Sympathetic Nervous System Activity Is Associated With Obesity-Induced Subclinical Organ Damage in Young Adults

Elisabeth Lambert, Carolina Ika Sari, Tye Dawood, Julie Nguyen, Mariee McGrane, Nina Eikelis, Reena Chopra, Chiew Wong, Kanella Chatzivlastou, Geoff Head, Nora Straznicky, Murray Esler, Markus Schlaich, Gavin Lambert

Abstract—Excess weight is established as a major risk factor for cardiovascular diseases, particularly in young individuals. To get a better understanding of the pathophysiology underlying increased cardiovascular disease risk, we evaluated early signs of organ damage and their possible relationship to sympathetic nervous activity. Eighteen lean (body mass index <25 kg/m²) and 25 overweight or obese (body mass index ≥25 kg/m²) healthy university students were included in the study. We comprehensively assessed subclinical target organ damage, including the following: (1) assessment of renal function; (2) left ventricular structure and systolic and diastolic function; and (3) endothelial function. Muscle sympathetic nervous activity was assessed by microneurography. Participants with excess weight had decreased endothelial function (P<0.01), elevated creatinine clearance (P<0.05), increased left ventricular mass index (P<0.05), increased left ventricular wall thickness (P<0.01), lower systolic and diastolic function (P<0.01), and elevated muscle sympathetic nervous activity (P<0.001) compared with lean individuals. In multiple regression analysis, endothelial function was inversely related to muscle sympathetic nervous activity (R²=0.244; P<0.05), whereas creatinine clearance and left ventricular mass index were positively related to muscle sympathetic nervous activity, after adjustment for body mass index, sex, and blood pressure (R²=0.318, P<0.01 and R²=0.312, P<0.05, respectively). Excess weight in young individuals is associated with subclinical alterations in renal and endothelial function, as well as in the structure of the heart, even in the absence of hypertension. Sympathetic activity is closely associated with cardiovascular and renal alterations observed in these subjects. (Hypertension. 2010;56:351-358.)

Key Words: sympathetic nervous system ■ obesity ■ cardiovascular risk factors ■ cardiac function ■ renal function ■ young adults

Obesity is an established risk factor for cardiovascular disease (CVD) development. Although excess adiposity is frequently linked with metabolic abnormalities such as elevated triglycerides, low levels of high-density lipoprotein (HDL), elevated glucose, elevated blood pressure (BP), insulin resistance, and a proinflammatory state, most likely contributing to excess CVD, large scale epidemiological studies have shown that the CVD risk associated with obesity remains appreciable even after correction for these factors. Perhaps surprising is the finding that the obesity-related relative risk of death from stroke and all of the CVDs combined is higher in younger than in older subjects, indicating that excess adiposity is likely to have deleterious effects on the cardiovascular system already at an early age, well before clinical manifestations of CVD become apparent. In agreement with this view, recent studies have demonstrated that the presence of obesity since childhood was the only consistent and significant determinant of adverse cardiac remodeling and that being overweight at age 20 years or obese at any time in life was linked with a 3-fold increased risk of developing chronic renal failure. Moreover, functional and structural abnormalities of the endothelium are already evident in obese children aged 9 to 12 years.

Given that the sympathetic nervous system (SNS) is an important regulatory mechanism of both metabolic and cardiovascular functions, altered SNS may likely play a role in the etiology and complications of obesity. It is now well established that obesity is associated with elevated SNS.

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activity, in particular in the outflow to the kidneys and the skeletal muscle vasculature. Animal and clinical studies convincingly demonstrated that high SNS activity is involved in the pathophysiology of altered cardiac structure and function in essential hypertension. In addition, there is growing evidence that obesity contributes to the development and progression of chronic kidney disease. Chronic kidney disease is characterized by an activated SNS, which adversely affects both renal and cardiovascular prognosis. Given the association between obesity and elevated CVD risk already at a very young age, we sought to assess subclinical target organ damage and its association with sympathetic activity in a cohort of young adults with excess body weight.

Methods

Subject Selection
All of the participants were recruited through 2 major universities in the Melbourne metropolitan area. We studied 18 lean (body mass index [BMI] <25 kg/m²) and 25 overweight (OW) or obese (Ob) subjects (BMI ≥25 kg/m²). All of the subjects were aged between 18 and 30 years. They were nonsmokers and not on any medication. None of the participants had a history of cardiovascular or cerebrovascular disease. Subjects attended at 9:00 AM, having fasted for 12 hours and abstained from caffeine for 18 hours and from alcohol for 36 hours. The study protocol was approved by the Alfred Hospital Ethics Committee, and all of the subjects gave written informed consent before participating in the study.

Clinical Assessment
Demographic details of age, sex, race, clinical status, and BP were obtained from standard measurements and questionnaires. A detailed history and physical examination were conducted to exclude obesity-related and cardiovascular comorbidities. Supine BP was measured after 5 minutes of rest by Dinamap monitor (Model 1846SX, Critikon Inc) as the average of the 3 consecutive measurements. Body weight was measured in light indoor clothes without shoes using a digital scale. Waist circumference was measured at the midpoint between the lowest rib and iliac crest and hip circumference at the level of the greater trochanters.

Dietary habits were assessed by prospective 7-day diet records, which were analyzed using Australian Food Composition Tables (FoodWorks Professional version 3.02, Xyris Software).

Before the study, all of the participants underwent ambulatory BP monitoring over 24 to 26 hours using an oscillometric monitor (model No. 90207, SpaceLabs Medical Inc) to measure brachial BP every 30 minutes. BP values were averaged over the total period of the recording.

Biochemistry
Fasting blood samples were drawn from a cannula placed in an antecubital vein for biochemical analysis of creatinine, electrolytes, insulin, total cholesterol, triglycerides, HDL, low-density lipoprotein cholesterol, glucose high-sensitivity C-reactive protein (h-CRP), and leptin.

Digital Vascular Function
Digital pulse amplitude was measured in the fasting state with a pulse amplitude tonometry (PAT) device placed on the tip of each index finger (Itamar Medical Ltd). PAT was assessed in response to reactive hyperemia. Measurements were obtained for 5 to 10 minutes at baseline followed by 5 minutes of occlusion of 1 arm, with the cuff inflated on the upper arm to suprasystolic pressure (60 mm Hg above systolic pressure or 200 mm Hg) and then released to induce reactive flow-mediated hyperemia, measured for 5 to 10 minutes. The PAT ratio was calculated as logₑ[(Xₜ/X₀)/(X₃₀/X₀)], with “X” representing pulse amplitude, “ₜ” denoting hyperemic finger, “₀” denoting the control finger, and “₃₀” denoting the 30-second time interval between 1.5 minutes and 2.0 minutes postdeflation, and “₀” denoting baseline. This calculation was made independent of the automatic algorithm provided by Itamar Medical Ltd and was implemented recently in endothelial function assessment in the Framingham Heart Study.

Muscle Sympathetic Nerve Activity
Recordings of multunit postganglionic muscle sympathetic nerve activity (MSNA) were made in the fasting state. A tungsten microelectrode (FHC) was inserted directly into the right peroneal nerve at the fibular head. A subcutaneous reference electrode was positioned 2 to 3 cm away from the recording site. The nerve signal was amplified (×50,000), filtered (bandpass: 700 to 2000 Hz), and integrated. BP, ECG, respiration, and MSNA were digitized with a sampling frequency of 1000 Hz (PowerLab recording system, model ML 785/8SP, ADI Instruments). Resting measurements were recorded over a 15-minute period and averaged. The MSNA was expressed as burst frequency (bursts per minute) and burst incidence (bursts per 100 heartbeats). In addition, the amplitude of each burst was determined, and the total MSNA was calculated by multiplying the mean burst amplitude per minute per burst rate and expressed as units per minute and units per 100 heartbeats.

Metabolic Measurements
After resting sympathetic neural measurements were completed, a standard 75-g oral glucose tolerance test was performed. Blood sample was withdrawn from a cannula placed in an antecubital vein 120 minutes after glucose administration (Glucaid, Fronine PTY, LTD). Insulin resistance was calculated from oral glucose tolerance test parameters according to the homeostatic model assessment method.

Renal Function
The creatinine clearance was used to assess renal function. All of the participants provided a 24-hour urine collection on the day of the test. Creatinine clearance was calculated using the following formula: 
\[C_{crea} = \left(\frac{U_{crea} \times V}{PCrecrea}\right),\] where “Ucrea” is the creatinine concentration in urine, “V” the urine flow rate, and “PCrecrea” the creatinine concentration in plasma. In addition, the creatinine clearance was estimated using the Cockcroft-Gault equation: 
\[(140−\text{age}) \times \text{weight}/(72 \times PCrecrea),\] not indexed per 1.73 m² of body surface area, because it may obscure the genuine association between renal function and total adiposity.

Echocardiographic Image Acquisition and Analysis
Images were gathered with a standard ultrasound machine (Vivid 7, GE Vingmed) with a 2.5-MHz, phased-array probe. Left ventricular (LV) diameter and wall thickness were measured from the 2D targeted M-mode echocardiographic tracings in the parasternal long axis, according to the criteria of the American Society of Echocardiography. LV end-diastolic diameter and LV ejection fraction at rest were computed from 2- and 4-chamber views, using a modified Simpson biplane method. Each representative value was obtained from the average of 3 measurements. LV mass was determined by Devereux formula and indexed to height to the power of 2.7. Cardiac output (CO) measurements were obtained using the LV outflow tract cross-sectional diameter and the velocity time integral measurements. Stroke volume was measured as CO/heart rate.

Tissue Doppler Imaging
Myocardial velocities were recorded by using color tissue Doppler to record low velocity, high-intensity myocardial signals at high frame rate (120 MHz), as described previously. Myocardial diastolic and systolic tissue velocities were obtained for the lateral and septal walls.

Statistics
Differences between lean and OW/Ob subjects were assessed using Student t test or the Mann–Whitney test if the data were not normally distributed. Associations between selected variables were assessed...
Table 1. Characteristics of Participants

<table>
<thead>
<tr>
<th>Characteristics of Participants</th>
<th>Lean (n=18)</th>
<th>OW/Ob (n=25)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male/Female</td>
<td>6/12</td>
<td>9/16</td>
</tr>
<tr>
<td>Age, y</td>
<td>22.6±0.7</td>
<td>22.6±0.7</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>20.9±0.4 (18.0 to 24.7)</td>
<td>30.8±1.1 (26.1 to 50.1)‡</td>
</tr>
<tr>
<td>Waist, cm</td>
<td>70±1 (60 to 79)</td>
<td>89±2 (77 to 118)‡</td>
</tr>
<tr>
<td>Hip, cm</td>
<td>84±2 (70 to 98)</td>
<td>103±2 (86 to 134)‡</td>
</tr>
<tr>
<td>Waist:hip ratio</td>
<td>0.83±0.02 (0.68 to 0.95)</td>
<td>0.87±0.02 (0.73 to 1.02)</td>
</tr>
<tr>
<td>Race</td>
<td></td>
<td></td>
</tr>
<tr>
<td>White</td>
<td>33</td>
<td>40</td>
</tr>
<tr>
<td>Asian</td>
<td>56</td>
<td>48</td>
</tr>
<tr>
<td>Other</td>
<td>11</td>
<td>12</td>
</tr>
<tr>
<td>Daily energy intake, calories</td>
<td>2238±171</td>
<td>2037±185</td>
</tr>
<tr>
<td>Physical activity, min/wk</td>
<td>318±78</td>
<td>191±39</td>
</tr>
<tr>
<td>Total cholesterol, mmol/L</td>
<td>4.29±0.16</td>
<td>4.53±0.17</td>
</tr>
<tr>
<td>LDL cholesterol, mmol/L</td>
<td>2.44±0.13</td>
<td>2.78±0.14</td>
</tr>
<tr>
<td>HDL cholesterol, mmol/L</td>
<td>1.46±0.06</td>
<td>1.26±0.06*</td>
</tr>
<tr>
<td>Triglycerides, mmol/L</td>
<td>0.87±0.08</td>
<td>1.08±0.09</td>
</tr>
<tr>
<td>Fasting glucose, mmol/L</td>
<td>4.35±0.07</td>
<td>4.62±0.11</td>
</tr>
<tr>
<td>2-h plasma glucose, mmol/L</td>
<td>4.63±0.3</td>
<td>5.87±0.38*</td>
</tr>
<tr>
<td>Fasting insulin, ng/mL</td>
<td>11.7±0.8</td>
<td>16.6±1.1†</td>
</tr>
<tr>
<td>2-h plasma insulin, ng/mL</td>
<td>47±13</td>
<td>64±8</td>
</tr>
<tr>
<td>HOMA</td>
<td>2.07±0.27</td>
<td>3.44±0.31†</td>
</tr>
<tr>
<td>Leptin, ng/mL</td>
<td>5.0±1.1</td>
<td>16.8±2.0‡</td>
</tr>
<tr>
<td>h-CRP</td>
<td>1.0±0.2</td>
<td>3.2±0.5†</td>
</tr>
<tr>
<td>Total protein, g/L</td>
<td>77±1</td>
<td>78±1</td>
</tr>
<tr>
<td>Albumin, g/L</td>
<td>42±1</td>
<td>41±1</td>
</tr>
<tr>
<td>Bilirubin, μmol/L</td>
<td>11.1±1.0</td>
<td>9.9±1.0</td>
</tr>
<tr>
<td>Creatinine, mmol/L</td>
<td>64.9±3.2</td>
<td>65.8±2.6</td>
</tr>
<tr>
<td>Urea, mmol/L</td>
<td>4.56±0.37</td>
<td>4.59±0.29</td>
</tr>
<tr>
<td>Sodium, mmol/L</td>
<td>137.9±4.0</td>
<td>138.6±0.4</td>
</tr>
<tr>
<td>Potassium, mmol/L</td>
<td>3.57±0.05</td>
<td>3.59±0.04</td>
</tr>
</tbody>
</table>

LDL indicates low-density lipoprotein; HOMA, homeostatic model assessment. Values are mean±SEM unless otherwise specified; numbers in bracket represent the range.

*P<0.05.
†P<0.01.
‡P<0.001.

Results

The characteristics of the subjects are presented in Table 1. There was no difference in age between the 2 groups. The OW/Ob group had higher BMI and waist and hip circumference (P<0.001). Subjects were matched for sex and ethnicity. There was no difference between the 2 groups in daily energy intake. Lean subjects tended to exercise more, but this did not reach significance (P=0.122 by Mann–Whitney test). OW/Ob subjects had significantly lower HDL cholesterol (P<0.05), higher 2-hour post–oral glucose tolerance test plasma glucose (P<0.05), higher fasting insulin (P<0.01), and higher homeostatic model assessment index values (P<0.01) compared with the lean subjects. OW/Ob subjects had higher leptin (P<0.001) and h-CRP (P<0.01) plasma concentration.

BP and SNS

Systolic BP (SBP), diastolic BP (DBP), and heart rate values are presented in Table 2. None of the participant had hypertension; however, OW/Ob subjects had slightly elevated BP compared with the lean subjects (P<0.05). Heart rate was also elevated in the OW/Ob participants (P<0.05).

Microneurographic recording was successfully obtained in 36 participants (15 lean and 21 OW/Ob). MSNA was significantly elevated in the OW/Ob group compared with the lean group (26±2 versus 14±2 bursts per 100 heartbeats; 1374 versus 1151 U/min; 217±2 versus 115±8 U per 100 heartbeats; P<0.001). In all of the subjects combined, univariate analysis indicated that MSNA correlated positively with weight, waist and hip circumference, BMI, heart rate, plasma triglycerides, and h-CRP. In forward stepwise regression, BMI remained the only predictor of MSNA.

Endothelial Function

Determination of endothelial function measurement was successfully obtained in 41 participants (16 lean and 25 OW/Ob). Indicative of impaired endothelial function, the PAT ratio was significantly decreased in the OW/Ob participants (P<0.01; Figure 1B). In univariate analysis, the PAT ratio was significantly correlated with SBP, DBP, and MSNA only. In forward stepwise regression, PAT ratio could be predicted by both DBP and MSNA (P=0.044; R²=0.244 [Figure 2A and Table 3]).

Kidney Function

Creatinine clearance, calculated by using the ratio of urine:plasma creatinine could not be evaluated in 5 participants who failed to provide adequate urine samples. Creatinine clearance was, therefore, also estimated in all of the participants using the Cockcroft-Gault equation. The values of
creatinine clearance, as assessed by both methods, were significantly higher in the OW/Ob subjects compared with their lean counterparts (P<0.001 with the Cockcroft-Gault equation [Figure 1C] and P<0.05 [Mann–Whitney test] for the direct calculation). In univariate analysis, Ccr estimated by the Cockcroft-Gault equation correlated significantly with BMI, MSNA, and plasma insulin concentration. In multiple regression analysis (adjusted for BMI), the dependent variable Ccr could still be predicted by MSNA and insulin (P<0.014, R^2=0.315 and P=0.025, R^2=0.416, respectively; Figure 2B). Ccr estimated by the direct calculation correlated significantly with SBP and MSNA. In multiple regression analysis, the dependent variable Ccr could still be predicted by these 2 parameters (P=0.031, R^2=0.367 and P=0.028, R^2=0.501, respectively [Table 3]).

**Echocardiographic Findings**

**CO, Stroke Volume, LV Outflow Tract Cross-Sectional Diameter, and Velocity Time Integral**

CO and stroke volume were higher in OW/Ob subjects compared with the lean subjects (5.24±0.31 versus 4.12±0.13 L/min, P<0.001, and 80.5±5.1 versus 65.8±3.0 mL, P<0.05, respectively). LV outflow tract cross-sectional diameters were similar between the 2 groups (2.02±0.04 and 2.11±0.05 cm in lean and OW/Ob, respectively). Velocity time integral measurements were slightly higher in OW/Ob subjects compared with the lean subjects (22.3±0.7 versus 20.4±0.6 cm; P=0.055).

**LV Structure**

LV wall thickness, diameter, and LV mass index (LVMI; Figure 1D) were elevated in the OW/Ob group compared with the lean participants (Table 4). In univariate analysis, LVMI showed a relation with sex, BMI, MSNA, CO, plasma insulin concentration, and SBP. LV septal and posterior wall thicknesses showed a relation with sex, BMI, SBP, MSNA, and CO. Forward stepwise regression (after adjustment for BMI and sex) indicated that both the CO and MSNA were significant predictors of LVMI and septal and posterior wall thickness (Figure 2C and Table 3).

**LV Systolic Function**

LV ejection fraction and FS were not different between the 2 groups. Tissue Doppler imaging–derived indices indicated significant reduction of systolic function (as assessed by systolic tissue velocity in the lateral wall) in OW/Ob subjects compared with the lean subjects (Table 4). Forward stepwise regression (after adjustment for sex) indicated that both plasma leptin concentration and age were significant predictors of the average systolic tissue velocity (Table 3).

**LV Diastolic Function**

Tissue Doppler imaging–derived indices indicated significantly decreased diastolic function (as assessed by diastolic tissue velocity in the lateral and septal wall) in OW/Ob subjects compared with the lean subjects (Table 4). Forward stepwise regression (after adjustment for age and BMI) indicated that plasma triglyceride concentration was a significant predictor of the average diastolic tissue velocity (Table 3).
Cardiac structure and function

Clinical manifestation of atherosclerosis. In fact, studies have shown that obesity is associated with endothelial dysfunction in adults, as well as in children. We used a newly developed noninvasive technique to measure endothelial function at the finger tip. This technique, which uses a PAT hyperemic response, has been shown to correlate with flow-mediated dilation in the brachial artery and was shown recently to be an independent predictor of subsequent adverse cardiovascular events. Our results demonstrate a significant reduction in endothelial function in the OW/Ob subjects and are in agreement with the results from the Framingham study. Interestingly, we found that the PAT ratio was also inversely related to the level of sympathetic nervous outflow to skeletal muscle, independent of sex, BMI, and BP. The link between SNS activity and endothelial dysfunction has not been fully investigated in humans, but data from a recent study also indicate an inverse relationship between MSNA and endothelial function in healthy individuals. Whether sympathetic activation contributes to endothelial dysfunction or vice versa is unknown. Central sympathoinhibition with moxonidine has been shown to improve endothelial dysfunction in patients with hypertension and in obese and insulin-resistant patients with the metabolic syndrome, suggesting a possible modulating role for the SNS on endothelial function. There is also some evidence that NO can influence sympathetic activity, but data are conflicting. Reduced NO release caused by inhibition of NO synthase has been shown to have no effect or to decrease MSNA in healthy subjects. Conversely, a positive linear relationship between the plasma nitrate concentration and the strength of MSNA has been shown to be more pronounced in younger subjects.

Endothelial dysfunction is often considered to predate the clinical manifestation of atherosclerosis. In fact, studies have shown that obesity is associated with endothelial dysfunction in adults, as well as in children. We used a newly developed noninvasive technique to measure endothelial function at the finger tip. This technique, which uses a PAT hyperemic response, has been shown to correlate with flow-mediated dilation in the brachial artery and was shown recently to be an independent predictor of subsequent adverse cardiovascular events. Our results demonstrate a significant reduction in endothelial function in the OW/Ob subjects and are in agreement with the results from the Framingham study. Interestingly, we found that the PAT ratio was also inversely related to the level of sympathetic nervous outflow to skeletal muscle, independent of sex, BMI, and BP. The link between SNS activity and endothelial dysfunction has not been fully investigated in humans, but data from a recent study also indicate an inverse relationship between MSNA and endothelial function in healthy individuals. Whether sympathetic activation contributes to endothelial dysfunction or vice versa is unknown. Central sympathoinhibition with moxonidine has been shown to improve endothelial dysfunction in patients with hypertension and in obese and insulin-resistant patients with the metabolic syndrome, suggesting a possible modulating role for the SNS on endothelial function. There is also some evidence that NO can influence sympathetic activity, but data are conflicting. Reduced NO release caused by inhibition of NO synthase has been shown to have no effect or to decrease MSNA in healthy subjects. Conversely, a positive linear relationship between the plasma nitrate concentration and the strength of MSNA has been shown to be more pronounced in younger subjects.

There is increasing evidence that obesity contributes to the development and progression of chronic kidney disease, independent of elevated BP and diabetes mellitus; the mechanisms by which renal injury develops in obesity, however, remain unclear. In the present study, none of the OW/Ob subjects had clinically demonstrable renal dysfunction; however, they presented with higher creatinine clearance, indicating the presence of increased filtration pressure. Glomerular “hyperfiltration” was demonstrated recently in a large cohort of young adult men to be commonly associated with elevated BMI and an unfavorable metabolic profile. Experimental and clinical data suggest that hyperfiltration

### Table 3. Stepwise Linear Regression Analysis (Adjusted for BMI and Sex)

<table>
<thead>
<tr>
<th>Dependent Variable</th>
<th>Step</th>
<th>Predictors</th>
<th>$R^2$</th>
<th>$P$</th>
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<tbody>
<tr>
<td>Endothelial function</td>
<td>1</td>
<td>DBP</td>
<td>0.140</td>
<td>0.028</td>
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<td></td>
<td>2</td>
<td>MSNA</td>
<td>0.244</td>
<td>0.044</td>
</tr>
<tr>
<td>Renal function</td>
<td>1</td>
<td>MSNA</td>
<td>0.318</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>SBP</td>
<td>0.403</td>
<td>0.037</td>
</tr>
<tr>
<td>Ccr (a)</td>
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<td>MSNA</td>
<td>0.315</td>
<td>0.014</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>Insulin</td>
<td>0.416</td>
<td>0.025</td>
</tr>
<tr>
<td>Cardiac structure and function</td>
<td>1</td>
<td>CO</td>
<td>0.199</td>
<td>0.03</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>MSNA</td>
<td>0.312</td>
<td>0.03</td>
</tr>
<tr>
<td>LVMI</td>
<td>1</td>
<td>CO</td>
<td>0.403</td>
<td>&lt;0.001</td>
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<tr>
<td></td>
<td>2</td>
<td>MSNA</td>
<td>0.496</td>
<td>0.021</td>
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<tr>
<td>LV septal thickness</td>
<td>1</td>
<td>CO</td>
<td>0.207</td>
<td>0.021</td>
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<tr>
<td></td>
<td>2</td>
<td>MSNA</td>
<td>0.299</td>
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<tr>
<td>Average diastolic tissue velocity</td>
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<td>Leptin</td>
<td>0.144</td>
<td>0.011</td>
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<tr>
<td></td>
<td>2</td>
<td>Age</td>
<td>0.240</td>
<td>0.035</td>
</tr>
<tr>
<td>Average systolic tissue velocity</td>
<td>1</td>
<td>Triglycerides</td>
<td>0.202</td>
<td>0.004</td>
</tr>
</tbody>
</table>

$Ccr$ (a) indicates creatinine clearance calculated as follows: $(U_c \times V)/P_c$, where “$U_c$” is the creatinine’s concentration in the urine, “$V$” the urine flow rate, and “$P_c$” the creatinine’s concentration in the plasma. $Ccr$ (b) indicates creatinine clearance calculated by using the Cockcroft-Gault equation.

### Table 4. LV Structure and Systolic and Diastolic Functions

<table>
<thead>
<tr>
<th>LV Structure and Systolic and Diastolic Functions</th>
<th>Lean</th>
<th>OW/Ob</th>
</tr>
</thead>
<tbody>
<tr>
<td>Structure</td>
<td></td>
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<tr>
<td>LVEDD, cm</td>
<td>45.6±0.8</td>
<td>48.7±0.8†</td>
</tr>
<tr>
<td>LVMI, g/m²</td>
<td>65.0±4.0</td>
<td>77.8±3.9*</td>
</tr>
<tr>
<td>Septal wall thickness, mm</td>
<td>7.76±0.21</td>
<td>8.80±0.28†</td>
</tr>
<tr>
<td>Posterior wall thickness, mm</td>
<td>7.75±0.39</td>
<td>8.84±0.39*</td>
</tr>
<tr>
<td>Systolic function</td>
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<tr>
<td>LVEF, %</td>
<td>67.7±1.0</td>
<td>69.3±1.0</td>
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<tr>
<td>FS, %</td>
<td>33.8±1.4</td>
<td>34.6±1.1</td>
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<tr>
<td>Systolic tissue velocity (lateral wall), cm/s</td>
<td>6.81±0.33</td>
<td>5.74±0.32*</td>
</tr>
<tr>
<td>Systolic tissue velocity (septal wall), cm/s</td>
<td>6.16±0.31</td>
<td>5.74±0.30</td>
</tr>
<tr>
<td>Average systolic tissue velocity, cm/s</td>
<td>6.14±0.20</td>
<td>5.59±0.21</td>
</tr>
<tr>
<td>Diastolic function</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diastolic tissue velocity (lateral wall), cm/s</td>
<td>10.41±0.42</td>
<td>9.09±0.41*</td>
</tr>
<tr>
<td>Diastolic tissue velocity (septal wall), cm/s</td>
<td>9.50±0.24</td>
<td>7.95±0.37†</td>
</tr>
<tr>
<td>Average diastolic tissue velocity, cm/s</td>
<td>9.32±0.26</td>
<td>8.24±0.26†</td>
</tr>
</tbody>
</table>

$LVEDD$ indicates LV end-diastolic diameter; $LVEF$, LV ejection fraction; $FS$, fractional shortening. *$P<0.05$. †$P<0.01$. 

Discussion

Excess adiposity is recognized as an independent risk factor of all-cause and cardiovascular mortality, with the relative risk associated with excess weight being particularly pronounced in younger subjects. The present study was carried out in young normotensive individuals with excess weight and no clinical cardiovascular or renal history. The main finding of this study is that young OW/Ob adults present with higher BP, a less favorable metabolic profile, high MSNA, and demonstrable manifestation of heart, kidney, and endothelial damage compared with their lean counterparts. Importantly, we found that SNS activity was closely related to the degree of subclinical organ damage.

Endothelial dysfunction is often considered to predate the clinical manifestation of atherosclerosis. In fact, studies have shown that obesity is associated with endothelial dysfunction in adults, as well as in children. We used a newly developed noninvasive technique to measure endothelial function at the finger tip. This technique, which uses a PAT hyperemic response, has been shown to correlate with flow-mediated dilation in the brachial artery and was shown recently to be an independent predictor of subsequent adverse cardiovascular events. Our results demonstrate a significant reduction in endothelial function in the OW/Ob subjects and are in agreement with the results from the Framingham study. Interestingly, we found that the PAT ratio was also inversely related to the level of sympathetic nervous outflow to skeletal muscle, independent of sex, BMI, and BP. The link between SNS activity and endothelial dysfunction has not been fully investigated in humans, but data from a recent study also indicate an inverse relationship between MSNA and endothelial function in healthy individuals. Whether sympathetic activation contributes to endothelial dysfunction or vice versa is unknown. Central sympathoinhibition with moxonidine has been shown to improve endothelial dysfunction in patients with hypertension and in obese and insulin-resistant patients with the metabolic syndrome, suggesting a possible modulating role for the SNS on endothelial function. There is also some evidence that NO can influence sympathetic activity, but data are conflicting. Reduced NO release caused by inhibition of NO synthase has been shown to have no effect or to decrease MSNA in healthy subjects. Conversely, a positive linear relationship between the plasma nitrate concentration and the strength of MSNA has also been reported. Other factors commonly present with excess adiposity have been shown to be involved in endothelial dysfunction. These include compounds secreted from adipose tissue, free fatty acids, hyperinsulinemia, elevated low-density lipoprotein, reduced HDL, and activation of the renin-angiotensin system. All of these factors, combined with activation of the SNS, may confer detrimental effects on endothelial function.

There is increasing evidence that obesity contributes to the development and progression of chronic kidney disease, independent of elevated BP and diabetes mellitus; the mechanisms by which renal injury develops in obesity, however, remain unclear. In the present study, none of the OW/Ob subjects had clinically demonstrable renal dysfunction; however, they presented with higher creatinine clearance, indicating the presence of increased filtration pressure. Glomerular “hyperfiltration” was demonstrated recently in a large cohort of young adult men to be commonly associated with elevated BMI and an unfavorable metabolic profile. Experimental and clinical data suggest that hyperfiltration...
may lead to a subsequent increase in urinary albumin excretion, which, over time, progresses to microalbuminuria, proteinuria, and, in the worst case scenario, end-stage renal failure. Recently, obesity-associated glomerular hyperfiltration was shown to lead to increased postglomerular oncotic pressure and enhanced proximal tubular sodium reabsorption, suggesting that glomerular hyperfiltration may play a role in the pathogenesis of hypertension in obesity. We found that both direct and indirect calculations for estimating renal function indicated that the degree of SNS activation could explain 31% of the variability of the creatinine clearance. Indeed, chronic bilateral renal denervation in experimental models has been shown to normalize the diabetes mellitus–induced glomerular hyperfiltration, indicating that sympathetic nerve stimulation is involved in the induction of glomerular hyperfiltration. More direct evidence from human studies indicates that mental stress-induced activation of the SNS aggravates the rise in glomerular filtration rate in young adults with mildly elevated BP, commensurate with the presence of glomerular hyperfiltration mediated via postglomerular vasoconstriction, likely to be a contributor to the development of essential hypertension in these subjects.

It is, however, important to note that, in the case of excess adiposity, other factors may also contribute to the initiation and/or exacerbation of renal dysfunction. In particular, adipokines, inflammatory cytokines, h-CRP, circulating free fatty acids, and activation of the renin-angiotensin system have all been associated with renal injury. Although we could not detect a direct relationship between plasma h-CRP or leptin concentration and creatinine clearance in our subjects, OW/Ob subjects did present markedly increased plasma leptin concentration and h-CRP levels that may further aggravate renal injury.

In line with previous studies, we found an alteration in LV structure and function in relatively young OW/Ob individuals. Indeed, LVMI, LV end-diastolic diameter, and wall thickness of OW/Ob individuals were all significantly different from the values observed in the lean subjects. In multiple regression analysis corrected for sex and BMI, the main determinants of the LV structure were the CO and the SNS. Increased CO, normally seen in obese individuals, is likely to represent an adaptive mechanism to supply adequate perfusion to an increased tissue mass; however, the role of the SNS on obesity-induced cardiac remodeling is less clear. A study in adolescents and young adults showed that high insulin coupled with increased sympathetic activity was a major determinant of LV wall growth and remodeling during adolescence. Our finding of a direct relationship between LVMI and MSNA in obesity is similar to that observed previously in hypertensive subjects. The present investigation indicates a possible role for the SNS in cardiac remodeling in obesity, even in the absence of hypertension. Tissue Doppler imaging–derived indices of diastolic function that are relatively load independent were all altered in OW/Ob subjects, as described previously. Such indices have been shown to be of important prognostic value of mortality in patients with cardiac disease and may predict worsening LV systolic and diastolic functions in subjects with increased BMI. Systolic and diastolic dysfunctions did not relate on SNS activity but were associated with plasma leptin and triglyceride concentration, perhaps underlying the importance of adipose tissue products and metabolic changes in generating LV dysfunction.

Limitations
Possible limitations of our study include the ethnic diversity and mixed sex of the participants, the lack of measures of fat distribution, reliance on measurement of sympathetic activity in the muscle vascular bed, and the use of the EndoPat device to assess endothelial function. Reflecting the population attending universities in Australia, half of the participants were of South Asian origin. In a previous report, Asian men were shown to be more insulin resistant and demonstrated endothelial dysfunction compared with whites, hence the possibly contributing to their increased CVD risk. Nevertheless, this was not the case in our study, with Asians and non-Asians displaying no significant difference in endothelial, renal, or cardiac function or sympathetic activity (data not shown). There was no effect of sex on SNS activity, endothelial function, or renal function, but sex did influence cardiac structure (data not shown). However, the relationships between SNS activity and cardiac structure remained significant after adjustment for sex. Previous reports by us and others have demonstrated sex differences in SNS regulation, with only males displaying a strong relation between MSNA and BMI and MSNA and waist:hip ratio, with the latter observation suggesting that fat distribution may be an important variable affecting MSNA. Indeed, abdominal visceral fat has been linked with MSNA in young lean and obese men. Fat distribution was not assessed in the present study, so we cannot exclude the possibility that fat distribution may also contribute to end-organ damage.

Sympathetic activation was determined using microneurography in the skeletal muscle vasculature, which represents ~20% of the total body sympathetic activity. Although in obesity SNS outflow to individual organs is not activated uniformly, it is important to note that preferential activation in the skeletal muscle and kidney is evident. With regard to endothelial function, the peripheral vascular beds located at the distal part of limbs are major sites of sympathetic vasoconstrictor activity and do play an important role in circulatory regulation, whereas endothelial cells along the major conduit vessels do not receive direct neural innervation from the autonomic nervous system. It is, therefore, possible that the relationship between SNS activity and endothelial function may differ depending on the vascular bed examined and the device/methodology used to assess endothelial function.

Although our results are suggestive of a possible association between SNS activation and the development of end-organ damage, it is important to note that a number of other factors or systems could also be operative. Despite being normotensive, our OW/Ob group did present with higher BP compared with the lean subjects. Hypertension and prehypertension in young American Indians have been demonstrated to be associated with LV hypertrophy and evidence of increased arterial stiffness. Interestingly, in subjects of African ancestry, Norton et al did demonstrate an association among prehypertension and LV mass, mean wall thick-
ness, and pulse wave velocity, although, after adjustment for age, sex, and other covariates, prehypertension was not independently associated with target organ changes. Activation of the renin-angiotensin-aldosterone system, which is common in obesity, is a well-known contributor of organ damage, and inhibition of this system is known to improve cardiac, renal, and endothelial functions.\textsuperscript{51,52} We cannot exclude that activation of the renin-angiotensin system or other factors, such as hyperinsulinemia, leptin, elevated low-density lipoprotein, reduced HDL, or inflammatory cytokines, contributes to or acts in concert with the SNS to initiate end-organ dysfunction in young OW or Ob subjects.

**Perspectives**

Whether alteration in SNS activity precedes and contributes to the development of obesity and associated CVD risk or is a consequence of it is still debated. Interestingly, the relationship between SNS activity and end-organ damage is not limited to obesity, because previous studies have demonstrated that high SNS activity was associated with the development of cardiac structural\textsuperscript{13,52} and functional\textsuperscript{52} abnormalities in patients with established hypertension, as well as in patients with chronic kidney disease.\textsuperscript{54} Because reduced renal function, endothelial dysfunction, and alteration in the LV structure and function all carry adverse prognostic significance, such a cluster of unfavorable features observed in a cohort of OW/Ob, otherwise healthy young individuals may place them at increased cardiovascular risk. Lifestyle changes, such as diet-induced weight loss and increased physical activity, are the cornerstones of the treatment of obesity. Moderate weight loss and/or weight loss and exercise are associated with decreased SNS activity and confer beneficial hemodynamic and metabolic effects in older individuals with the metabolic syndrome.\textsuperscript{55,56} Whether intervention aiming at decreasing SNS activity in young obese subjects would confer similar beneficial cardiovascular protection remains to be established.

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**Disclosures**

M.E. serves on scientific advisory boards of Servier Australia, Abbott (formerly Solvay) Pharmaceuticals, and ARDIAN Inc. M.S. serves on scientific advisory boards for Abbott (formerly Solvay) Pharmaceuticals and Novartis Pharmaceuticals. The laboratories of M.E., M.S., and E.L. currently receive research funding from ARDIAN Inc. and Allergan. The investigators report no conflicts of interest with regards to this article.

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