

## ORIGINAL ARTICLE

# Sleep, ghrelin, leptin and changes in body weight during a 1-year moderate-intensity physical activity intervention

AJ Littman<sup>1,2</sup>, MV Vitiello<sup>3</sup>, K Foster-Schubert<sup>4,5</sup>, CM Ulrich<sup>1,2</sup>, SS Tworoger<sup>6,7</sup>, JD Potter<sup>1,2</sup>, DS Weigle<sup>4,8</sup> and A McTiernan<sup>1,2</sup>

<sup>1</sup>Division of Public Health Sciences, Fred Hutchinson Cancer Research Center, Seattle, WA, USA; <sup>2</sup>Department of Epidemiology, University of Washington, Seattle, WA, USA; <sup>3</sup>Department of Psychiatry and Behavioral Sciences, University of Washington School of Medicine, Seattle, WA, USA; <sup>4</sup>Department of Medicine, Division of Metabolism, Endocrinology, and Nutrition, University of Washington School of Medicine, Seattle, WA, USA; <sup>5</sup>Veterans Affairs Puget Sound Health Care System, Seattle, WA, USA; <sup>6</sup>Channing Laboratory, Department of Medicine, Brigham and Women's Hospital, Boston, MA, USA; <sup>7</sup>Department of Epidemiology, Harvard School of Public Health, Boston, MA, USA and <sup>8</sup>Division of Endocrinology, Harborview Medical Center, Seattle, WA, USA

**Objective:** To investigate cross-sectional and longitudinal relationships among exercise, sleep, ghrelin and leptin.

**Methods:** We randomly assigned 173 post-menopausal sedentary overweight (body mass index  $\geq 24.0$  kg/m<sup>2</sup> and  $> 33\%$  body fat) women aged 50–75 years living in western Washington State to either a facility- and home-based moderate-intensity physical activity intervention or a stretching control group. Fasting plasma ghrelin, leptin, measured height, weight and self-reported sleep were assessed at baseline and 12 months.

**Results:** There were no consistent cross-sectional patterns between self-reported sleep measures and ghrelin or leptin at baseline. The weight loss differences between exercisers and stretchers were greater for those who slept less at follow-up than at baseline compared to those whose sleep duration did not change ( $-3.2$  kg, 95% confidence interval (CI)  $-5.8, -0.5$ ). Improvements in sleep quality were associated with significantly greater differences between exercisers and stretchers for ghrelin increases (improved vs same sleep quality:  $+115$  pg/ml, 95% CI  $+25, +206$ ) and leptin decreases (improved vs worsened sleep quality:  $-5.7$  ng/ml, 95% CI  $-9.5, -1.5$ ).

**Conclusion:** There was only limited evidence that changes in sleep duration or quality modified exercise-induced changes in weight, ghrelin or leptin. Moreover, the observed differences were not in the directions hypothesized. Future longitudinal studies including population-based samples using objective measures of sleep and long follow-up may help to clarify these relationships.

*International Journal of Obesity* advance online publication, 15 August 2006; doi:10.1038/sj.ijo.0803438

**Keywords:** physical activity; randomized trial; ghrelin; leptin; sleep; weight change

## Introduction

Average sleep duration has declined over the past 40 years in the US.<sup>1–3</sup> At the same time, obesity rates have increased dramatically.<sup>4</sup> In addition to increased consumption of energy-dense food and physical inactivity,<sup>5</sup> recent evidence points to sleep deprivation<sup>6–10</sup> as a possible contributor to

the increase in obesity.<sup>4</sup> Studies indicate that sleep may moderate hunger and satiety through hormones including ghrelin and leptin.<sup>7–9,11,12</sup> Ghrelin is a gut-derived peptide that stimulates appetite and increases food intake.<sup>13,14</sup> Leptin, in contrast, is an adipocyte-derived factor that circulates in proportion to body fat stores and induces satiety in normal-weight persons.<sup>15,16</sup> Previous studies have indicated that sleep deprivation leads to increases in ghrelin and decreases in leptin, and that these changes are sufficient to affect appetite and hunger.<sup>11,12</sup>

It is not well understood how sleep curtailment might lead to changes in plasma levels of ghrelin and leptin or to changes in body weight. One proposed hypothesis is

Correspondence: Dr AJ Littman, Division of Public Health Sciences, Fred Hutchinson Cancer Research Center, 1100 Fairview Avenue North M4 B402, PO Box 19024, Seattle, WA 98109-1024, USA.

E-mail: alittman@fhcrc.org

Received 30 December 2005; revised 7 May 2006; accepted 6 June 2006

through changes in hypothalamic control of autonomic nervous system activity,<sup>7</sup> including changes in the balance of cholinergic (parasympathetic) and sympathetic tone, which have been shown to influence leptin and ghrelin secretion.<sup>17,18</sup>

We used data from a randomized trial of moderate-intensity physical activity among obese, sedentary post-menopausal women to investigate associations between exercise, sleep, body mass index (BMI), ghrelin, leptin and weight change. First, we examined whether sleep measures were associated with BMI, ghrelin and leptin at baseline. Based on findings from other studies,<sup>11,12</sup> we expected that participants who reported less sleep, and possibly more restless sleep, or more daytime sleepiness, would have higher plasma ghrelin levels and lower leptin levels. We also hypothesized that the relationship between sleep and BMI would be inverse or U-shaped.<sup>8,12,19</sup> Second, we examined whether changes in sleep modified the effects of exercise on changes in weight, ghrelin and leptin during the 12-month trial. We hypothesized that improvements in sleep might lead to greater weight loss, perhaps by influencing satiety and hunger hormones such as ghrelin and leptin; these effects would presumably be stronger among women in the exercise arm. We also hypothesized that exercise-induced decreases in leptin and increases in ghrelin would tend to be greater among those whose sleep worsened compared to those whose sleep stayed the same or improved. To our knowledge, no previous investigations have examined how sleep might modify the associations between weight, ghrelin or leptin changes in individuals randomized to a moderate-intensity physical activity intervention vs a control (stretching) intervention.

## Methods

### Study population

Participants were post-menopausal women, aged 50–75 years, who were sedentary at baseline, characterized as less than 60 min/week of moderate or vigorous intensity physical activity and maximal oxygen consumptions (VO<sub>2</sub> max) of less than 25.0 ml kg<sup>-1</sup> min<sup>-1</sup> (Table 1). To be eligible, women were required to have a BMI >25.0 kg/m<sup>2</sup> (or a BMI between 24.0 and 25.0 kg/m<sup>2</sup> if percent body fat as measured by bioelectrical impedance was >33.0%). Major exclusion criteria included tobacco use, a clinical diagnosis of diabetes or a fasting blood glucose level of 140 mg/day or more, a history of any potentially cachectic state such as malignancy (except non-melanoma skin cancer) within 10 years or liver or kidney disease. Details of the recruitment procedures have been reported elsewhere.<sup>20</sup> We obtained written informed consent from participants before enrollment. Eligible women were then block-randomized by BMI (<27.5 vs ≥27.5 kg/m<sup>2</sup>) to ensure balance of heavier and lighter women in both study arms, to an exercise intervention

**Table 1** Baseline demographic, anthropometric, hormone and sleep measures of study participants

Characteristic	Exercisers (n = 87)	Stretchers (n = 86)
	Mean (s.d.) or %	
Age (years)	60.7 (6.7)	60.6 (6.8)
% with a college or graduate degree	48.8	47.1
% White participants	85.1	87.2
BMI (kg/m <sup>2</sup> )	30.4 (4.1)	30.5 (3.7)
Ghrelin (pg/ml)	599 (355)	629 (331)
Leptin <sup>a</sup> (ng/ml)	28.4 (7.9)	27.9 (8.5)
Sleep measures		
%		
Sleep duration (h/night)		
5–6	19.5	24.4
7	34.5	29.1
8	35.6	40.7
9	10.3	5.8
Sleep quality		
Very restless/restless	6.9	9.3
Average	36.8	40.7
Sound/ very sound	56.3	50.0
Use of sleep medications or alcohol to help sleep		
No	82.8	80.2
Yes	17.2	19.8
Trouble falling asleep (times/week)		
No	72.4	62.8
<1	13.8	19.8
≥1	13.8	17.4
Falling asleep during quiet activities (times/week)		
No	41.4	31.4
<1	21.8	16.3
≥1	36.8	52.3
Napping during the day (times/week)*		
No	60.9	41.9
<1	26.4	34.8
≥1	12.6	23.3

Abbreviations: BMI, body mass index; s.d., standard deviation. <sup>a</sup>One stretcher whose baseline leptin was below the limits of detection (<0.5 ng/ml) was not included in any leptin analyses. \* $P < 0.05$  for the  $\chi^2$  test for heterogeneity.

(exercisers,  $n = 87$ ) or a control group (stretchers,  $n = 86$ ). Five women randomized to the exercise arm did not have complete information on sleep, body weight and ghrelin/leptin at 12 months, and were excluded from analyses involving 12-month measures. One stretcher did not have body weight measured at 12 months and was excluded from analyses on weight change. Finally, one stretcher had baseline leptin levels that were below the limits of detection (<0.5 ng/ml) and was excluded from all leptin analyses.

### Baseline and follow-up measure, including sleep, leptin and ghrelin

Participants completed questionnaires before randomization ('baseline') and at 3 and 12 months after randomization,

which inquired about their medical histories, demographic information, usual food intake, sleep patterns, job status and depression using the short version of the Center for Epidemiologic Studies Depression Scale.<sup>21</sup> At the baseline and 3- and 12-month visits, weight was measured to the nearest 0.1 kg on a balance-beam scale, and height was measured to the nearest 0.1 cm on a stadiometer. These measures were used to calculate BMI as an estimate of adiposity, calculated as weight in kilograms divided by the square of height in meters.

The Women's Health Initiative Insomnia Rating Scale Questionnaire was used to assess sleep over the previous month.<sup>22,23</sup> In our analyses, we collapsed upper and sometimes lower categories because of small numbers. The measures included sleep duration ( $\leq 6, 7, 8, \geq 9$  h/night), sleep quality (very restless/restless, average, sound/very sound), use of sleep medications or alcohol to help sleep (yes, no), trouble falling asleep (no,  $< 1$  time/week,  $\geq 1$  time/week), falling asleep during quiet activities (no,  $< 1$  time/week,  $\geq 1$  time/week) and napping during the day (no,  $< 1$  time/week,  $\geq 1$  time/week) during the previous 4 weeks. We included the first three items because they are similar to scales of the commonly used Pittsburgh Sleep Quality Index.<sup>24</sup> The last two questions reflect the daytime consequences of night time sleep quality. To calculate sleep-change measures, we compared responses at baseline and 12 months and created variables with three categories, depending on whether individuals reported improved sleep (e.g., longer sleep duration, more sound sleep or less napping), no change, or worsened sleep (e.g., shorter sleep duration, more restless sleep or more napping).

We collected morning blood samples after a 12-h overnight fast at the baseline and 3- and 12-month visits. Blood was processed within 1 h of collection, and plasma was aliquoted and stored at  $-70^{\circ}\text{C}$ . Storage time for plasma samples ranged from 3 months to 3 years, an interval that in our experience does not affect measurements of total ghrelin or leptin levels. The total plasma immunoreactive ghrelin concentration was measured in duplicate with samples that had never been previously thawed, using our modification of a commercial radioimmunoassay (RIA) that employs a polyclonal antibody raised against full-length acylated human ghrelin and  $^{125}\text{I}$ -labeled ghrelin as a tracer (Phoenix Pharmaceuticals, Belmont, CA, USA). This assay detects both acylated and des-acyl ghrelin. Although only acylated ghrelin is bioactive, levels of total ghrelin appear to be a reasonable surrogate for those of the acylated form because the ratio of these measures remains constant under a wide variety of conditions that affect ghrelin.<sup>25–27</sup> The lower and upper levels of detection for this assay were 80 and 2500 pg/ml, respectively. The intra-assay coefficient of variation (CV) was 3.5% and the inter-assay CV was 4.9%. Plasma leptin was measured using a commercial RIA with lower and upper detection limits of 0.5 and 100 ng/ml, respectively (Linco Research Inc., St Charles, MO, USA). The intra-assay CV was 8.7% and the inter-assay CV was 11.2%. In each of the

hormone assays, all samples (baseline, 3 months and 12 months) from a participant were run in the same batch. Samples were placed into batches such that, within each batch, the number of exercise and control participants was approximately equal, the randomization dates of participants were similar and the sample order was random. Laboratory personnel were blinded to intervention status and were also unaware of which samples belonged to the same woman.

#### *Study interventions*

The exercise intervention goals were to perform a minimum of 45 min of moderate-intensity aerobic exercise, 5 days per week for 12 months. During the first 3 months of the intervention, exercisers completed three supervised sessions per week at one of the training facilities, and exercised on their own 2 additional days per week. In months 4 through 12, exercisers performed one to three supervised exercise sessions, and exercised on their own for a total of 5 days per week. The training program started at 40% of maximal heart rate for 16 min/session and gradually increased to 60–75% of maximal heart rate for 45 min/session by week 8. Women maintained this level of exertion and duration for the remainder of the study. Walking and use of a stationary bicycle were the most commonly performed activities. Adherence to the exercise regimen was assessed using daily exercise logs and measurements of cardiopulmonary fitness ( $\text{VO}_2$  max, procedure described elsewhere<sup>28</sup>) at baseline and 12 months. Women randomized to the control group attended a 45-min stretching session once per week for a year, and were asked to not change other exercise habits during the study. Exercisers and stretchers were strictly instructed to maintain their usual diet.

#### *Statistical analyses*

In analyses examining cross-sectional associations between baseline sleep and weight, ghrelin and leptin measures, we combined values for exercisers and stretchers. Because distributions were skewed, we log transformed hormone values for analyses, and back transformed them for the tables. Analyses were adjusted for age (weight, ghrelin and leptin) and BMI (ghrelin and leptin only), and additionally for intervention assignment for the 3- and 12-month analyses.

We used linear regression to estimate the difference in 12-month changes in the outcomes of interest (i.e., changes in weight, ghrelin and leptin) between exercisers and stretchers associated with sleep change, after adjusting for age, baseline values of the predictor (the given sleep measure) and baseline values of the outcome of interest (e.g., baseline weight, ghrelin and leptin, respectively). As weight change might be on the causal pathway between exercise and changes in ghrelin or leptin, we conducted analyses both unadjusted and adjusted for baseline weight and weight

change to evaluate how changes in sleep might modify changes in these hormones, beyond any effects caused by weight loss or gain.<sup>29</sup> We also analyzed whether differences across sleep-change categories between exercisers and stretchers were significantly different from each other. Statistical significance was defined as at or below the 0.05 level.

## Results

Participants had a mean age of 61 years and 48% had a college or graduate degree; 86% were non-Hispanic white (Table 1). Baseline demographic and body weight measures in the exercise and stretching groups were similar and have been reported in detail elsewhere.<sup>28</sup> There were no baseline differences in ghrelin, leptin or sleep measures between the groups except frequency of napping, which was greater in the women randomized to stretching ( $P=0.03$ ).

There was only borderline evidence that BMI was associated with sleep measures at baseline, and no evidence that

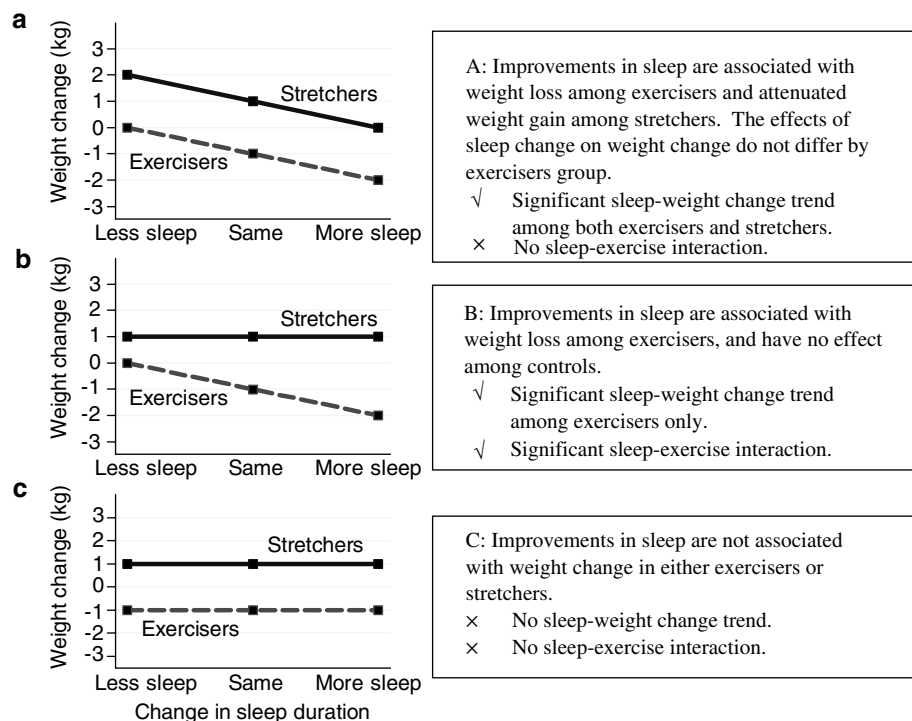
ghrelin or leptin levels were associated with sleep. Mean BMIs in women who slept 9 h per night or napped more than once per week were higher than in women who slept 8 or fewer hours per night or napped less frequently ( $P$  for trend = 0.06 and 0.08, respectively; Table 2). Although ghrelin levels tended to increase with increasing restlessness of sleep (489, 552 and 559 pg/ml for sound/very sound sleep, average and very restless/restless sleep, respectively,  $P=0.20$ ), and leptin levels tended to increase with increasing frequency of trouble falling asleep (26.4, 26.9 and 28.9 ng/ml for 0, <1 and  $\geq 1$  time(s) per week, respectively,  $P=0.13$ ), these differences were not statistically significant.

The remaining tables and figures present results for the longitudinal analyses. To make these complex results clearer, we will take a moment to orient the reader. In the following analyses, we examine whether changes in sleep modified the effects of exercise on weight, ghrelin and leptin. Our focus is on the interaction between sleep and exercise on these outcomes; both sleep and the outcomes (weight, ghrelin and leptin) involve changes from baseline to 12 months. The comparisons of interest are differences between exercisers

**Table 2** Adjusted mean (95% CI) body mass index (BMI, kg/m<sup>2</sup>), plasma ghrelin (pg/ml) and leptin (ng/ml) levels at baseline stratified by baseline sleep measures

Self-reported baseline sleep measures	n	Mean BMI <sup>a</sup> (95% CI)	Mean pg/ml ghrelin <sup>b</sup> (95% CI)	Mean ng/ml leptin <sup>b,c</sup> (95% CI)
<i>Sleep duration (h/night)</i>				
5–6	38	30.0 (28.8, 31.1)	507 (419, 613)	26.4 (24.3, 28.8)
7	55	30.0 (29.0, 31.0)	540 (461, 633)	27.0 (25.2, 29.1)
8	66	30.7 (29.8, 31.6)	485 (420, 560)	26.9 (25.2, 28.6)
9	14	32.2 (30.3, 34.2)	633 (462, 868)	27.4 (23.8, 36.1)
<i>P for trend</i>		0.06	0.77	0.72
<i>Sleep quality</i>				
Very restless/restless	14	29.6 (27.6, 31.6)	559 (408, 765)	27.8 (24.2, 32.0)
Average	67	30.8 (29.9, 31.7)	552 (478, 636)	26.9 (25.2, 28.7)
Sound/very sound	92	30.3 (29.5, 31.1)	489 (433, 553)	26.7 (25.3, 28.2)
<i>P for trend</i>		0.98	0.20	0.63
<i>Use of sleep medications or alcohol to help sleep</i>				
No	141	30.3 (29.7, 30.9)	512 (464, 566)	26.7 (25.5, 27.9)
Yes	32	31.0 (29.7, 32.3)	543 (441, 668)	27.8 (25.3, 30.5)
<i>P-value</i>		0.33	0.62	0.42
<i>Trouble falling asleep (times/week)</i>				
No	117	30.4 (29.7, 31.2)	514 (461, 573)	26.4 (25.2, 27.7)
<1	29	30.4 (29.2, 31.6)	515 (414, 641)	26.9 (24.4, 29.6)
$\geq 1$	27	30.4 (28.8, 32.0)	538 (429, 675)	28.9 (26.1, 31.9)
<i>P for trend</i>		0.88	0.74	0.13
<i>Falling asleep during quiet activities (times/week)</i>				
No	63	31.0 (30.1, 31.9)	503 (433, 583)	27.5 (25.7, 29.3)
<1	33	29.4 (28.1, 30.7)	552 (449, 678)	27.7 (25.3, 30.4)
$\geq 1$	77	30.4 (29.6, 31.2)	516 (452, 590)	26.0 (24.5, 27.6)
<i>P for trend</i>		0.38	0.82	0.21
<i>Napping during the day (times/week)</i>				
No	89	29.9 (29.1, 30.7)	512 (452, 580)	27.1 (23.3, 28.0)
<1	53	30.9 (29.8, 31.9)	508 (432, 597)	26.7 (24.0, 30.4)
$\geq 1$	31	31.1 (29.8, 32.4)	554 (448, 684)	26.5 (23.1, 31.5)
<i>P for trend</i>		0.08	0.60	0.45

Abbreviations: BMI, body mass index; s.d., standard deviation. <sup>a</sup>Adjusted for age at baseline. <sup>b</sup>Adjusted for age and BMI at baseline. <sup>c</sup>One stretcher whose baseline leptin was below the limits of detection (<0.5 ng/ml) was not included in any leptin analyses.



**Figure 1** Hypothetical associations between changes in sleep duration and weight change among exercisers and stretchers and what they tell us about how sleep, exercise and weight are related. We would expect similar patterns for ghrelin and leptin changes, except exercisers would have lower ghrelin values than stretchers, and improvements in sleep would be associated with increases in leptin.

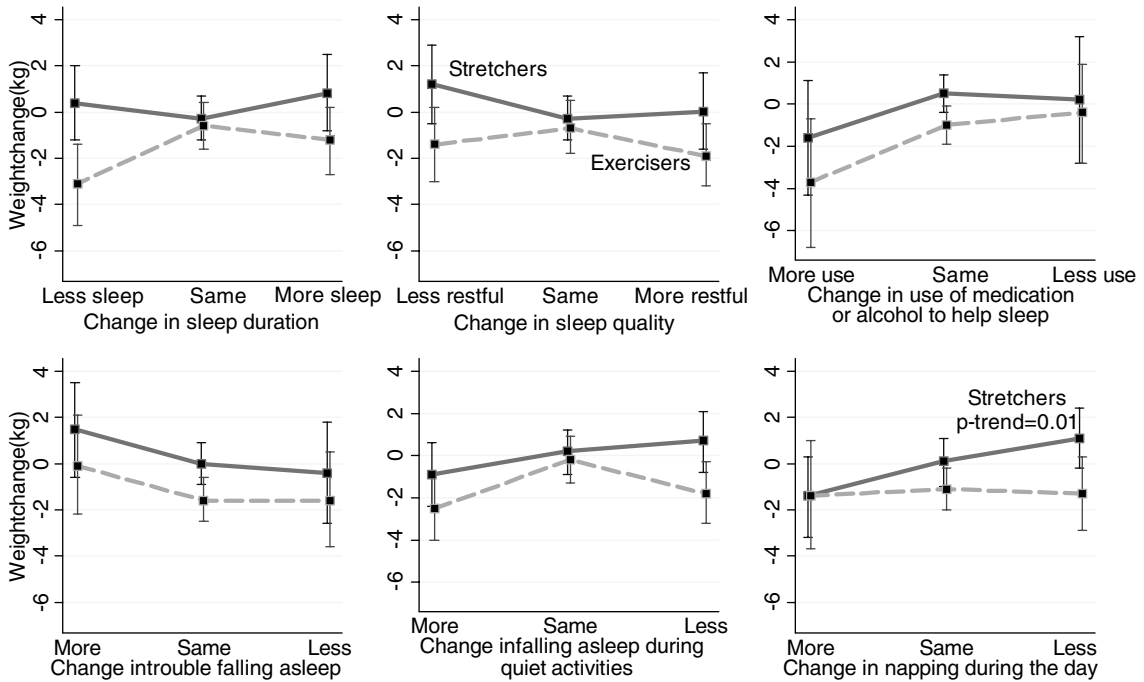
and stretchers for these changes (e.g., within a given sleep-change category), and furthermore, for the differences in the differences between exercisers and stretchers for these changes (e.g., between sleep-change categories), as well as the trends across sleep changes within an intervention group (e.g., among exercisers or controls, separately). Figure 1 presents our hypothesized results for weight change; hypothesized results for ghrelin are the same. Because we expected that improved sleep might lead to increases in leptin, we reversed the scale on the y axis of Figure 4 so that the hypothesized associations resemble those of weight and ghrelin changes. Figures 2–4 present changes in weight, ghrelin and leptin, respectively, among exercisers and stretchers, with any statistically significant trends noted. Tables 3 and 4 give the differences between exercisers and stretchers for weight, ghrelin and leptin changes by sleep-change categories. Statistically significant differences between levels of a sleep-change measure are noted with superscripts, and the differences are given in the text only.

Overall, stretchers did not gain or lose weight during the trial (+0.1 kg, 95% confidence interval (CI) -0.6, +0.8), whereas exercisers lost 1.3 kg (95% CI -2.1, -0.6), resulting in a 1.4 kg difference between the intervention groups (95% CI -2.5, -0.4; Table 3). Among stretchers, increased napping was associated with increased weight gain ( $P$  for trend = 0.01; Figure 2). No other trends among exercisers or stretchers across sleep-change categories were statistically significant.

Compared to those whose sleep duration did not change, the weight-loss difference between exercisers and stretchers was greater among those who slept less at follow-up (less sleep vs same: -3.2 kg, 95% CI -5.8, -0.5; Table 3). No other sleep-change measures modified exercise-induced weight changes (data for selected sleep measures are not presented).

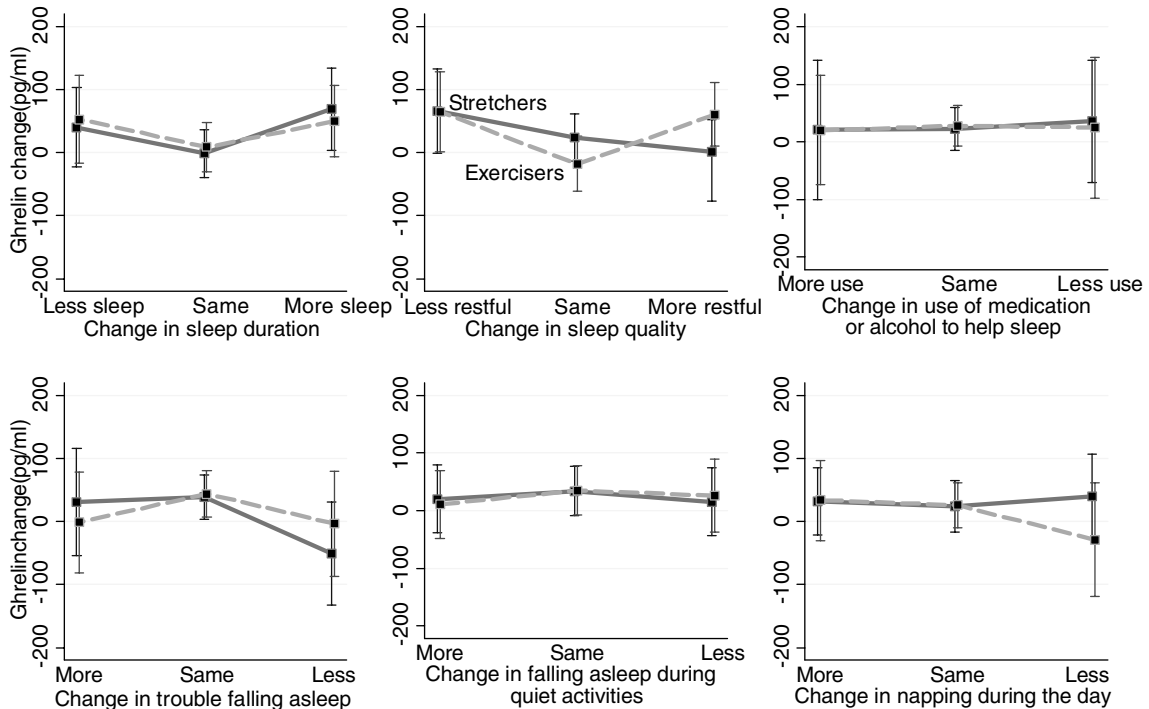
Next, we examined exercise-induced changes in ghrelin and leptin, stratified by sleep-change measures (Figures 3 and 4, and Table 4). As we reported previously,<sup>29</sup> ghrelin levels increased during the 12-month period both among exercisers and stretchers. The increase in ghrelin was mainly explained by weight loss. We observed no statistically significant trends for either exercisers or stretchers for any sleep-change measures (Figure 3). Differences between exercisers and stretchers in ghrelin levels were significantly greater (+115 pg/ml, 95% CI +25, +206) among those who reported improved vs the same sleep quality.

Leptin decreased significantly more among exercisers than stretchers (-1.6 ng/ml, 95% CI -3.1, -0.1), although these differences were attenuated after additional adjustment for weight and weight change (-0.7 ng/ml, 95% CI -2.0, +0.7). Among exercisers only, leptin increased with decreasing sleep duration or worsening sleep quality ( $P$  for trend  $\leq 0.01$ ; Figure 4). Exercise-induced decreases in leptin were greater among women whose sleep duration increased compared to decreased (-4.2, 95% CI -8.1, -0.2;  $P$  for trend = 0.02). Differences between exercisers and stretchers in leptin levels



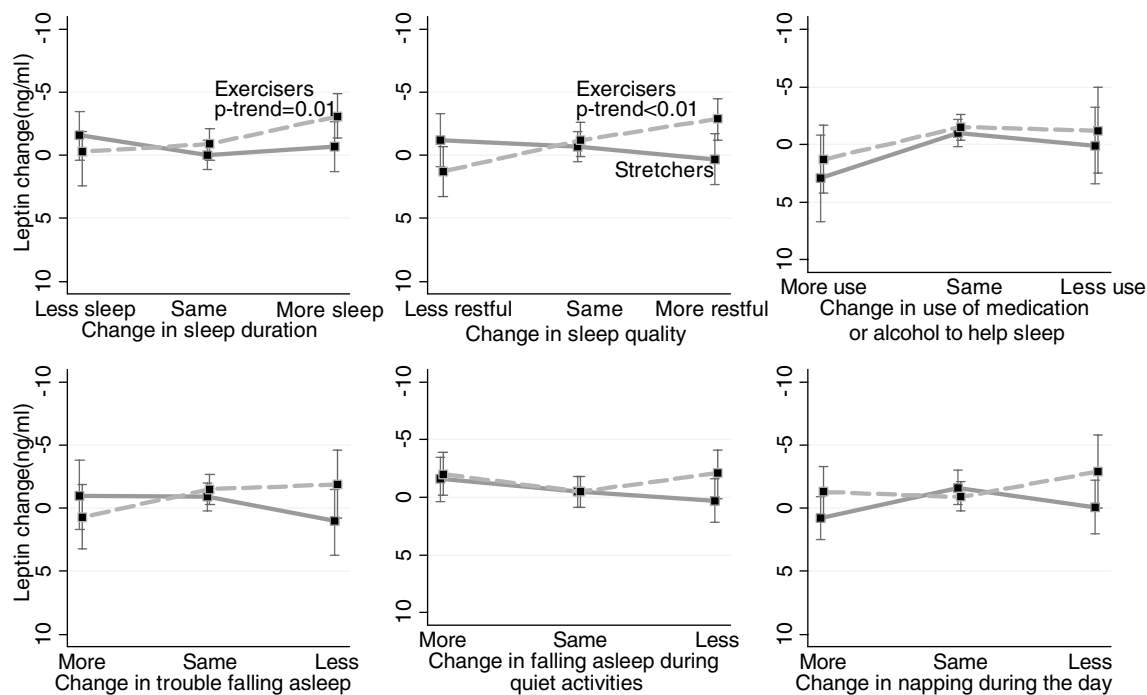
Dark gray solid lines represent stretchers.  
 Light gray dashed lines represent exercisers.  
 Adjusted for baseline age, respective sleep measure, and weight.

**Figure 2** Sleep change and 12-month adjusted weight changes (kg): exercisers vs stretchers.



Dark gray solid lines represent stretchers.  
 Light gray dashed lines represent exercisers.  
 Adjusted for baseline age, respective sleep measure, weight, and weight change.

**Figure 3** Sleep change and 12-month adjusted ghrelin changes (pg/ml): exercisers vs stretchers.



Dark gray solid lines represent stretchers. Light gray dashed lines represent exercisers. Adjusted for baseline age, respective sleep measure, weight, and weight change. Note: y-axis is reversed to correspond with other figures.

**Figure 4** Sleep change and 12-month adjusted leptin changes (ng/ml): exercisers vs stretchers.

**Table 3** Differences in adjusted<sup>a</sup> changes in body weight (kg) from baseline to 12 months among exercisers vs stretchers, stratified by sleep-change measures

Changes in self-reported sleep from baseline to 12 months	Exercisers <sup>b</sup> , n	Stretchers <sup>b</sup> , n	Difference in body weight change (95% CI): exercisers–stretchers
Overall	82	85	-1.4 (-2.5, -0.4)
<i>Sleep duration</i>			
More	23	18	-2.0 (-4.2, +0.1) <sup>c,d</sup>
Same	43	49	-0.4 (-1.8, +1.0) <sup>d</sup>
Less	16	18	-3.5 (-5.8, -1.2) <sup>c</sup>
<i>Sleep quality</i>			
More restful	28	19	-1.9 (-3.9, +0.2)
Same	35	50	-0.4 (-1.9, +1.1)
Less restful	19	16	-2.6 (-4.9, -0.4)
<i>Falling asleep during quiet activities</i>			
Less	20	24	-1.5 (-3.6, +0.5)
Same	40	39	-0.4 (-1.9, +1.1)
More	22	22	-2.4 (-4.4, -0.4)
<i>Napping during the day</i>			
Less	9	19	+0.1 (-2.7, +2.9)
Same	56	41	-1.2 (-2.6, +0.2)
More	17	25	-2.4 (-4.5, -0.3)

<sup>a</sup>Adjusted for baseline age, sleep measures and weight. <sup>b</sup>Five exercisers and one control have no follow-up data on sleep and/or weight. <sup>c,d</sup>Categories with different superscripts are statistically significantly different from each other ( $P < 0.05$ ).

were significantly less (-5.7 ng/ml, 95% CI -9.5, -1.5) among those who reported improved sleep quality compared to those with worsened sleep quality. Leptin decreases were also significantly greater for decreases or increases in daytime sleepiness, as measured by falling asleep during quiet activities (less vs same: -3.6 ng/ml, 95% CI -7.2, 0.0) and frequency of napping (more vs same: -3.8 ng/ml, 95% CI -7.4, -0.3). After adjustment for weight and weight change, results, however, were no longer statistically significant.

## Discussion

In the cross-sectional analyses, there were no consistent associations between self-reported sleep measures and ghrelin or leptin and only a weak and nonsignificant positive association between sleep duration or napping and BMI. In the longitudinal analyses, we hypothesized that improved sleep might increase weight loss and attenuate exercise-induced increases in ghrelin and decreases in leptin (Figure 1). A finding such as this might suggest a damping of the compensatory metabolic mechanisms that lead to weight regain after weight loss. Nevertheless, despite these plausible hypotheses, changes in sleep were not strongly associated with changes in weight, ghrelin and leptin, and observed trends were typically in the direction opposite to our hypotheses. Specifically, the weight-loss difference

**Table 4** Differences in adjusted<sup>a</sup> changes in plasma ghrelin (pg/ml) and leptin (ng/ml) from baseline to 12 months among exercisers vs stretchers<sup>b</sup>, stratified by sleep-change measures

Changes in self-reported sleep from baseline to 12 months	Difference in <i>ghrelin</i> change (95% CI): exercisers–stretchers		Difference in <i>leptin</i> change (95% CI): exercisers–stretchers	
	Model 1 <sup>a</sup>	Model 2 <sup>a</sup>	Model 1 <sup>a</sup>	Model 2 <sup>a</sup>
Overall	+16 (–26, +59)	+2 (–39, +43)	–1.6 (–3.1, –0.1)	–0.7 (–2.0, +0.7)
<i>Sleep duration</i>				
More	+6 (–82, +93)	–19 (–103, +66)	–4.1 (–7.1, –1.0)	–2.4 (–5.1, +0.2) <sup>d,f</sup>
Same	+10 (–46, +67)	+10 (–44, +64)	–1.0 (–3.0, +1.0)	–0.8 (–2.5, +0.9) <sup>c,d</sup>
Less	+49 (–45, +143)	+13 (–80, +105)	–0.8 (–4.1, +2.5)	+1.8 (–1.1, +4.7) <sup>c</sup>
<i>Sleep quality</i>				
More sound	+98 (+16, +180) <sup>d</sup>	+73 (–8, +153) <sup>d</sup>	–4.6 (–7.5, –1.7) <sup>e,f</sup>	–3.2 (–5.7, –0.6) <sup>d,f</sup>
Same	–46 (–104, +13) <sup>c</sup>	–42 (–99, +15) <sup>c</sup>	–0.6 (–2.7, +1.4) <sup>c,d</sup>	–0.5 (–2.3, +1.3) <sup>c,d</sup>
More restless	+35 (–55, +125) <sup>c,d</sup>	–1 (–90, +89) <sup>c,d</sup>	+0.6 (–2.6, +3.8) <sup>c</sup>	+2.6 (–0.3, +5.4) <sup>c</sup>
<i>Falling asleep during quiet activities</i>				
Less	+31 (–55, +117)	+11 (–71, +93)	–3.8 (–6.7, –0.8) <sup>c</sup>	–2.4 (–5.0, +0.2)
Same	+2 (–60, +64)	+1 (–59, +61)	–0.2 (–2.3, +2.0) <sup>d</sup>	0.0 (–1.9, +1.9)
More	+17 (–68, +102)	–9 (–92, +73)	–2.0 (–5.0, +0.9) <sup>c,d</sup>	–0.5 (–3.1, +2.1)
<i>Napping during the day</i>				
Less	–68 (–182, +46)	–69 (–178, +39)	–2.8 (–6.8, +1.2) <sup>c,d</sup>	–2.8 (–6.3, +0.7)
Same	+10 (–45, +66)	+1 (–53, +55)	–0.0 (–2.0, +1.9) <sup>c</sup>	+0.7 (–1.1, +2.4)
More	+33 (–53, +119)	+2 (–82, +85)	–3.8 (–6.9, –0.8) <sup>d</sup>	–2.1 (–4.7, +0.6)

Abbreviation: CI, confidence interval. <sup>a</sup>Model 1: Adjusted for baseline age, sleep measures and ghrelin or leptin, respectively. Model 2: Adjusted for factors in model 1 in addition to baseline weight and change in weight from baseline to follow-up. <sup>b</sup>Five exercisers have no follow-up data on sleep and/or ghrelin or leptin. One stretcher whose baseline leptin was below the limits of detection (<0.5 ng/ml) was not included in any leptin analyses. <sup>c,d,e</sup>Categories with different superscripts are statistically significantly different from each other ( $P < 0.05$ ). <sup>f</sup> $P$  for trend <0.01.

between exercisers and stretchers was greater for those who slept less at follow-up than at baseline compared to those whose sleep duration did not change. Similarly, ghrelin levels *increased* significantly more and leptin levels *decreased* more among those who reported improved sleep quality compared to those with the same or worsened sleep quality. When we examined sleep quality and duration together, the basic pattern of findings did not change (data not presented). These findings might be explained by the fact that exercise was much more predictive of weight, ghrelin and leptin changes, than was sleep. There was a positive correlation between fitness increases and sleep improvements in exercisers ( $r = 0.27$  for sleep duration and  $r = 0.33$  for sleep quality, both  $P \leq 0.02$ ), with no association in stretchers, suggesting that sleep improvements might effectively be a proxy for exercise adherence.

We also considered baseline sleep duration, sleep quality and a variable combining duration and quality as predictors of our outcomes. Exercise-induced weight loss was greatest among those who slept  $\leq 6$  h per night (exercisers vs stretchers:  $-2.8$  kg, 95% CI  $-5.0, -0.6$ ) or reported poor sleep quality ( $-3.6$ , 95% CI  $-7.2, +0.1$ ) or, when combined, short duration but good quality ( $-7.3$  kg, 95% CI  $-11.2, -3.3$ ). These differences appeared to be caused by greater weight gain in stretchers with these sleep patterns (e.g., short sleep duration and poor quality or short sleep and good quality), rather than simply by greater weight loss in exercisers with these sleep patterns. There were no

statistically significant differences in ghrelin change between exercisers and stretchers for any baseline sleep duration or quality measure, nor were there any statistically significant differences in ghrelin changes across any sleep duration or quality measures. Leptin decreased significantly more in exercisers who slept 7 h per night ( $-3.0$  pg/ml, 95% CI  $-5.7, -0.3$ ) and who reported very restless/restless sleep quality at baseline ( $-5.5$  ng/ml, 95% CI  $-10.8, -0.3$ ), again, mainly because leptin increased the most among stretchers with these sleep patterns. Regardless, there were no significant differences in leptin changes across categories of sleep duration, quality or the combination. These findings are generally the reverse of the sleep-change findings (where exercise-induced decreases in leptin were greater among women with improved sleep quality compared to those with worsened sleep quality). This is in part because poor sleep quality at baseline could only stay the same or improve, but could not worsen.

To our knowledge, no previous study has examined whether sleep modifies the effect of exercise on weight, ghrelin or leptin. However, several studies have evaluated associations between sleep and ghrelin and leptin levels independent of exercise. A recent study investigated whether partial sleep curtailment (4 vs 10 h) for two consecutive nights altered ghrelin and leptin levels in 12 young men (mean age 22 years) using an experimental design.<sup>11</sup> Sleep deprivation was associated with a 28% mean increase in ghrelin ( $P < 0.05$ ) and an 18% mean decrease in leptin



( $P=0.04$ ) and led to increases in hunger and appetite. However, owing to the short duration of the trial, the investigators could not evaluate weight changes associated with sleep deprivation.<sup>11</sup> A notable difference between that study and the present one is that the investigators studied extremes of sleep duration (4 vs 10 h), whereas the current study had few individuals at the extremes or making large changes from baseline to follow-up. Specifically, of those whose sleep duration changed, only 9% increased or decreased by more than 1 h. In addition, it is unclear whether the biologic effects of sleep deprivation seen in healthy normal-weight young men would be the same as those in overweight, sedentary post-menopausal women.

In an observational study of 1024 men and women 30–60 years old (mean 52.7 years), Taheri *et al.*<sup>12</sup> evaluated cross-sectional relationships between objectively measured sleep duration and levels of leptin and ghrelin, as well as other hormones and lipid profiles. Short sleep (defined as <6 h per night), as measured by polysomnography, was positively associated with ghrelin, but not leptin. Conversely, sleep as measured by questionnaire was inversely associated with leptin, but not ghrelin. Based on regression models, the authors estimated that reducing sleep from 8 to 5 h per night would be associated with a 15.5% decrease in leptin and a 14.9% increase in ghrelin. Besides having objectively measured sleep, other key differences between that study and the present one are that that study had a sample size over five times as large, including men and individuals under 50 years of age, with more subjects in the 'restricted-sleep' range.<sup>12</sup>

Numerous other studies have observed a linear or U-shaped curve between sleep and BMI in adults and children,<sup>6,8,10,30–34</sup> such that BMIs tended to increase with decreasing sleep duration, with some studies also finding higher mean BMIs among those sleeping the most hours per night.<sup>8,19</sup> To our knowledge, only two studies have used a longitudinal study design.<sup>8,19</sup> With a cross-sectional design, it is unclear whether shorter sleep leads to weight gain and obesity or whether obesity and weight gain predispose to shorter sleep. Despite the longitudinal nature of the current data, it is nevertheless unclear whether sleep changes associated with weight change caused, or were caused by, the weight change. To determine this, a trial with a sleep intervention would be required. Nonetheless, there is accumulating evidence that sleep may be a potentially important behavioral factor involved in weight gain and obesity, but the relationships are not straightforward or simple.

Several other limitations of our study should be mentioned. It is unclear how sleeping less relates to sleep quality or deprivation, as the report of shortened sleep time could be representative of either less sleep or more consolidated sleep, the former indicative of sleep deprivation, the latter, not. Furthermore, data on sleep were collected via self-report, not measured objectively by actigraphy or polysomnography. Although there is some relationship between self-reported

sleep patterns and objective measures,<sup>24,35</sup> the two measurement strategies may assess different aspects of sleep.<sup>36</sup> In any case, it is likely that misclassification of sleep would be non-differential in relation to outcomes and exercise group, so this would most plausibly attenuate results. In addition, because this study involved six different sleep measures, and three different outcomes, multiple comparisons and type I errors (error of rejecting a true null hypothesis) are a concern. Consequently, caution must be exercised in interpreting observed associations. All women in the current study were sedentary and overweight or obese. If associations of interest vary by BMI, adiposity or even duration of excess adiposity or high BMI, the nature of the study population limited our ability to examine them. An additional limitation is that the exercise intervention led to only small changes in our outcomes of interest. Particularly for weight and ghrelin changes, we had limited power to detect associations. Finally, as studies indicate that ghrelin and leptin levels are responsive to acute changes in sleep, it is unclear whether a single morning fasting sample would closely reflect usual hormone levels over the previous month.<sup>11</sup> However, we have previously shown that a single morning fasting sample correlates very well with 24-h profiles for plasma ghrelin;<sup>37</sup> thus it is likely that it is also correlated with ghrelin levels over the previous month. Two strengths of this study were its randomized-controlled-trial design and its excellent subject adherence and retention. Furthermore, body weight was objectively measured, avoiding possible biases introduced by self-report.

In conclusion, self-reported sleep was not associated cross-sectionally with ghrelin or leptin. Furthermore, in this sample of overweight and obese post-menopausal women, sleep improvements did not cause greater weight loss in exercisers, nor did they moderate exercise-induced increases in ghrelin or decreases in leptin. Although our findings with baseline sleep are far from definitive, they are intriguing, and beg the question of whether exercise can ameliorate weight gain in women with sleep problems. Future longitudinal studies that include a population-based sample, employing objective measures of sleep and a long follow-up, may help to clarify these relationships.

## Acknowledgements

Dr Littman was partially funded by a training grant in cancer epidemiology R25 CA94880 through the National Cancer Institute. Dr Tworoger was partially supported by a training grant in cancer epidemiology T32 CA090001 from the National Cancer Institute.

## References

- 1 Foundation NS. *Sleep in America' Poll*. National Sleep Foundation: Washington, DC, 2002.

- 2 Foundation NS. *Sleep in America Poll*. National Sleep Foundation: Washington, DC, 2005.
- 3 Kripke DF, Simons RN, Garfinkel L, Hammond EC. Short and long sleep and sleeping pills. Is increased mortality associated? *Arch Gen Psychiatry* 1979; **36**: 103–116.
- 4 Hedley AA, Ogden CL, Johnson CL, Carroll MD, Curtin LR, Flegal KM. Prevalence of overweight and obesity among US children, adolescents, and adults, 1999–2002. *JAMA* 2004; **291**: 2847–2850.
- 5 Hill JO, Wyatt HR. Role of physical activity in preventing and treating obesity. *J Appl Physiol* 2005; **99**: 765–770.
- 6 Vioque J, Torres A, Quiles J. Time spent watching television, sleep duration and obesity in adults living in Valencia, Spain. *Int J Obes Relat Metab Disord* 2000; **24**: 1683–1688.
- 7 Flier JS, Elmquist JK. A good night's sleep: future antidote to the obesity epidemic? *Ann Intern Med* 2004; **141**: 885–886.
- 8 Hasler G, Buysse DJ, Klaghofer R, Gamma A, Ajdacic V, Eich D *et al*. The association between short sleep duration and obesity in young adults: a 13-year prospective study. *Sleep* 2004; **27**: 661–666.
- 9 Prinz P. Sleep, appetite, and obesity – what is the link? *PLoS Med* 2004; **1**: e61.
- 10 von Kries R, Toschke AM, Wurmser H, Sauerwald T, Koletzko B. Reduced risk for overweight and obesity in 5- and 6-y-old children by duration of sleep – a cross-sectional study. *Int J Obes Relat Metab Disord* 2002; **26**: 710–716.
- 11 Spiegel K, Tasali E, Penev P, Van Cauter E. Brief communication: sleep curtailment in healthy young men is associated with decreased leptin levels, elevated ghrelin levels, and increased hunger and appetite. *Ann Intern Med* 2004; **141**: 846–850.
- 12 Taheri S, Lin L, Austin D, Young T, Mignot E. Short sleep duration is associated with reduced leptin, elevated ghrelin, and increased body mass index. *PLoS Med* 2004; **1**: e62.
- 13 Cummings DE, Frayo RS, Marmonier C, Aubert R, Chapelot D. Plasma ghrelin levels and hunger scores in humans initiating meals voluntarily without time- and food-related cues. *Am J Physiol Endocrinol Metab* 2004; **287**: E297–E304.
- 14 Cummings DE, Shannon MH. Roles for ghrelin in the regulation of appetite and body weight. *Arch Surg* 2003; **138**: 389–396.
- 15 Badman MK, Flier JS. The gut and energy balance: visceral allies in the obesity wars. *Science* 2005; **307**: 1909–1914.
- 16 Leibel RL. The role of leptin in the control of body weight. *Nutr Rev* 2002; **60**: S15–S19; discussion S68–S84, 85–87.
- 17 Williams DL, Grill HJ, Cummings DE, Kaplan JM. Vagotomy dissociates short- and long-term controls of circulating ghrelin. *Endocrinology* 2003; **144**: 5184–5187.
- 18 Mantzoros CS, Qu D, Frederich RC, Susulic VS, Lowell BB, Maratos-Flier E *et al*. Activation of beta(3) adrenergic receptors suppresses leptin expression and mediates a leptin-independent inhibition of food intake in mice. *Diabetes* 1996; **45**: 909–914.
- 19 Gangwisch JE, Malaspina D, Boden-Albala B, Heymsfield SB. Inadequate sleep as a risk factor for obesity: analyses of the NHANES I. *Sleep* 2005; **28**: 1289–1296.
- 20 McTiernan A, Ulrich C, Yancey D, Slate S, Nakamura H, Oestreicher N *et al*. The Physical Activity for Total Health (PATH) Study: rationale and design. *Med Sci Sports Exerc* 1999; **31**: 1307–1312.
- 21 Burnam MA, Wells KB, Leake B, Landsverk J. Development of a brief screening instrument for detecting depressive disorders. *Med Care* 1988; **26**: 775–789.
- 22 Levine DW, Kripke DF, Kaplan RM, Lewis MA, Naughton MJ, Bowen DJ *et al*. Reliability and validity of the Women's Health Initiative Insomnia Rating Scale. *Psychol Assess* 2003; **15**: 137–148.
- 23 Levine DW, Kaplan RM, Kripke DF, Bowen DJ, Naughton MJ, Shumaker SA. Factor structure and measurement invariance of the Women's Health Initiative Insomnia Rating Scale. *Psychol Assess* 2003; **15**: 123–136.
- 24 Buysse DJ, Reynolds III CF, Monk TH, Berman SR, Kupfer DJ. The Pittsburgh Sleep Quality Index: a new instrument for psychiatric practice and research. *Psychiatry Res* 1989; **28**: 193–213.
- 25 Murakami N, Hayashida T, Kuroiwa T, Nakahara K, Ida T, Mondal MS *et al*. Role for central ghrelin in food intake and secretion profile of stomach ghrelin in rats. *J Endocrinol* 2002; **174**: 283–288.
- 26 Ariyasu H, Takaya K, Hosoda H, Iwakura H, Ebihara K, Mori K *et al*. Delayed short-term secretory regulation of ghrelin in obese animals: evidenced by a specific RIA for the active form of ghrelin. *Endocrinology* 2002; **143**: 3341–3350.
- 27 Nakai Y, Hosoda H, Nin K, Ooya C, Hayashi H, Akamizu T *et al*. Plasma levels of active form of ghrelin during oral glucose tolerance test in patients with anorexia nervosa. *Eur J Endocrinol* 2003; **149**: R1–R3.
- 28 Irwin ML, Yasui Y, Ulrich CM, Bowen D, Rudolph RE, Schwartz RS *et al*. Effect of exercise on total and intra-abdominal body fat in postmenopausal women: a randomized controlled trial. *JAMA* 2003; **289**: 323–330.
- 29 Foster-Schubert KE, McTiernan A, Frayo RS, Schwartz RS, Rajan KB, Yasui Y *et al*. Human plasma ghrelin levels increase during a one-year exercise program. *J Clin Endocrinol Metab* 2005; **90**: 820–825.
- 30 Kripke DF, Garfinkel L, Wingard DL, Klauber MR, Marler MR. Mortality associated with sleep duration and insomnia. *Arch Gen Psychiatry* 2002; **59**: 131–136.
- 31 Locard E, Mamelie N, Billette A, Miginiac M, Munoz F, Rey S. Risk factors of obesity in a five year old population. Parental versus environmental factors. *Int J Obes Relat Metab Disord* 1992; **16**: 721–729.
- 32 Sekine M, Yamagami T, Handa K, Saito T, Nanri S, Kawaminami K *et al*. A dose-response relationship between short sleeping hours and childhood obesity: results of the Toyama Birth Cohort Study. *Child Care Health Dev* 2002; **28**: 163–170.
- 33 Shigeta H, Shigeta M, Nakazawa A, Nakamura N, Yoshikawa T. Lifestyle, obesity, and insulin resistance. *Diabetes Care* 2001; **24**: 608.
- 34 Vorona RD, Winn MP, Babineau TW, Eng BP, Feldman HR, Ware JC. Overweight and obese patients in a primary care population report less sleep than patients with a normal body mass index. *Arch Intern Med* 2005; **165**: 25–30.
- 35 Coates TJ, Killen JD, George J, Marchini E, Silverman S, Thoresen C. Estimating sleep parameters: a multitrait-multimethod analysis. *J Consult Clin Psychol* 1982; **50**: 345–352.
- 36 Carskadon MA, Dement WC, Mitler MM, Guilleminault C, Zarcone VP, Spiegel R. Self-reports versus sleep laboratory findings in 122 drug-free subjects with complaints of chronic insomnia. *Am J Psychiatry* 1976; **133**: 1382–1388.
- 37 Cummings DE, Purnell JQ, Frayo RS, Schmidova K, Wisse BE, Weigle DS. A preprandial rise in plasma ghrelin levels suggests a role in meal initiation in humans. *Diabetes* 2001; **50**: 1714–1719.