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 cardiac14 sympathetic activation. Modest weight gain has been associated with increased muscle sympathetic nerve activity in nonobese men.15 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.

Polysonomography
Patients underwent nocturnal, laboratory-based attended digital polysonomography 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 method29 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 (nus) using the equation LFnus=LF/(LF+HF), and the HF component was corrected to normalized units as HFnus=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 (Mediagnost). Homeostatic model assessment of insulin resistance was calculated with the formula as plasma insulin (in microunits per milliliter) × fasting glucose (in milligrams per deciliter)/405.30 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 (LFnu, HFnu, 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 μU/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;
homeostatic model assessment of insulin resistance. Data are presented as mean ± SEM. HOMA-IR indicates homeostatic model assessment of insulin resistance.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Fat Gainers (n=20)</th>
<th>Weight Maintainers (n=16)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Baseline</td>
<td>Weight Gain</td>
</tr>
<tr>
<td>Age, y</td>
<td>29.7±1.4</td>
<td>...</td>
</tr>
<tr>
<td>Women, %</td>
<td>40</td>
<td>...</td>
</tr>
<tr>
<td>Body weight, kg</td>
<td>73.9±3.4</td>
<td>...</td>
</tr>
<tr>
<td>Body fat, %</td>
<td>31.7±1.9</td>
<td>...</td>
</tr>
<tr>
<td>Body mass index, kg/m²</td>
<td>24.4±0.9</td>
<td>...</td>
</tr>
<tr>
<td>Waist circumference, cm</td>
<td>82.3±2.3§</td>
<td>...</td>
</tr>
<tr>
<td>Hip circumference, cm</td>
<td>98.2±1.8</td>
<td>...</td>
</tr>
<tr>
<td>Systolic blood pressure, mm Hg</td>
<td>118.3±2.9</td>
<td>119.4±3.3</td>
</tr>
<tr>
<td>Diastolic blood pressure, mm Hg</td>
<td>73.7±2.5</td>
<td>74.6±2.2</td>
</tr>
<tr>
<td>Resting heart rate, bpm</td>
<td>67.7±2.7</td>
<td>68.3±2.6</td>
</tr>
<tr>
<td>Volume of oxygen peak, mL/kg per min</td>
<td>36.0±2.3</td>
<td>36.2±2.0</td>
</tr>
<tr>
<td>Total sleep time, min</td>
<td>353.0</td>
<td>367.8±8.5</td>
</tr>
<tr>
<td>AHI, events per h</td>
<td>1.1±0.3</td>
<td>0.9±0.3</td>
</tr>
<tr>
<td>No. of arousals, events per h</td>
<td>19.1±2.4</td>
<td>21.9±2.8</td>
</tr>
<tr>
<td>Glucose, mg/dL</td>
<td>98.0±2.1</td>
<td>100.2±5.0</td>
</tr>
<tr>
<td>Insulin, μU/mL</td>
<td>5.3±0.7</td>
<td>7.1±0.9*</td>
</tr>
<tr>
<td>HOMA-IR</td>
<td>1.4±0.2</td>
<td>2.0±0.3</td>
</tr>
<tr>
<td>Leptin, ng/mL</td>
<td>6.5±1.0</td>
<td>10.9±1.5§</td>
</tr>
<tr>
<td>Adiponectin, ng/mL</td>
<td>8129±1218</td>
<td>9339±1477‡</td>
</tr>
</tbody>
</table>

Data are presented as mean ± SEM. HOMA-IR indicates homeostatic model assessment of insulin resistance.

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 HFn u 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 HFn u, 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 HFn u (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 significantly increased (0.23 versus 0.29 nu; \( P < 0.01 \)), LFnu was slightly decreased (0.73 versus 0.68 nu; \( P = 0.04 \)), 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 nu, \( P = 0.05 \) and 0.54 versus 0.59 nu, \( P = 0.13 \), respectively) or after weight loss (0.40 versus 0.46 nu, \( P = 0.07 \) and 0.59 versus 0.55 nu, \( P = 0.11 \), 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 ($r = 0.59$; \( P = 0.02 \) Table 4).

### Table 3. HR Data During the Fat-Gain and Weight-Maintenance Protocols

<table>
<thead>
<tr>
<th>Heart Rate, bpm</th>
<th>Fat Gainers (n=20)</th>
<th>Weight Maintainers (n=16)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Baseline</td>
<td>Weight Gain</td>
</tr>
<tr>
<td>During wakefulness</td>
<td>60.3±2.3</td>
<td>64.5±1.8‡</td>
</tr>
<tr>
<td>During REM sleep</td>
<td>58.8±1.9‡</td>
<td>62.1±1.8*‡</td>
</tr>
<tr>
<td>During NREM sleep</td>
<td>56.9±2.1</td>
<td>61.0±1.8‡‡</td>
</tr>
</tbody>
</table>

Data are presented as mean±SEM. There was no significant difference at baseline between fat gainers and weight maintainers.  
*\( P < 0.05 \) when compared with baseline (within-group comparison).
†\( P < 0.01 \) when compared with baseline (within-group comparison).
‡\( P < 0.01 \) when compared with recovery (within-group comparison).

### Table 4. Correlation Coefficients ($\rho$) Between Changes in HRV Measurements and Changes in Metabolic Markers

<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>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>-0.26</td>
<td>-0.45</td>
</tr>
<tr>
<td>Change LFnu during wakefulness</td>
<td>0.17</td>
<td>0.26</td>
<td>0.45</td>
</tr>
<tr>
<td>Change LF/HF during wakefulness</td>
<td>-0.25</td>
<td>0.10</td>
<td>0.30</td>
</tr>
<tr>
<td>Change HFnu during REM sleep</td>
<td>-0.31</td>
<td>-0.48</td>
<td>-0.28</td>
</tr>
<tr>
<td>Change LFnu during REM sleep</td>
<td>0.31</td>
<td>0.59</td>
<td>0.34</td>
</tr>
<tr>
<td>Change LF/HF during REM sleep</td>
<td>0.24</td>
<td>0.31</td>
<td>0.01</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>-0.04</td>
<td>0.03</td>
</tr>
</tbody>
</table>

From weight gain to recovery

| Change HFnu during wakefulness   | 0.27                | -0.44             | -0.59                  |
| Change LFnu during wakefulness   | -0.27               | 0.43              | 0.58                   |
| Change LF/HF during wakefulness  | -0.09               | 0.34              | 0.73                   |
| Change HFnu during REM sleep     | 0.23                | 0.18              | -0.20                  |
| Change LFnu during REM sleep     | -0.23               | -0.19             | 0.28                   |
| Change LF/HF during REM sleep    | -0.19               | 0.05              | 0.47                   |
| Change HFnu during NREM sleep    | ...*                | ...*              | ...*                   |
| Change LFnu during NREM sleep    | ...*                | ...*              | ...*                   |
| Change LF/HF during NREM sleep   | -0.50               | -0.07             | 0.45                   |

*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,33–35 that weight loss is associated with a reduction in sympathetic nerve activity in obese subjects,36 and that fat gain influences both the sympathetic and parasympathetic nervous systems in humans,37 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 attributed 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.38 and although this seems unlikely to explain our results, because we observed neither an increase in the number of arousals or hypopnea index during the study, it is possible that more subtle changes in respiratory mechanics occurred.

As leptin increases with weight gain,39,40 it has been speculated that the effect of increased body fat on sympathetic drive is mediated by this adipokine.41 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.42 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 concentration43 and, therefore, may indirectly influence sympathetic activity. We noted a correlation be-

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**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.
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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.

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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


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