Heart, Lung and Circulation (2016) **25,** 1218–1225
1443-9506/04/$36.00
http://dx.doi.org/10.1016/j.hlc.2016.04.012

ORIGINAL ARTICLE

Non-Dipping and Cardiometabolic Profile: A Study on Normotensive Overweight Middle-Aged Men

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Received 14 December 2015; received in revised form 16 March 2016; accepted 8 April 2016; online published-ahead-of-print 17 May 2016

Background We aimed to assess insulin sensitivity and other metabolic features of dippers and non-dippers among overweight middle-aged men.

Methods We studied 73 men (45.8 ± 5.3 years) who were overweight but normotensive. Participants were separated into dippers and non-dippers based on the magnitude of the nocturnal decline of blood pressure, with dippers experiencing an overnight decline ≥10% as per standard definition. Our study included 51 dippers and 22 non-dippers. All participants underwent 24-hour ambulatory blood pressure monitoring. Insulin sensitivity was assessed by the Matsuda method from an oral glucose tolerance test; other assessments included carotid artery intima-media thickness (CIMT), body composition derived from dual-energy X-ray absorptiometry, lipid profiles, and a physical activity questionnaire.

Results Non-dippers had lower daytime systolic (-5.0 mmHg; p = 0.022) and diastolic (-3.3 mmHg; p = 0.035) blood pressure than dippers. Conversely, during sleep, non-dippers had higher systolic (+6.5 mmHg; p = 0.003) and diastolic (+5.6 mmHg; p = 0.001) blood pressure. In continuous associations, increasing CIMT was associated with decreasing systolic (p = 0.012) and diastolic (p = 0.042) dipping. Thus, non-dippers had CIMT that was 9% greater than that of dippers (749 vs 820 µm; p = 0.036). Importantly, there was no association between non-dipping status or the magnitude of the nocturnal dip with insulin sensitivity.

Conclusions Non-dippers had lower blood pressure in the daytime, but higher blood pressure in the night time compared to dippers. Non-dippers had increased CIMT, which suggests that normotensive men with a non-dipping ambulatory blood pressure profile may be at increased cardiovascular risk. However, it appears that the non-dipping profile is unrelated to dysfunction of glucose homeostasis in overweight normotensive men.

Keywords Dipping • Systolic • Diastolic • Insulin sensitivity • Carotid artery intima-media thickness

Introduction

The role of hypertension in the development of atherosclerotic cardiovascular disease has long been recognised [1]. The advent of 24-hour ambulatory blood pressure monitoring has allowed the assessment of the circadian profile and, in particular, the nocturnal blood pressure and the changes that occur during sleep [2].

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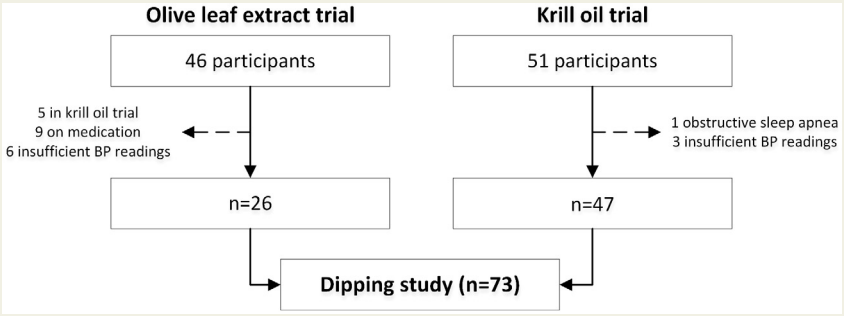

Figure 1 Summary of study recruitment. All participants from the olive leaf extract [31] and krill oil [32] trials were overweight middle-aged men recruited in Auckland, New Zealand.

Nocturnal hypertension is associated with greater arterial stiffness [3], carotid artery intima-media thickness [3], and urinary albumin excretion [4], as well as excessive inotropic response to exercise [3]. These translate to greater risk of cardiovascular events and mortality in association with increased nocturnal blood pressure in population cohorts [5–7], participants with hypertension [5,7–10], and even those with isolated nocturnal hypertension [8]. Importantly, the nocturnal blood pressure is more strongly predictive of cardiovascular events than daytime blood pressure [5,11].

A related measure, the magnitude of the nocturnal decline of blood pressure (i.e. dipping) is also an important cardiovascular risk factor, which is independent of the average blood pressure [12]. Non-dippers (whose blood pressure does not decrease by 10% or more at night) who are hypertensive have greater cardiovascular mortality [7,12–16], greater prevalence of left ventricular hypertrophy [17–19], and increased carotid intima-media thickening [17]. Further, non-dipping and nocturnal hypertension have an additive effect on cardiovascular risk and end-organ damage [4]. Importantly, even in normotensive individuals, a non-dipping pattern is associated with greater urinary albumin excretion [20], elevated left ventricular mass index [20,21], and increased rate of cardiovascular events [13,22–24].

Essential hypertension and other aspects of the metabolic syndrome share a common cause: pathological reduction of insulin sensitivity (insulin resistance) with hyperinsulinaemia [25–27]. However, while there is evidence to suggest that the non-dipping pattern is associated with dysfunction of the autonomic nervous system [28–30], the relationship between insulin sensitivity and dipping remains unclear. Thus, we aimed to assess insulin sensitivity and other metabolic features of dippers and non-dippers in a cohort of overweight middle-aged men.

Methods

Ethics Approval

Ethics approval was provided by the Central and Northern Y Regional Ethics Committees (Ministry of Health, New Zealand). Written and verbal informed consent was obtained from all participants. This study was performed in accordance with all appropriate institutional and international guidelines and regulations for medical research, in line with the principles of the Declaration of Helsinki.

Participants and Recruitment

Participants were recruited for two clinical trials investigating the metabolic effects of nutritional supplementation (ACTRN12610001082099 [31] and ACTRN12611000602921 [32]; Figure 1). This study encompasses a *post hoc* analysis of their pre-trial baseline data.

Volunteers were recruited in 2011 and 2012 using advertisements in local newspapers that circulate freely in the central Auckland metropolitan area (New Zealand). Men who were overweight (body mass index (BMI) 25–30 kg/m^2) and middle-aged (35–55 years) were eligible to participate. Only males were recruited to the clinical trials to avoid the effects of the menstrual cycle and/or oral contraceptives on insulin sensitivity (the primary outcome). Exclusion criteria included obstructive sleep apnoea, diabetes mellitus, pre-diagnosed hypertension or elevated clinic blood pressure at the time of recruitment (systolic blood pressure >145 mmHg or diastolic blood pressure >95 mmHg), known dyslipidaemia (formal diagnosis or current treatment with lipid-lowering drugs), tobacco use, or intake of medication likely to affect blood pressure, lipid profile, or insulin sensitivity. Note that a relatively high 'clinic' blood pressure cut-off was used as the clinical trials aimed to enrol participants with mild metabolic dysfunction. From this group, all participants born at term (37–41 weeks of gestation) from singleton pregnancies were included. Five subjects participated in both trials, and only the data from the most recent trial were used (Figure 1).

Clinical Assessments

Insulin sensitivity was assessed via a 75 g oral glucose tolerance test using the Matsuda method, with glucose and insulin samples collected at 0, 30, 60, 90, and 120 minutes [33]. The Matsuda index has a strong correlation with the hyperinsulinaemic euglycaemic clamp ($r = 0.77$) [34], and excellent reproducibility during multiple measures [35].

Lipid profile (triglycerides, total cholesterol, HDL-C, and LDL-C) was assessed from fasting blood samples. Highly

sensitive C-reactive protein (hsCRP) was also assessed. Height was measured using a Harpenden stadiometer. Weight and body composition were examined using whole-body dual-energy X-ray absorptiometry (DXA, Lunar Prodigy 2000, General Electric, Madison, USA).

24-hour ambulatory blood pressure monitoring was performed before the clinical assessment. Participants were fitted with a Spacelabs 90207 or 90217 (Spacelabs Medical Inc., Redmond, USA). Measurements were performed every 20 minutes between 07:00 and 22:00, and every 30 minutes from 22:00 to 07:00. Only profiles with >14 daytime and >7 night time readings over a 24-hour period were analysed. Nocturnal blood pressure dipping was defined as a reduction ≥10% in mean systolic or diastolic blood pressure during sleep (recorded between 22:00 and 06:00) compared with the mean daytime systolic or diastolic blood pressure [36]. Based on this definition, participants were separated into 'dippers' and 'non-dippers'. Note that participants were aware that 22:00 was the predefined sleep period and were encouraged to retire by that time. In addition they were asked about their sleep, and where it had been unusually disturbed the blood pressure profile was repeated.

Carotid artery intima-media thickness (CIMT) is a validated and reproducible measure that is predictive of cardiovascular and cerebrovascular risks [37,38]. It was assessed using a M-Turbo ultrasound system (Sonosite, Bothell, USA) by trained investigators, with longitudinal images attained using a standard protocol [39]. The right common carotid artery was scanned from both posterolateral and anterolateral views. Digitally-stored images were analysed using computer software automated calipers to measure the far wall (SonoCalc^{tm} v.4.1, Sonosite). Maximal measurement from both views (~10 mm proximal to the carotid bulb) was used for comparative analysis. SonoCalc^{tm} has been shown to produce measurements that are equivalent to those from manual electronic calipers, with an estimated mean difference of 7 µm (95% confidence interval: -12 to 26 µm) [40]. Further, the SonoCalc^{tm} method was significantly more reproducible than the calipers [40]. In our study, we assessed reproducibility by taking triplicate measures from seven healthy volunteers over a seven-day interval, which resulted in an intra-observer CV of 3.7% (unpublished data).

Physical activity levels were assessed using the International Physical Activity Questionnaire (IPAQ) [41], covering four domains of physical activity: work-related, transportation, housework/gardening, and leisure time. Socioeconomic status was estimated by geo-coded deprivation scores derived from current address using the New Zealand Index of Deprivation 2006 (NZDep2006) [42]. The Berlin Questionnaire [43] was adopted to classify participants as low-risk or high-risk for obstructive sleep apnoea.

Assays were performed as per Albert et al. [44].

Statistical Analysis

Demographic characteristics and prevalence of hypertension in dippers and non-dippers were compared using one-way ANOVA or Fisher's exact tests in Minitab (v.16, Pennsylvania State University, State College, PA, USA). Multivariable linear regression models were carried out in SAS v.9.3 (SAS Institute, Cary, NC, USA). All models accounted for important confounders (factors likely to affect metabolic outcomes of interest based on published evidence), namely age, socioeconomic status, physical activity levels, birth order, and total body fat percentage. Height was also included as a covariate when comparing blood pressure outcomes. Where necessary, outcomes were log-transformed to approximate a normal distribution. Statistical tests were two-tailed and significance level maintained at 5%. Demographic data are presented as means ± standard deviations, while other data are means and 95% confidence intervals adjusted for confounders in multivariable models.

Power Calculations

Post-hoc power calculations were performed for the 73 participants studied, with a ratio of 2.32 subjects between groups. Based on an observed standard deviation of 0.61 for the log-transformed Matsuda index, this study was powered to detected a 25% difference in means between groups, with 80% power and α=0.05. With the same power and α, and an observed standard deviation of 0.14, the study was also able to detect a 13% difference in CIMT. It is important to note however, that our models controlling for important confounding factors increased our study's power to detect statistically significant differences between groups.

Results

Study Participants

There were 97 subjects in both trials, but since five men took part in both studies, there were 92 individuals enrolled (Figure 1). Nine participants on anti-hypertensive or lipid-lowering medication and one man subsequently diagnosed with obstructive sleep apnoea were excluded from this study; nine of the remaining 82 participants had insufficient 24-hour blood pressure readings (Figure 1). We consequently studied a total of 73 men (Figure 1), who were aged 45.8 ± 5.3 years and with BMI of 27.4 ± 1.8 kg/m^2. Most participants were of European descent (86%).

Across the cohort, nine participants (12%) had systolic and/or diastolic hypertension during the daytime (>138.2/86.4 mmHg) [45], so that most were normotensive during the day. There were 51 dippers and 22 non-dippers. In the latter group, all participants were systolic non-dippers, while 13 men were both systolic and diastolic non-dippers. Non-dippers were on average 3.3 years older than dippers (p = 0.013). However, there were no differences in anthropometry, ethnic composition, socioeconomic status, and physical activity levels (Table 1). In addition, the proportion of participants at high risk of obstructive sleep apnoea was not different in the two groups (Table 1).

Table 1 **Demographic data among dippers and non-dippers in a cohort of middle-aged overweight men recruited in Auckland, New Zealand. Where appropriate, data are means ± standard deviations, except for physical activity levels where the medians and interquartile ranges are provided. Risk of obstructive sleep apnoea was assessed using the Berlin Questionnaire [43]. P-value statistically significant at p < 0.05 is shown in bold**

	Dippers	Non-dippers	p-value
n	51	22	
Age (years)	44.8 ± 5.4	48.1 ± 4.3	**0.013**
Socioeconomic status (NZDep2006)	4.0 ± 2.3	3.9 ± 2.3	0.97
Physical activity levels (MET-minutes per week)	2880 [3003]	2933 [6458]	0.68
Ethnicity (New Zealand European)	88%	82%	0.48
Height (cm)	179.3 ± 6.5	178.4 ± 6.7	0.59
Weight (kg)	88.1 ± 9.4	87.4 ± 10.7	0.73
BMI (kg/m^2)	27.3 ± 1.7	27.4 ± 2.0	0.64
Total body fat (%)	28.1 ± 5.5	28.9 ± 6.7	0.60
Android fat to gynoid fat ratio	1.27 ± 0.19	1.31 ± 0.19	0.36
High risk of obstructive sleep apnoea	12%	21%	0.65

Blood Pressure

Nocturnal systolic and diastolic blood pressure dippings were on average 14.1 and 19.9% (respectively) among dippers, but 5.3 and 9.2% among non-dippers. There were considerable cardiovascular differences between the two groups (Table 2). Non-dippers had lower daytime systolic (-5.0 mmHg; p = 0.022) and diastolic (-3.3 mmHg; p = 0.035) blood pressure than dippers (Table 2). Conversely, during sleep, non-dippers had higher systolic (+6.5 mmHg; p = 0.003) and diastolic (+5.6 mmHg; p = 0.001) blood pressure (Table 2). Non-dippers tended to have a lesser reduction in heart rate during sleep compared to dippers (8.0 vs 10.9%; p = 0.066) and had a greater rate of nocturnal diastolic hypertension (Table 2).

Table 2 **Study outcomes among dippers and non-dippers in a cohort of middle-aged overweight men. Data are means and 95% confidence intervals adjusted for other confounding factors in the multivariable models, except for hypertension data that are numbers of participants and percentages in parentheses. Cut-offs for the diagnosis of hypertension were systolic >138.2 mmHg and diastolic >86.4 mmHg during the daytime, and systolic >119.5 mmHg and >70.8 mmHg at night [45]. P-values statistically significant at p < 0.05 are shown in bold**

		Dippers	Non-dippers	p-value
n		51	22	
24-hour ambulatory blood pressure	Daytime systolic (mmHg)	127.6 (124.2–131.1)	122.6 (118.5–126.8)	**0.022**
	Daytime diastolic (mmHg)	81.0 (78.5–83.4)	77.7 (75.0–80.7)	**0.035**
	Nocturnal systolic (mmHg)	109.7 (106.4–113.0)	116.2 (112.1–120.4)	**0.003**
	Nocturnal diastolic (mmHg)	64.7 (62.1–67.2)	70.3 (67.1–73.4)	**0.001**
	24-hour mean systolic (mmHg)	123.3 (120.0–126.7)	121.1 (117.1–125.1)	0.29
	24-hour mean diastolic (mmHg)	76.7 (74.4–79.1)	75.6 (72.8–78.4)	0.44
	Daytime systolic hypertension	4 (8%)	0	0.31
	Daytime diastolic hypertension	7 (14%)	1 (5%)	0.42
	Nocturnal systolic hypertension	6 (12%)	5 (23%)	0.24
	Nocturnal diastolic hypertension	6 (12%)	10 (45%)	**0.002**
Carotid intima-media thickness (μm)		749 (697–800)	820 (757–884)	**0.036**
Glucose homeostasis	Insulin sensitivity (Matsuda index)	5.85 (4.76–7.19)	5.45 (4.23–7.04)	0.60
	Fasting insulin (mU/l)	5.55 (4.59–6.71)	5.66 (4.48–7.15)	0.88
Lipid profile	Total cholesterol (mmol/l)	4.88 (4.53–5.24)	4.91 (4.49–5.34)	0.81
	LDL-C (mmol/l)	3.29 (2.97–3.61)	3.27 (2.88–3.66)	0.90
	HDL-C (mmol/l)	1.06 (0.93–1.19)	1.12 (0.96–1.28)	0.47
	Total cholesterol: HDL-C	4.65 (4.18–5.19)	4.39 (3.85–5.02)	0.42
	Triglycerides (mmol/l)	1.09 (0.94–1.26)	0.99 (0.83–1.19)	0.33
Inflammatory marker	Highly-sensitive CRP (mg/l)	1.05 (0.71–1.55)	1.12 (0.69–1.80)	0.80

Table 3 **Pearson's correlation coefficients for the associations between ambulatory blood pressure parameters with insulin sensitivity (Matsuda index) and carotid intima-media thickness. P-values statistically significant at p < 0.05 are shown in bold. Note that correlations are reported for the log-transformed Matsuda index**

		Matsuda index		Carotid intima-media thickness	
		r	p-value	r	p-value
Daytime	Systolic	-0.15	0.20	-0.04	0.74
	Diastolic	-0.26	**0.025**	-0.03	0.83
	Mean arterial pressure	-0.23	**0.049**	-0.03	0.78
Night time	Systolic	-0.25	**0.034**	0.24	**0.038**
	Diastolic	-0.31	**0.007**	0.21	0.074
	Mean arterial pressure	-0.31	**0.009**	0.24	**0.043**
Dipping	Systolic	0.15	0.20	-0.32	**0.005**
	Diastolic	0.13	0.28	-0.26	**0.025**

Metabolic Parameters

There were no observed differences in glucose homeostasis between groups (Table 2). There were also no differences in lipid profile or hsCRP concentrations (Table 2).

Pearson's correlation coefficients showed no associations between insulin sensitivity and blood pressure dipping (Table 3). However, insulin sensitivity was negatively correlated with daytime diastolic (r = -0.26; p = 0.025), nocturnal systolic (r = -0.25; p = 0.034), and nocturnal diastolic (r = -0.31; p = 0.007) blood pressures (Table 3). Insulin sensitivity was also correlated with mean arterial pressure in both the daytime and night time (Table 3).

There were no associations between triglyceride and hsCRP levels with the main outcomes of interest, in particular CIMT or the magnitude of nocturnal dipping.

Carotid Intima-media Thickness

Non-dippers had CIMT that was 9% greater (approximately 71 µm thicker) than dippers (p = 0.036; Table 2). Note that age was the strongest predictor of CIMT (ß = 0.011; p = 0.0005), and its exclusion from the multivariable model would increase the difference between groups to 99 µm (p = 0.007).

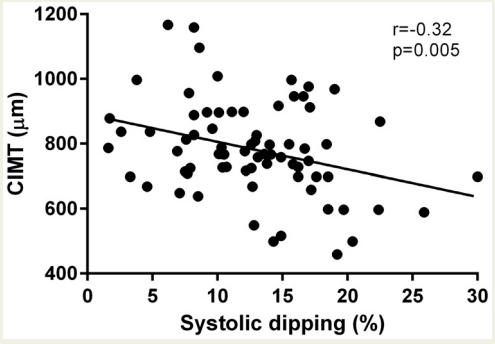

Figure 2 The association between systolic blood pressure dipping and carotid intima-media thickness (CIMT).

Daytime blood pressures were not associated with CIMT (Table 3). However, both systolic (r = -0.32; p = 0.005; Figure 2) and diastolic (r = -0.26; p = 0.025) dippings were negatively correlated with CIMT, while a positive association was observed for nocturnal systolic blood pressure (r = 0.24; p = 0.038) (Table 3). These patterns were corroborated by multivariable models showing greater CIMT to be associated with lower systolic (β=-0.007; p = 0.012) and diastolic (β=-0.004; p = 0.042) dipping, but with increased nocturnal systolic blood pressure (p = 0.042).

Discussion

This study showed that in a cohort of predominantly normotensive overweight males, non-dippers had lower blood pressure in the daytime, but higher blood pressure in the night time compared to dippers. Despite these differences, non-dippers and dippers had similar average blood pressure and rates of hypertension during the day. Importantly, we observed no association between blood pressure dipping and insulin sensitivity, triglycerides, or hsCRP. However, non-dippers had CIMT that was 9% greater, which is consistent with the increased cardiovascular risk reported in non-dippers and in association with higher nocturnal blood pressure [7]. Notably, these findings were observed despite adjustment for the important confounders known to influence blood pressure, such as age, height, total body fat percentage, ethnicity, and physical activity levels. Further, there were no differences in socioeconomic status between groups.

The differences in blood pressure profile observed between dippers and non-dippers are consistent with previous studies of normotensive individuals [13,21] and some studies of hypertensive subjects [13,18,46]. However, one investigation on hypertensive participants has shown increased daytime blood pressure in non-dippers [47]. Nonetheless, it appears that the presence or absence of the dynamic dipping pattern is independent of average blood pressure.

Insulin resistance and hyperinsulinaemia (to which obesity and inactivity are major contributors) have a causative role in the pathogenesis of essential hypertension [25–27]. This occurs through three major effects: 1) increased sympathetic activation [48,49], 2) increased sodium retention [50], and 3) impaired insulin signalling in endothelial cells [51]. The latter leads to reduced nitric oxide synthesis and subsequent vasoconstriction [51]. Consistent with this, across our entire cohort, lower insulin sensitivity correlated with higher systolic and diastolic blood pressures, but was not associated with dipping. In addition, a range of other factors linked to metabolic health did not differ between dippers and non-dippers, including body composition, physical activity, triglycerides, and hsCRP. This suggests that within overweight middle-aged men, insulin sensitivity does not appear to be an important factor affecting nocturnal blood pressure dipping.

However, the reported relationship between insulin sensitivity and dipping is conflicting [19,52–59]. Blood pressure dipping is dependent on cardiac innervation [60], and non-dippers have been shown to have dysfunction of the autonomic nervous system [3,28–30]. Consistent with autonomic dysfunction, we observed a trend to a lesser fall in nocturnal pulse rate in non-dippers. It is plausible that insulin resistance could contribute to a reduced nocturnal decline in blood pressure and heartrate, by increasing sympathetic activity [48,49].

The relationship between dipping and insulin sensitivity has revealed conflicting results depending upon the characteristics of the group studied, and is complicated by methodological flaws. In the context of more severe insulin resistance there is evidence for an association [57–59]. However, in studies of subjects who are only overweight or hypertensive (similar to our participants), there are inconsistent reports of this relationship [19,52–55]. In agreement with some studies [52–54], we report a lack of association between insulin sensitivity and nocturnal dipping whereas others found non-dippers had poorer insulin sensitivity [19,55]. Importantly, we used the Matsuda Index, a more accurate measure of insulin sensitivity than HOMA-IR [33,34] which was used in these previous studies. Whilst Björklund et al. used the gold standard hyperinsulinaemic euglycaemic clamp technique to directly measure insulin sensitivity in elderly hypertensives, the study was flawed by an incorrect definition of nocturnal dipping [56].

In more insulin resistant groups such as those with non-alcoholic fatty liver disease (NAFLD) and type 2 diabetes mellitus, an association is more clearly seen between reduced insulin sensitivity and nocturnal non-dipping. NAFLD subjects have severe insulin resistance [61] and hyperinsulinaemia [62], and frequently have autonomic dysfunction [62,63]. Notably, among subjects with NAFLD [57] and in a group with a high prevalence of NAFLD [58], insulin sensitivity was associated with non-dipping. Further, non-dipping appears to be more common in those with type 2 diabetes mellitus, which is characterised by severe insulin resistance and hyperglycaemia [59]. However in type 2 diabetics, the relative contributions of insulin resistance and hyperglycaemia are unclear, as hyperglycaemia even without significant insulin resistance (e.g. in type 1 diabetes mellitus) leads to autonomic dysfunction [64]. Thus, we speculate that while the association between non-dipping and insulin sensitivity is weak or absent in patient groups where insulin resistance is mild, there may be a stronger association in more severely insulin resistant groups, probably mediated through hyperinsulinaemia and its effects on sympathetic activity.

In our predominantly normotensive cohort, non-dippers had increased CIMT, which is consistent with previous evidence in hypertensive non-dippers [17,65]. In contrast, a previous study of normotensive individuals in Japan showed no difference in CIMT between dippers and non-dippers [21]. However, they studied a rural and relatively elderly Japanese population, compared to our urban, overweight, middle-aged, male, and predominantly European participants. Importantly, while greater nocturnal blood pressure was associated with increased CIMT, daytime blood pressures were not, underscoring the importance of the nocturnal decline in blood pressure. In addition, we observed a continuous association between decreasing nocturnal blood pressure dipping and increasing CIMT. As CIMT is a marker of atherosclerosis and an independent predictor of vascular events across the adult age range [38], our findings suggest that overweight but otherwise healthy non-dippers may have a greater rate of atherosclerosis and be at increased cardiovascular risk.

Perhaps surprisingly, despite increased CIMT in non-dippers, there were no differences in lipid profile between groups. This suggests that within our cohort the difference in CIMT between groups was not mediated through dyslipidaemia. Nonetheless, a larger study in non-dippers who were hypertensive did find features suggestive of a more atherogenic lipid profile [65]. Thus, it is unclear whether the lack of an association between CIMT and lipid profile in our study was a result of inadequate statistical power or because this relationship does not exist amongst normotensive non-dippers.

It is important to consider the reproducibility of the dipping blood pressure profile. Ambulatory blood pressure profiles have been shown to be reliable [66–69] and more reproducible than clinic blood pressure [67,68]. However, although dipping status has been described as reliable [70], there is evidence that 40% of people will change their dipping status during a second 24-hour ambulatory blood pressure measurement [46,71]. Further, the definition of dipping based on a $\geq$10% nocturnal blood pressure decline is arbitrary [29]. Thus, during re-test, a small change in the nocturnal decline could change an individual's classification, although there is no compelling reason to believe that, for example, a 9% dip should carry a substantially greater risk than an 11% dip. Our data support the arbitrary nature of dipping status, as there was a continuous negative association between the nocturnal reduction in blood pressure and CIMT without a threshold (Figure 2). Further, this continuous relationship corroborates our stratified analysis, suggesting that the difference in CIMT we have demonstrated

between dippers and non-dippers is not simply an artefact of our group classification.

The major strengths of this study are the detailed metabolic assessment, which used a robust method to measure insulin sensitivity in comparison to other studies that have used only fasting insulin and glucose (e.g. HOMA-IR), and the statistical models that adjusted for important confounders (such as height, body fat, and physical activity levels). In particular, our analysis controlled for the small difference in age between dippers and non-dippers, as CIMT has been shown to increase with age [72]. However, our study has limitations, including the *post hoc* analysis. Our sample size was small with a relatively low number of non-dippers, and relied on a single 24-hour blood pressure profile. In addition, we defined night time using specific time points rather than sleep diaries, so that our nocturnal recordings could have included measurements taken when the participant was still awake and active. We also studied a relatively narrow range of individuals (overweight males living in a large urban centre, mostly of New Zealand European ethnicity), which may limit wider applicability of our findings, especially to females and those who are obese (BMI $\geq$30 kg/m^2) or of non-European ethnicity. Lastly, as this was a cross-sectional study, the actual rate of atherosclerosis could not be assessed, and it is not possible to determine causation.

Nonetheless, this study in overweight normotensive men adds to the evidence that amongst subjects expected to have only mild metabolic dysfunction, the non-dipping blood pressure pattern is independent of insulin sensitivity. However, as previous studies in similar groups are conflicting and most have used HOMA-IR (a surrogate index solely derived from fasting values), further studies using more accurate measures of insulin sensitivity are required to better characterise this association. In addition, our study highlights the importance of the nocturnal decline in blood pressure. Even in the context of a normal average blood pressure, non-dipping status was associated with increased CIMT in overweight middle-aged men. Thus, non-dipping, which is known to be associated with increased cardiovascular risk in normotensive men, men may be associated with a greater rate of atherosclerosis.

Acknowledgements

This study was supported by a TECHNZ grant (University of Auckland – UniS 30475.001) through the New Zealand Ministry of Science and Innovation, and by The Douglas Charitable Trust. The authors have no financial or non-financial conflicts of interest to disclose that may be relevant to this work. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of this manuscript.

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