Effect of Weight Gain on Cardiac Autonomic Control During Wakefulness and Sleep

Taro Adachi, Fatima H. Sert-Kuniyoshi, Andrew D. Calvin, Prachi Singh, Abel Romero-Corral, Christelle van der Walt, Diane E. Davison, Jan Bukartyk, Tomas Konecny, Snigdha Pusalavidyasagar, Justo Sierra-Johnson, Virend K. Somers

Abstract—Obesity has been associated with increased cardiac sympathetic activation during wakefulness, but the effect on sleep-related sympathetic modulation is not known. The aim of this study was to investigate the effect of fat gain on cardiac autonomic control during wakefulness and sleep in humans. We performed a randomized, controlled study to assess the effects of fat gain on heart rate variability. We recruited 36 healthy volunteers, who were randomized to either a standardized diet to gain ≈4 kg over 8 weeks followed by an 8-week weight loss period (n=20) or to serve as a weight-maintainer control (n=16). An overnight polysomnogram with power spectral analysis of heart rate variability was performed at baseline, after weight gain, and after weight loss to determine the ratio of low-frequency to high-frequency power and to examine the relationship between changes in heart rate variability and changes in insulin, leptin, and adiponectin levels. Mean weight gain was 3.9 kg in the fat gain group versus 0.1 kg in the maintainer group. Low frequency/high frequency increased both during wakefulness and sleep after fat gain and returned to baseline after fat loss in the fat gain group and did not change in the control group. Insulin, leptin, and adiponectin also increased after fat gain and fell after fat loss, but no clear pattern of changes was seen that correlated consistently with changes in heart rate variability. Short-term fat gain in healthy subjects is associated with increased cardiac sympathetic activation during wakefulness and sleep, but the mechanisms remain unclear. (Hypertension. 2011;57:723-730.)

Key Words: weight gain ■ heart rate variability ■ sympathetic nerve activity ■ obesity ■ insulin ■ leptin ■ adiponectin

There is considerable evidence that overweight or obesity increases cardiovascular morbidity and mortality.1–3 A number of mechanisms, including sympathetic activation,4 have been proposed to explain this association.5–8

Obesity has been linked with increased peripheral9–13 and cardiac sympathetic activation. Modest weight gain has been associated with increased muscle sympathetic nerve activity in nonobese men.13 Mechanisms linking obesity to alterations in neural circulatory control are not well defined, but it has been postulated that the increased circulating leptin and insulin and decreased adiponectin16 are associated with increased cardiac sympathetic activity and vasoconstriction in obese people.17

Sleep accounts for approximately one third of our lives and is accompanied by significant changes in autonomic and circulatory regulation. Rapid eye movement (REM) sleep in particular is associated with enhanced muscle sympathetic nerve activity,18 striking fluctuations in heart rate, and alterations in coronary artery blood flow.19 Meanwhile, the early morning transition from sleep to wakefulness is associated with an increased risk of sudden cardiac death,20 stroke,21 and myocardial infarction.22 Assessment of sympathetic activation during periods of sleep and wakefulness may be clinically relevant and can be enabled by power spectral analysis of heart rate variability (HRV).23–25 Furthermore, there are no data regarding the effects of weight gain and related changes in insulin, leptin, and adiponectin on sleep-related cardiac sympathetic modulation. The aim of this study, therefore, was to investigate the effects of weight gain and subsequent weight loss on cardiac autonomic control during sleep as measured by HRV in healthy subjects and to examine the relationship between these changes and changes in insulin, leptin, and adiponectin concentration.

Methods

Subjects

This study was approved by the Mayo Clinic Institutional Review Board, and written informed consent was obtained from each subject. We recruited 36 volunteers, and after a weight maintenance period of 3 days, subjects were randomly assigned to be in the fat-gainer (n=20) or weight-maintainer group (n=16). Exclusion criteria...
included use of any tobacco products, employment in shift work, previous diagnosis of any disease including any sleep-related disorder, and use of any prescription medications other than oral contraceptives. Findings from this study relating to endothelial dysfunction have been published elsewhere.26

Weight-Gain and Weight-Loss Protocols
Each subject received weight-maintenance meals from our metabolic kitchen for 3 days before each phase. The menus were based on the standardized foods available in the metabolic kitchen at the Mayo Clinic Clinical Research Unit and each subject’s food preferences. Weight maintenance caloric needs were calculated per the Harris-Benedict equation,27 plus an additional 30% to 60% to match occupational activity needs. After the weight maintenance period of 3 days, those randomized to gain weight received a diet with 1000 kcal/d beyond their weight maintenance requirements for 8 weeks, whereas those randomized to maintain weight continued to receive the same diet for 8 weeks. The goal was to gain ~3 to 4 kg of total body fat (~5% increase in weight), and weight was measured ±5 days per week. After the fat gain period, subjects underwent a supervised diet program for 8 weeks to return to their basal weight. The diet composition throughout the study was 40% carbohydrate, 40% fat, and 20% protein. Cardiopulmonary exercise testing at baseline, after weight gain, and after weight loss was conducted to assess levels of physical fitness. The study outline is shown in Figure 1.

Polysomnography
Patients underwent nocturnal, laboratory-based attended digital polysomnography in the Center for Translational Science Activities Sleep Facility at the Mayo Clinic Clinical Research Unit. Polysomnograms were recorded using a Compumedics E-Series Comprehensive Networked-Linked Amplifier (Compumedics). Polysomnograms were scored by an experienced registered polysomnographic technologist in accordance with current American Academy of Sleep Medicine guidelines.28

HRV Spectral Analysis
HRV was measured during wakefulness, non-REM (NREM) sleep, and REM sleep. The data obtained during wakefulness were recorded for 5 minutes at 10:00 pm before sleep onset. The data during NREM and REM sleep were visually identified from the polysomnographic recordings, and whole segments from the first and second epoch of each sleep stage were selected for analysis with the results averaged. Measurements were taken only during established sleep stages during periods of stable breathing not associated with any arousals. ECG signals from bipolar leads were transformed to digital signals to calculate the R-R intervals at a sampling rate of 512 Hz. Power spectral analysis of HRV was performed by the MemCalc power spectral density method using a commercial software package (MemCalc/Win, Suwa Trust) that used the maximum entropy method for spectral analysis and the nonlinear least-squares method for fitting analysis.

Low frequency (LF) was defined as 0.04 to 0.15 Hz, and high frequency (HF) was defined as 0.15 to 0.40 Hz. The LF component was corrected to normalized units (nu) using the equation \( LF_{nu} = LF/(HF+LF) \), and the HF component was corrected to normalized units as \( HF_{nu} = HF/(LF+HF) \).

Measurements of Body Composition
Body composition was measured at baseline, after weight gain, and after recovery and included height measured by wall stadiometer, weight by an electronic scale, waist and hip circumferences by nonelastic tape, and body fat by dual-energy x-ray absorptiometry (Lunar Radiation).

Blood Measurements
Fasting blood samples were obtained by venipuncture immediately after polysomnography at 6:00 AM and assayed in the immunochemical core laboratory at the Mayo Clinic Clinical Research Unit. Plasma glucose levels were measured using the standard turbidimetric method using a Hitachi 912 (Roche Diagnostic); plasma insulin levels were measured using a 2-site immune enzymatic assay (Beckman Instruments); plasma leptin levels were measured with commercially available radioimmunoassay kits (Linco Research); and plasma adiponectin levels were measured using ELISA kits (Mediangost). Homeostatic model assessment of insulin resistance was calculated with the formula as plasma insulin (in microunits per milliliter) \( \times \) fasting glucose (in milligrams per deciliter)/405.29 This index is considered to be a useful marker for simple assessment of insulin resistance.

Statistical Analysis
Data are summarized as number and percentage for categorical variables and means with SEM for continuous variables. Changes in HRV between baseline and after weight gain, between weight gain and after recovery, and between baseline and after recovery were prespecified analyses evaluated by Wilcoxon sign-rank test. As an exploratory analysis, the correlation among circulating insulin, leptin, and adiponectin and those HRV parameters that changed significantly during the study was assessed using the Spearman correlation coefficient. Analyses were performed with JMP version 8 (SAS Institute). A 2-sided \( P \) value of <0.05 was considered statistically significant, and a Bonferroni correction was used to correct for multiple comparisons involving the 3 measures of spectral power (\( LF_{nu} \), \( HF_{nu} \), and \( LF/HF \); \( P<0.016 \)).

Results
We recruited 36 healthy volunteers, 22 men and 14 women, between the ages of 18 and 50 years (mean: 29.6±1.3 years).

Glucose levels were significantly different between fat gainers (n=20) and weight maintainers (n=16) at baseline (98.0 versus 88.5 mg/dL; \( P<0.01 \)). Baseline body fat percentage in the fat gainers and weight maintainers was not significantly different (31.7% versus 29.6%; \( P=0.15 \)). There were no differences in any variable measured between baseline and at the 8-weeks time point in the weight-maintainer group. In the fat-gainer group, subjects gained an average of 3.9±0.2 kg in the weight gain period, which was also reflected by increases in body fat and waist and hip circumference. However, blood pressure, volume of oxygen peak, apnea-hypopnea index, total sleep time, and number of arousals did not change during the study in the fat-gainer group (Table 1).

Weight gain was associated with increased circulating concentrations of both insulin (5.3 versus 7.1 \( \mu \)U/mL; \( P<0.05 \)) and leptin (5.2 versus 9.8 ng/mL; \( P<0.01 \)) and a trend toward increased adiponectin concentration (8129 versus 9339 ng/mL; \( P=0.17 \)). After weight loss, circulating levels of insulin, leptin, and adiponectin fell toward baseline levels. Fasting plasma glucose concentrations did not significantly change after weight gain (98.0 versus 100.2 mg/dL; \( P=0.92 \)) or after weight loss (100.2 versus 95.3 mg/dL;
Changes in HRV during wakefulness, during REM sleep, and during NREM sleep are presented in Table 2 and Figure 2. During wakefulness (Figure 2A), there was a significant decrease in HFnu along with an increase in LFnu and LF/HF ratio (0.39 versus 0.31 nu, P<0.01; 0.61 versus 0.69 nu, P<0.01; and 2.00 versus 2.99, P<0.01, respectively) after weight gain. Moreover, changes in HFnu, LFnu, and LF/HF ratio resolved with weight loss and returned toward baseline levels (0.38 versus 0.39 nu, P=0.88; 0.62 versus 0.61 nu, P=0.88; and 2.39 versus 2.00, P=0.48, respectively). During REM sleep (Figure 2B), there was a significant decrease in HFnu (0.29 versus 0.23 nu; P<0.01), and a slight increase in LFnu and LF/HF ratio (0.71 versus 0.73 nu, P=0.05 and 3.22 versus 4.16, P=0.02, respectively) after weight gain. On the other hand,
Figure 2. Changes in HRV during wakefulness (A), during REM sleep (B), and during NREM sleep (C). Data are presented as mean ± SEM.
HFnu was slightly decreased (0.73 versus 0.68, *P* < 0.01), and LF/HF ratio was significantly decreased (4.16 versus 2.95, *P* < 0.01) after weight loss. During NREM sleep (Figure 2C), no significant changes were observed in HFnu and LFnu, either after weight gain (0.46 versus 0.40, *P* = 0.05 and 0.54 versus 0.59, *P* = 0.13, respectively) or after weight loss (0.40 versus 0.46, *P* = 0.07 and 0.59 versus 0.55, *P* = 0.11, respectively), although the LF/HF ratio trended up after weight gain (1.57 versus 2.48, *P* = 0.02) and returned to approximately baseline values after recovery (2.48 versus 1.65, *P* = 0.03). None of the HRV parameters changed between baseline and follow-up in the weight-maintainer group (Table 2).

Changes of heart rate during wakefulness, during REM sleep, and during NREM sleep are presented in Table 3. During wakefulness, during REM sleep, and during NREM sleep, heart rate was significantly increased after weight gain (60.3 versus 64.5 bpm, *P* = 0.03; 58.8 versus 62.1 bpm, *P* = 0.02; and 56.9 versus 61.0 bpm, *P* < 0.01, respectively) and decreased after weight loss (64.5 versus 57.6 bpm, *P* < 0.01; 62.1 versus 54.5 bpm, *P* < 0.01; and 61.0 versus 54.7 bpm, *P* < 0.01, respectively). Heart rate in recovery decreased slightly from baseline during wakefulness and during NREM sleep (57.6 versus 60.3 bpm, *P* = 0.05 and 54.7 versus 56.9 bpm, *P* = 0.06, respectively) and significantly decreased during NREM sleep (54.5 versus 58.8 bpm, *P* < 0.01). Heart rate did not change between baseline and follow-up in the weight-maintainer group (Table 3).

Changes in HRV measurements after weight gain were not associated with changes in insulin, leptin, or adiponectin levels during wakefulness or during NREM sleep. During REM sleep, the only significant correlation was between LFnu and leptin level (*r* = 0.59; *P* = 0.021 Table 4).

<table>
<thead>
<tr>
<th>Variable</th>
<th>Changes in Insulin</th>
<th>Changes in Leptin</th>
<th>Changes in Adiponectin</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td><em>ρ</em></td>
<td><em>P</em></td>
<td><em>ρ</em></td>
</tr>
<tr>
<td>From Baseline to Weight Gain</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Change HFnu during wakefulness</td>
<td>−0.17</td>
<td>0.67</td>
<td>−0.26</td>
</tr>
<tr>
<td>Change LFnu during wakefulness</td>
<td>0.17</td>
<td>0.67</td>
<td>0.26</td>
</tr>
<tr>
<td>Change LF/HF during wakefulness</td>
<td>−0.25</td>
<td>0.51</td>
<td>0.10</td>
</tr>
<tr>
<td>Change HFnu during REM sleep</td>
<td>−0.31</td>
<td>0.46</td>
<td>−0.48</td>
</tr>
<tr>
<td>Change LFnu during REM sleep</td>
<td>0.31</td>
<td>0.46</td>
<td>0.59</td>
</tr>
<tr>
<td>Change LF/HF during REM sleep</td>
<td>0.24</td>
<td>0.57</td>
<td>0.31</td>
</tr>
<tr>
<td>Change HFnu during NREM sleep</td>
<td>…*</td>
<td>…*</td>
<td>…*</td>
</tr>
<tr>
<td>Change LFnu during NREM sleep</td>
<td>…*</td>
<td>…*</td>
<td>…*</td>
</tr>
<tr>
<td>Change LF/HF during NREM sleep</td>
<td>−0.04</td>
<td>0.89</td>
<td>−0.04</td>
</tr>
<tr>
<td>From weight gain to recovery</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Change HFnu during wakefulness</td>
<td>0.27</td>
<td>0.49</td>
<td>−0.44</td>
</tr>
<tr>
<td>Change LFnu during wakefulness</td>
<td>−0.27</td>
<td>0.49</td>
<td>0.43</td>
</tr>
<tr>
<td>Change LF/HF during wakefulness</td>
<td>−0.09</td>
<td>0.81</td>
<td>0.34</td>
</tr>
<tr>
<td>Change HFnu during REM sleep</td>
<td>0.23</td>
<td>0.56</td>
<td>0.18</td>
</tr>
<tr>
<td>Change LFnu during REM sleep</td>
<td>−0.23</td>
<td>0.56</td>
<td>−0.19</td>
</tr>
<tr>
<td>Change LF/HF during REM sleep</td>
<td>−0.19</td>
<td>0.62</td>
<td>0.05</td>
</tr>
<tr>
<td>Change HFnu during NREM sleep</td>
<td>…*</td>
<td>…*</td>
<td>…*</td>
</tr>
<tr>
<td>Change LFnu during NREM sleep</td>
<td>…*</td>
<td>…*</td>
<td>…*</td>
</tr>
<tr>
<td>Change LF/HF during NREM sleep</td>
<td>0.50</td>
<td>0.20</td>
<td>−0.07</td>
</tr>
</tbody>
</table>

*Data not presented as this HRV variable did not change with weight gain or recovery.*
Changes in HRV measurements from weight gain to recovery were not associated with changes in insulin or leptin during wakefulness, during REM sleep, or during NREM sleep. Changes in adiponectin concentration correlated with changes in HFnu (r = -0.59; P = 0.03), LFnu (r = 0.58; P = 0.03), and LF/HF (r = 0.73; P < 0.01) only during wakefulness but not during REM sleep or NREM sleep (Table 4).

Discussion

The novel finding of this study is that modest short-term weight gain is associated with changes in cardiac sympathovagal balance favoring sympathetic drive not only during wakefulness but also during sleep, and this increased sympathetic activation resolves with weight loss. In the same way, modest short-term weight gain is associated with parasympathetic attenuation, during wakefulness and REM sleep, which resolves with weight loss. To the best of our knowledge, this is the first report of the effect of short-term weight gain followed by weight loss on cardiac autonomic control during wakefulness and sleep in healthy humans.

The increase in LFnu and decrease in HFnu suggest an increase in cardiac sympathetic activation together with a reduction in parasympathetic (vagal) activation. Although previous studies have suggested that weight gain and obesity are associated with increased sympathetic nerve activity, that weight loss is associated with a reduction in sympathetic nerve activity in obese subjects, and that fat gain influences both the sympathetic and parasympathetic nervous systems in humans, the cross-sectional or observational nature of these previous studies limited the ability to assess causality. The prospective, randomized, longitudinal nature of our study, on the other hand, allows us to conclude that the increase in sympathetic activity is likely attributable to fat gain. Moreover, our study shows that increased cardiac sympathetic activity associated with weight gain and the decreased cardiac sympathetic activity associated with weight loss are evident not only during wakefulness but also during sleep.

There is evidence linking changes in cardiac autonomic drive to arousals from sleep and obstructive events, and although this seems unlikely to explain our results, because we observed neither an increase in the number of arousals or apnea-hypopnea index during the study, it is possible that more subtle changes in respiratory mechanics occurred.

As leptin increases with weight gain, it has been speculated that the effect of increased body fat on sympathetic drive is mediated by this adipokine. We confirmed that leptin increased after short-term experimental weight gain and found that changes in leptin correlated with changes in LF, but not LF/HF, during REM sleep. Similarly, it has been reported that hyperinsulinemia increases sympathetic activity. However, our data do not show a relationship between changes in circulating insulin and changes in HRV. Adiponectin is a protein secreted from adipose tissue that activates the AMP-activated protein kinase in the peripheral tissues. Adiponectin increases insulin sensitivity and decreases insulin concentration and, therefore, may indirectly influence sympathetic activity. We noted a correlation between changes in adiponectin concentration and changes in HRV between weight gain and weight loss only during wakefulness.

The major strengths of our study include its longitudinal experimental design and inclusion of normal healthy subjects without medical conditions or medications that might have confounded our results. Furthermore, our rigorous laboratory-based polysomnography and HRV analyses strengthen our conclusions. Changes in physical conditioning are also unlikely to explain our findings, because exercise tolerance was unchanged at the different stages of the protocol. However, some limitations should be considered. The magnitude, rate, and duration of fat gain in this study likely do not reflect the long-term severe and chronic weight gain, and so our results may not be readily extrapolated to obese people in the general population. Moreover, we were not able to determine whether the observed changes were related to changes in the diet or to weight gain. Finally, we examined just 3 potential mechanisms that may link weight gain with cardiac sympathetic activity, namely, circulating concentrations of insulin, leptin, and adiponectin. It is unclear whether the lack of clear association between changes in these hormones and cardiac sympathetic activity was because of our sample size and the short-term nature of our study of a chronic process or reflective of a more complex relationship among insulin, insulin resistance, leptin, leptin resistance, adiposity, and neurohormonal changes that occur with fat gain.

The increase in sympathetic balance during sleep associated with weight gain may have important clinical implications. The conversion from sleep to wakefulness is associated with an increased risk of sudden cardiac death, stroke, and myocardial infarction. Hence, fluctuation of autonomic nervous activity during both wakefulness and sleep is likely of clinical importance. During REM sleep there is normally intense vascular sympathetic activation associated with wide fluctuations in cardiac autonomic drive. These changes are associated with a reduction in coronary artery blood flow in the setting of coronary stenosis and with variant angina in some patients.

Increased cardiac sympathetic balance during sleep may exacerbate this phenomenon and perhaps contribute to increased cardiovascular morbidity and mortality associated with obesity. The reduction of cardiac sympathetic drive after weight loss suggests that the changes associated with short-term weight gain are reversible. Whether reduction of body fat after years of chronic severe obesity results in a similar effect is unknown and would have significant implications for global public health.

Perspectives

Our findings suggest that modest, short-term weight gain is associated with increased cardiac sympathetic activity not only during wakefulness but also during sleep, which is reversible by weight loss in healthy individuals. These data may be relevant to our understanding of mechanisms underlying the association between weight gain and cardiovascular morbidity and mortality.
Acknowledgments
We greatly thank Debra L. Pfeifer and Ann B. Peterson for administrative assistance and Toru Suzuki and Wataru Hayashi of GMS, Co, for guidance with HRV measurements.

Sources of Funding
T.A. was supported by the Japanese Heart Foundation and the Japanese Society of Electrocardiology. F.H.S.-K. was supported by American Heart Association grant 09-20069G. A.D.C. is supported by the Mayo Clinic Clinician-Investigator Training Program. T.K. was partially supported from the scientific grant of the Czech Ministry of Health #NT 11401-5/2011. V.K.S. is supported by National Institutes of Health grants HL73211, HL65176, R21 HL96071-01, and 1 UL1 RR024150.

Disclosures
V.K.S. has served as a Consultant for ResMed, Cardiac Concepts, Apnex Medical, and Sova Pharmaceuticals and has been a principal investigator or coinvestigator on research grants funded by the Respironics Foundation and the Sorin Corporation. F.H.S.-K. became an employee of Philips Respironics, Inc, after the collection of the data presented in this article.

References
27. Harris JA, Benedict FG. A biometric study of basal metabolism in man. 1919;27.
Effect of Weight Gain on Cardiac Autonomic Control During Wakefulness and Sleep
Taro Adachi, Fatima H. Sert-Kuniyoshi, Andrew D. Calvin, Prachi Singh, Abel Romero-Corral, Christelle van der Walt, Diane E. Davison, Jan Bukartyk, Tomas Konecny, Snigdha Pusalavidyasagar, Justo Sierra-Johnson and Virend K. Somers

Hypertension. 2011;57:723-730; originally published online February 28, 2011; doi: 10.1161/HYPERTENSIONAHA.110.163147

Hypertension is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2011 American Heart Association, Inc. All rights reserved.
Print ISSN: 0194-911X. Online ISSN: 1524-4563

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://hyper.ahajournals.org/content/57/4/723

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Hypertension can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Hypertension is online at:
http://hyper.ahajournals.org//subscriptions/