
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract	2
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found	2
Introduction			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	3
Objectives	3	State specific objectives, including any prespecified hypotheses	3 & 4
Methods			
Study design	4	Present key elements of study design early in the paper	4
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	4
Participants	6	(*a*) Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up	4
		(*b*) For matched studies, give matching criteria and number of exposed and unexposed	N/A
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	4 & 5
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	4
Bias	9	Describe any efforts to address potential sources of bias	4
Study size	10	Explain how the study size was arrived at	4
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	5
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding	5
		(*b*) Describe any methods used to examine subgroups and interactions	N/A
		(*c*) Explain how missing data were addressed	5
		(*d*) If applicable, explain how loss to follow-up was addressed	N/A
		(*e*) Describe any sensitivity analyses	N/A
Results			

Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	4
		(b) Give reasons for non-participation at each stage	N/A
		(c) Consider use of a flow diagram	N/A
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	4 & 6
		(b) Indicate number of participants with missing data for each variable of interest	N/A
		(c) Summarise follow-up time (eg, average and total amount)	4
Outcome data	15*	Report numbers of outcome events or summary measures over time	N/A
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	6 & 7 & Tables 1-4
		(*b*) Report category boundaries when continuous variables were categorized	N/A
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	N/A
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	N/A
Discussion			
Key results	18	Summarise key results with reference to study objectives	7-10
Limitations			
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	9 & 10
Generalisability	21	Discuss the generalisability (external validity) of the study results	10
Other information			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	1 & 10

*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at www.strobe-statement.org.

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STROBE 2007 (v4) Statement—Checklist of items that should be included in reports of *cohort studies*

Section/Topic	Item #	Recommendation	Reported on page #
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract	2
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found	2
Introduction			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	3
Objectives	3	State specific objectives, including any prespecified hypotheses	3 & 4
Methods			
Study design	4	Present key elements of study design early in the paper	4
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	4
Participants	6	(*a*) Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up	4
		(*b*) For matched studies, give matching criteria and number of exposed and unexposed	N/A
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	4 & 5
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	4
Bias	9	Describe any efforts to address potential sources of bias	4
Study size	10	Explain how the study size was arrived at	4
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	5
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding	5
		(*b*) Describe any methods used to examine subgroups and interactions	N/A
		(*c*) Explain how missing data were addressed	5
		(*d*) If applicable, explain how loss to follow-up was addressed	N/A
		(*e*) Describe any sensitivity analyses	N/A
Results			

Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	4
		(b) Give reasons for non-participation at each stage	N/A
		(c) Consider use of a flow diagram	N/A
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	4 & 6
		(b) Indicate number of participants with missing data for each variable of interest	N/A
		(c) Summarise follow-up time (eg, average and total amount)	4
Outcome data	15*	Report numbers of outcome events or summary measures over time	N/A
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	6 & 7 & Tables 1-4
		(*b*) Report category boundaries when continuous variables were categorized	N/A
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	N/A
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	N/A
Discussion			
Key results	18	Summarise key results with reference to study objectives	7-10
Limitations			
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	9 & 10
Generalisability	21	Discuss the generalisability (external validity) of the study results	10
Other information			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	1 & 10

*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at www.strobe-statement.org.