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REVIEW

Biomarkers of cardiovascular risk in sleep-deprived people

MA Miller and FP Cappuccio

The global increase in both coronary heart disease (CHD) and cardiovascular disease (CVD) and the associated increase in undetected subclinical cardiovascular pathology highlight the continuing need for improved risk prediction. Traditional risk factors fail to identify all 'at-risk' individuals. Although new risk factors, associated with endothelial function, inflammatory and oxidative stress pathways, for example, have been identified, studies have often observed only minimal improved risk classification when such markers are added. We examine the emerging evidence that short sleep may be a risk factor for obesity, type 2 diabetes and hypertension, and an independent predictor of stroke, CHD and CVD. We examine the underlying mechanisms and the evidence to suggest that short sleep may modulate the association between established factors and CVD. We consider whether the levels of markers of obesity and appetite control, energy metabolism, glucose homoeostasis, inflammation, thrombosis and haemostasis, which are affected by short duration of sleep, might be useful predictors of the risk of developing CVD. Finally, the usefulness of such markers for disease detection, management and prevention is considered.

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Keywords: sleep; coronary heart disease; cardiovascular disease; biomarker; inflammation

INTRODUCTION

The use of biomarkers for the prediction of coronary heart disease (CHD) and cardiovascular disease (CVD) risk has been a subject of debate. Conventional risk factors have failed to identify all individuals who will subsequently develop CVD. More recently, investigators have examined the possibility of using a combination of biomarkers to improve detection. In a recent analysis from the Woman's Health Initiative trial,[1] among a panel of 18 markers, five (interleukin-6 (IL-6), D-dimer, factor VIII, homocysteine and von-Willebrand factor (vWF)) were significantly associated with CHD following adjustment for conventional risk factors, whereas high-sensitivity C-reactive peptide (hs-CRP), which has been shown in many epidemiological studies to predict cardiovascular outcomes,[2] was only of borderline significance ($P = 0.08$). Inclusion of the five significant risk factors, however, led to a 6.45% improvement in risk classification. There are many important issues, which need to be taken into consideration when assessing the utility of a biomarker. These include cost, measurement specificity and precision, and applicability to different groups (for example, different ethnic or gender groups). Sleep quantity is also associated with variations in the level of cardiovascular risk markers, including IL-6, hs-CRP and vWF,[3,4] the latter offering plausible mechanisms of actions to explain the risk associations. Differences in sleep quantity and quality may therefore be either causal factors or important modifiers of the association between biomarkers of cardiovascular risk and observed cardiovascular events. It is of great interest to consider the recent evidence for the role of short duration of sleep as a risk factor for cardiovascular events.[5–8] In this short review, we examine the observed relationship between quantity and quality of sleep and CVD, the underlying mechanisms and the utility of the measurement of both sleep and of various molecules within these pathways as possible future cardiovascular biomarkers.

SHORT SLEEP AND INCREASED RISK OF ADVERSE HEALTH OUTCOMES

A variety of cultural, social, psychological, behavioural, pathophysiological and environmental factors influence an individual's sleep pattern and associated quantity and quality of sleep. In recent times, we have seen the introduction of longer working hours, more shift work and 24/7 availability of commodities. These changes have been paralleled by secular trends of curtailed duration of sleep to fewer hours per day across westernised populations.[9] Moreover, there is now accumulating evidence to suggest that short sleep is associated with self-reported ill health[10] and a number of adverse health outcomes.[11] A summary of the outcomes from a number of recent meta-analyses[5–8,12,13] is shown in Table 1. These meta-analyses include a large number of participants and cases or events. In prospective studies, self-reported short duration of sleep is associated with an increased risk of developing or dying from CHD, stroke and total CVD. There is also an increased risk of developing type 2 diabetes. In adults and children, there is also evidence from cross-sectional studies, and some prospective study, that short sleep is associated with an increased risk of obesity.[5,14–16] Finally, a variety of studies do indicate a significant risk of developing hypertension in short sleepers,[17–22] particularly in relation to a reduction of slow-wave sleep.[23]

MECHANISMS

The association between chronic short sleep and the observed adverse health outcomes may be explained by different possible mechanisms. Short-term sleep-deprivation studies have shown that a lack of sleep is associated with changes in appetite control, insulin resistance, glucose homoeostasis, endothelial function, sympathetic nervous system activation and inflammatory and haemostatic pathways. Sleep deprivation is also associated with a

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Table 1. Meta-analyses of the risks associated with short duration of sleep and cardio-metabolic risk factors and cardiovascular outcomes in population studies worldwide

Author (year)	*Outcome*	*Number of participants*	*Number of events*	*Relative risk (95% CI)*	*Combined effect (P)*
Prospective studies					
Gallicchio and Kalesan (2009)	All-cause mortality	1 082 457	106 833	1.10 (1.06–1.15)	
Cappuccio *et al.* (2010)	All-cause mortality	1 381 324	112 324	1.12 (1.06–1.18)	<0.01
Cappuccio *et al.* (2011)	CHD	249 324	4141 (fatal and non-fatal)	1.48 (1.22–1.80)	<0.0001
Cappuccio *et al.* (2011)	Stroke	210 978	3478 (fatal and non-fatal)	1.15 (1.00–1.31)	<0.047
Gallicchio and Kalesan (2009)	CVD	105 192		1.06 (0.94–1.18)	
Cappuccio *et al.* (2011)	CVD	222 253	8250	1.03 (0.93–1.15)	0.52
Cappuccio *et al.* (2010)	Type 2 diabetes	90 623	3079	1.28 (1.03–1.60)	0.024
Cross-sectional studies					
Cappuccio *et al.* (2008)	Obesity	29 502	Age 2–20	1.89 (1.46–2.43)	<0.001
Cappuccio *et al.* (2008)	Obesity	603 519	Age 15–102	1.55 (1.43–1.68)	<0.0001
Chen *et al.* (2008)	Obesity	48 104[a]	Age 3–19	1.58 (1.26–1.98)	<0.001

Abbreviations: CHD, coronary heart disease; CVD, cardiovascular disease. [a]$n = 38\,065$ cross-sectional; $n = 10\,039$ prospective.

30% reduction in the phosphorylation of Akt, an important step in the insulin-signaling pathway, in human adipocytes,[24] supporting the notion that sleep is not an idle state or a sole function of the brain but has wider influence on bodily functions, including metabolism, adipose tissue and cardiovascular function.[25] Although there is evidence to suggest that sleep may be causally associated with the development of obesity,[14,15] a bi-directional pathway may also exist between sleep and obesity[26] and, likewise, between sleep and inflammation. Obese individuals are at increased risk of obstructive sleep apnoea, a condition that is associated with disturbed and short sleep,[27] but it is possible that the associated lack of sleep may also have adverse metabolic effects, leading to further weight gain and glucose dysregulation.[27] Likewise, the innate immune system responds to invading foreign bodies and viral and bacterial antigens by activation of the Toll-like receptor pathways. Initially, this leads to an increase in body temperature and longer periods of slow-wave sleep and reduced wakefulness. However, in advanced stages of inflammation, the sleep-promoting effects are diminished, and reduced non rapid-eye-movement sleep and increased wakefulness may ensue.[28]

MEASUREMENT OF BIOMARKERS OF CARDIOVASCULAR RISK IN SLEEP-DEPRIVED PEOPLE

There are a number of important factors that need to be considered. Often, biomarkers are taken at a single time point, which means they may be confounded by endogenous circadian rhythms and the influence of sleep-wake cycles and by exposure to light, which could affect levels of circulating biomarker. Cortisol, for example, is high in the morning but low at night. Adjusting for the time of day or performing venepuncture at a particular time can help to alleviate this problem, but not all individuals will have the same circadian timing, especially if they are shift workers.[29] When evaluating markers of obesity or inflammation in the context of cardiovascular risk prediction, the acute and time-course reciprocal relationships need to be considered. Likewise, the associations between traditional risk factors and sleep need to be considered when evaluating the efficacy of individual risk markers and risk equations so as to remove potential confounding. For example, men are twice as likely to develop obstructive sleep apnoea as women, and the menopause may be a risk factor for sleep-disordered breathing. Lipoprotein lipase, a key enzyme involved in the hydrolysis of triglycerides, is reduced in obstructive sleep apnoea patients. Increased exercise reduces cardiovascular risk and is considered as a non-pharmacological treatment for sleep disorders. The time of day when the exercise occurs is important as it can affect cytokine levels that in turn may inhibit sleep if it occurs late in the day.

MEASUREMENT OF SLEEP

The recognised gold-standard for the measurement of sleep is polysomnography as this provides an objective assessment of the sleep-wake cycle over the entire sleep period.[30] However, it is recognised that undertaking detailed sleep studies on large populations is difficult and much of the data collection is limited to subjective self-administered questionnaires or sleep diaries. A simple and cost-effective alternative to polysomnography is to use actigraphy and sleep diaries. Actigraphs are activated by movement and can differentiate when a person is awake or asleep. They are useful in identifying night-time awakenings and for determining their subsequent duration. When used in conjunction with self-recorded sleep diaries, actigraphs can help to establish a very detailed sleep pattern. Alongside the actual sleep measurements, the conditions under which the sleep measurements are obtained are important. Laboratory studies of sleep restriction provide essential insight into potential mechanisms because they are typically conducted in well-controlled environments and involve detailed physiological measurements. These are often short-term, however, and this raises the question as to whether the associations observed in the laboratory persist in the real world when sleep restriction becomes chronic. Observational studies, especially large population-based epidemiological studies, can provide some insight into the associations between sleep and health outside of the laboratory. The detailed recording of naps and confounding factors or mediators, such as depression, can improve the measurements. Likewise, questionnaires administered to a bed partner can also help to establish a diagnosis of sleep-disordered breathing.

POTENTIAL BIOMARKERS

Obesity and appetite control

Short sleep may lead to obesity through the activation of hormonal responses, leading to an increase in appetite and caloric intake. In the first randomized cross-over clinical trial of short-term sleep deprivation, individuals were subjected to an extreme acute sleep deprivation (<4 h per night) for 2 days and compared with 2 days with 10 h of sleep.[31] The caloric intake of these individuals was controlled through a constant glucose infusion. Sleep deprivation was associated with decreased leptin

and increased ghrelin levels.[31] The resulting change in the ghrelin to leptin ratio was associated with a positive change in hunger. This suggests that if the individuals had had access to food, they may have increased their food intake. If this mechanism was activated on a long-term basis, it could lead to the development of obesity. In adults, however, although cross-sectional data support an association between sleep and obesity as yet, there is inconsistent prospective evidence to support this association, but it is possible that sleep duration may confound the observed relationships between body mass index and cardiovascular risk. In children, recent prospective data support the association between sleep and obesity,[14,15] and it is likely that sleep is causally associated with the development of obesity in children. The ultimate proof of principle may be obtained from a randomized trial of the effect of sleep extension on weight. It remains to be determined if the measurement of leptin and ghrelin may prove to be clinically useful as good biomarkers of the risk of development of obesity and associated CVD in short-sleeping children.

Energy metabolism

Studies on the effect of sleep deprivation on energy metabolism in humans come mainly from short-lived severe sleep-deprivation experiments in young volunteers, and as such cannot be directly extrapolated to longer-term effects of sustained sleep curtailment in the general population. In animal models, acute sleep loss leads to changes to the circadian clock and altered metabolism, especially in relation to energy stores.[32] Individuals with sleep problems may have a reduction in their energy expenditure but it remains to be determined whether this is true for all short sleepers.[33] In the Nurses Health Study, short sleep duration was associated with the rate of weight gain with time. However, in this study, there was no evidence to suggest that this resulted from an increase in appetite. It has therefore been suggested that the effects observed may have been the result of changes in energy metabolism.[34] The brain tracks wakefulness via extracellular adenosine triposphate released during neuro- and glio-transmission.[35] Dynamic changes in adenosine triposphate act upon the production of sleep-regulatory substances, which act on known sleep-regulatory circuits, to determine the sleep-wake state of the individual.[36] Further studies are required to determine if the energy metabolism pathways could potentially provide biomarkers of sleep deprivation and to determine if chronic changes in such components are related to CVD risk.

Glucose homoeostasis

A recent meta-analysis has estimated that there is a 28% increased risk of development of type 2 diabetes in short-sleeping individuals (Table 1).[5] There are a number of potential mechanisms that may underlie this association, including those that control appetite, caloric intake and energy expenditure,[37,38] as well as those regulating insulin and glucose.[39] Within these pathways, there are many molecules that are potential biomarkers for the risk of type 2 diabetes, including insulin, glucose and haemoglobin A1c. Positron emission tomography studies[40] suggest that the increase in blood glucose levels in individuals subjected to total sleep deprivation is a result of decreased brain glucose utilisation. Sleep restriction (6 days) has also been associated with an extended duration of elevated night-time growth hormone concentrations,[41] with an increase in evening cortisol levels.[42] Following sleep restriction, elevated evening cortisol concentrations may result in reduced insulin sensitivity the following morning, leading to an additional increase in blood glucose.[43] In the Swedish Obese Subjects cohort, self-reported loud snoring and observed breathing pauses, which are highly suggestive of the presence of obstructive sleep apnoea, were associated with adverse levels of components of the metabolic syndrome, including high blood pressure and fasting insulin.[44]

Markers of sympathetic nervous system activation and endothelial dysfunction

The endothelium is responsible for maintaining vascular tone through the release of various substances, including vasoconstrictors such as Endothelin-1 (ET-1), vasodilators such as nitric oxide (NO) and other biologically active substances such as the soluble adhesion molecules. Endothelial dysfunction is independently associated with increased risk of CVD and has an important role in the development of atherosclerosis.[45] Recent studies have indicated that habitual short-sleep duration is associated with increased ET-1 vasoconstrictor tone.[46] The mechanisms of this relationship, however, are not known. It is possible that short sleep may lead to the production of inflammatory cytokines, which augment ET-1 production,[47] or that chronic short sleep leads to an increase in sympathetic nervous system activity, which leads to an increase in ET-1. Partial sleep deprivation increases sympathetic activation and endothelial dysfunction.[48] Studies indicate that the endothelial dysfunction may precede the increase in sympathetic activity.[49] Shift work, which is associated with increased cardiovascular risk,[50] is also associated with changes in endothelial function and heart rate variability, suggesting that there may be an increase in sympathetic and a decrease in parasympathetic activity in these individuals.[51]

In the early phase of atherosclerosis, inflammatory cells are recruited from the circulation and migrate through the endothelium. This process is predominantly mediated by cellular adhesion molecules, which are expressed on the vascular endothelium. The inflammatory response to sleep deprivation is discussed in more detail below.

Inflammatory markers

Short-term sleep deprivation is associated with an increase in hs-CRP,[52] but to date, the potential impact of chronic sleep deprivation on the inflammatory system, immune responses and cardiovascular risk markers has only been examined in a limited number of studies. In the Whitehall II study, we examined the relationship between sleep and markers of inflammation in over 4000 individuals. There were no overall linear or non-linear trends between sleep duration and IL-6 after multiple adjustments. However, in women but not men, the levels of IL-6 tended to be lower in individuals who slept 8 h as compared with 7 h per night.[3] These results are in contrast with the Cleveland family study in which increasing sleep was associated with an increase in IL-6.[53]

Significant gender differences in the association with hs-CRP and sleep were also observed in the Whitehall II study. Interestingly, although no significant association between hs-CRP and sleep was observed in men, there was a significant non-linear association in women.[3] The observed gender interactions in the relationship between sleep and inflammation may account for why Taheri *et al.*[54] failed to find an association. In the latter study, the number of females studied was much smaller, and a gender-adjusted analysis, as opposed to a sex-stratified analysis, was used. To fully investigate possible temporal relationships between short sleep and markers of inflammation in both male and female individuals, further large longitudinal studies are required.

Other factors that might affect the observed associations between sleep and markers of inflammation include the effect of sampling time on the measured markers. In the Whitehall II study, we investigated the effect of sampling time and demonstrated that there was no major effect of time on these levels over the observed sampling time period. The proportion of the variability due to 'time of sampling' for hs-CRP was 0.03% and for IL-6 was 0.3%.[3]

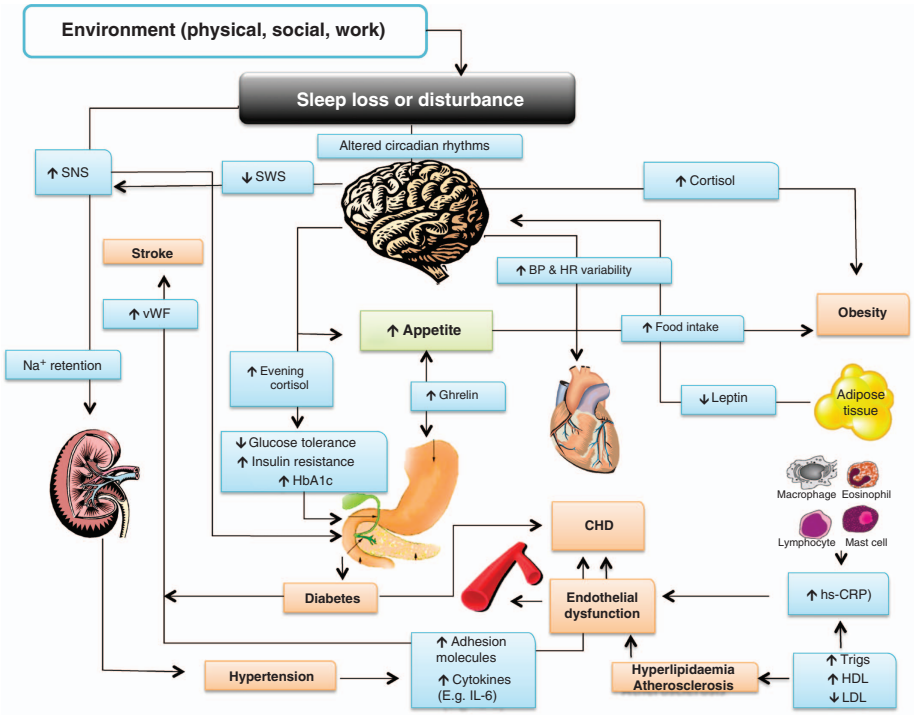

Figure 1. Possible mechanistic pathways linking short duration of sleep and adverse cardiovascular health. BP, blood pressure; HbA1c, haemoglobin A1c; HDL, high-density lipoprotein cholesterol; HR, heart rate; LDL, low-density lipoprotein cholesterol; PAI-1, plasminogen activator inhibitor-1; SNS, sympathetic nervous system; SWS, slow-wave sleep; Trigs, triglycerides.

There are a number of factors that need to be considered before inflammatory markers can be used as biomarkers for short-sleep duration and associated cardiovascular risk. In particular, in some studies, a non-linear 'U'-shaped relationship between sleep duration and the level of the inflammatory marker has been observed. This phenomenon was observed in the association between sleep and hs-CRP and in women participants of the Whitehall II study.[3] However, the mechanisms underlying the association between short sleep and markers of inflammation are likely to differ from those possibly explaining the association between long sleep and some markers of inflammation. Factors such as specificity, feedback mechanisms, diurnal rhythm, stability of marker with storage and the accessibility of marker for measurement along with its precision and accuracy of measurement are all important sources of variation. Likewise, possible confounding factors (for example, age, sex, ethnicity, alcohol, smoking, exercise, socio-economic status, marital status, pre-existing disease and so on) need to be considered when interpreting these findings. The acute effect on the inflammatory marker levels of undiagnosed infections present at the time of sampling must be considered, and ultimately the cost of the measurement will also determine its utility.

Thrombosis and haemostasis

Endothelial activation can lead to activation of haemostatic, coagulation and thrombotic pathways, which may lead to the formation of a thrombus, impaired blood flow and possibly a stroke or a coronary event. In the Whitehall II study, we examined the relationship between self-reported sleep duration and three important factors in these pathways (vWF, fibrinogen and factor VII) in approximately 6400 individuals. A significant gender interaction was observed for vWF: following multiple adjustments, vWF levels were significantly higher in men with both short sleep duration ($\leqslant$6 h per night) and long sleep duration ($\geqslant$8 h per night) as compared with those who slept 7 h ($P<0.05$ for both). In women, irrespective of menopausal status, levels of vWF were significantly higher in individuals who slept 8 h or longer compared with 7 h. This difference was observed in pre-menopausal and post-menopausal women. In women, the association was non-linear ($P=0.02$), but not in men ($P=0.09$). No statistically significant associations between sleep duration and fibrinogen or factor VII were observed.[4] Longitudinal studies are required to investigate possible causality but it is of interest that, in a recent study, the risk of developing or dying of stroke among short sleepers and long sleepers is increased.[8] Moreover, increased levels of vWF have been previously shown to be associated with an increased risk of CHD.[1]

PERSPECTIVES

Sleep is a fundamental and natural process, and yet the exact purpose of sleep and its effects on health and disease are poorly understood. However, in recent years, researches in the fields of chronobiology, physiology, biochemistry, metabolism, nutrition and clinical epidemiology have begun to depict strong and complex links between short duration of sleep and cardiovascular (ill) health (Figure 1). Further research is required to fully investigate the mechanisms underlying the relationship between sleep and both CHD and CVD and to determine if markers of short sleep might be useful markers of cardiovascular risk and whether sleep may confound the association between known biomarkers of CVD and risk prediction. This might be of particular importance in that it might help explain, at least in part, gender differences in CVD risk. Although it is now recognised that sleep disorders and sleep deprivation are important determinants of morbidity and mortality, and despite the recent advances made by sleep and circadian science in recent years, it is recognised that a simple, accurate and easy-to-measure biomarker of sleepiness still remains a major challenge.[55] Nevertheless, individuals are currently being recruited into the first randomised clinical trial of the effect of sleep extension.[56] In this study, the feasibility of increasing sleep duration to a healthy length (approximately

71/2 h) is being assessed. In addition, the effect of sleep extension on body weight and both endocrine (leptin and ghrelin) and immune (cytokines) parameters is being determined, along with the prevalence of metabolic syndrome. Such proofs of concept studies are required to determine if alteration in sleep and associated physiological and metabolic markers might have beneficial effects on health.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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