Effect of obstructive sleep apnea on glucose metabolism

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Abstract

Background: Obstructive sleep apnea (OSA) is prevalent in people with obesity and is a major risk factor for type 2 diabetes (T2D). The effect of OSA on metabolic function and the precise mechanisms (insulin resistance, β-cell dysfunction, or both) responsible for the increased T2D risk in people with OSA are unknown.

Design and methods: We used a two-stage hyperinsulinemic–euglycemic clamp procedure in conjunction with stable isotopically labeled glucose and palmitate tracer infusions and 18F-fluorodeoxyglucose injection and positron emission tomography to quantify multi-organ insulin action and oral and intravenous tolerance tests to evaluate glucose-stimulated insulin secretion in fifteen people with obesity and OSA and thirteen people with obesity without OSA.

Results: OSA was associated with marked insulin resistance of adipose tissue triglyceride lipolysis and glucose uptake into both skeletal muscles and adipose tissue, whereas there was no significant difference between the OSA and control groups in insulin action on endogenous glucose production, basal insulin secretion, and glucose-stimulated insulin secretion during both intravenous and oral glucose tolerance tests.

Conclusions: These data demonstrate that OSA is a key determinant of insulin sensitivity in people with obesity and underscore the importance of taking OSA status into account when evaluating metabolic function in people with obesity. These findings may also have important clinical implications because disease progression and the risk of diabetes-related complications vary by T2D subtype (i.e. severe insulin resistance vs insulin deficiency). People with OSA may benefit most from the targeted treatment of peripheral insulin resistance and early screening for complications associated with peripheral insulin resistance.

Introduction

Obesity is a major risk factor for insulin resistance and type 2 diabetes (T2D), but the reasons why some people with obesity develop metabolic dysfunction while others do not are unclear (1, 2, 3). We speculate that obesity-associated comorbidities, such as obstructive sleep apnea (OSA) contribute to the development of metabolic dysfunction. OSA affects ~50% (or even more) of people with obesity and is a major risk factor for T2D (4). However, the effect of OSA on the pathophysiological mechanisms involved in causing T2D (namely muscle, liver, and adipose tissue insulin resistance and β-cell dysfunction) is not well understood (4, 5). Studies that evaluated insulin sensitivity in people with and without OSA assessed the relationship between plasma glucose and insulin concentrations (5, 6, 7, 8). This approach assumes differences in the relationship between plasma glucose and insulin concentrations are solely due to differences in insulin action on glucose metabolism. However, insulin-independent mechanisms (e.g. glucose itself and glucagon) are also involved in regulating plasma glucose concentration (9, 10, 11). In
addition, plasma insulin could be high and low glucose level because of insulin hypersecretion with normal insulin sensitivity, and vice versa plasma insulin could be low and high glucose level because of β-cell dysfunction with normal insulin sensitivity (12, 13). Results from studies that evaluated the effect of positive airway pressure therapy on insulin sensitivity in people with OSA are equivocal, and even the studies that assessed insulin sensitivity by using the gold-standard hyperinsulinemic–euglycemic clamp technique (14) found discrepant results (15, 16, 17, 18, 19). The discrepancy in results is likely due to differences in study design. In studies that did not include a control group, positive airway pressure therapy improved insulin sensitivity (15, 16, 18). On the other hand, the results from a randomized controlled trial suggest positive airway pressure therapy does not improve insulin sensitivity even though it improves sleep (19). However, the randomized controlled trial may have lacked statistical power to detect significant differences. The effect of OSA on β-cell function has been evaluated by using the homeostasis model assessment of β-cell function (HOMA-%B) index or the insulinogenic index, or by assessing the acute insulin response during an intravenous glucose tolerance test (5, 7, 8). These methods assume plasma insulin concentration reflects insulin secretion from β-cells. However, plasma insulin concentration is determined by both insulin secretion and plasma insulin clearance rates (20, 21).

The goal of the present study was to evaluate the effects of OSA on insulin sensitivity of glucose metabolism and insulin kinetics. In addition, we evaluated insulin sensitivity of adipose tissue lipolysis and adipose tissue oxygenation, because OSA causes hypoxemia and poor tissue oxygenation presumably causes excess fatty acid release into the circulation and subsequently lipid-induced insulin resistance in the liver and muscles (22). We hypothesized that OSA impairs both multi-organ insulin action (the ability of insulin to inhibit lipolysis of adipose tissue triglycerides, suppress hepatic glucose production, and stimulate muscle and adipose tissue glucose uptake) and glucose-stimulated insulin secretion. Basal substrate kinetics and multi-organ insulin action were assessed by using a two-stage (low- and high-dose insulin infusion) hyperinsulinemic–euglycemic pancreatic clamp procedure in conjunction with stable isotopically labeled glucose and palmitate tracer infusions, [18F]fluorodeoxyglucose ([18F]FDG) and [13C]water injections and PET, and mathematical modeling of the substrate and C-peptide kinetics. Plasma insulin concentrations during basal conditions and the low- and high-dose insulin infusions spanned the physiological (fasted-fed) range and were optimal to simultaneously assess insulin action on adipose tissue triglyceride lipolysis and glucose production and muscle and adipose tissue glucose uptake (23). Glucose-stimulated insulin secretion by β-cells was evaluated after both oral and i.v. glucose administration to assess the potential importance of incretins in the acute insulin response.

Subjects and methods
Study participants

Fifteen participants with obesity and OSA took part in this study, which was approved by the Institutional Review Board at Washington University, St. Louis, MO, USA, and registered as NCT03408613 at Clinicaltrials.gov. Written informed consent was obtained from all participants before their participation in this study. Participants then completed a comprehensive medical examination, including a history and physical examination, a resting ECG, standard blood tests, and an oral glucose tolerance test to assess the following inclusion criteria: (i) age ≥30 and ≤70 years; (ii) BMI ≥30.0 and <45.0 kg/m²; (iii) no evidence of diabetes or other chronic illness; (iv) no use of medications or treatments for previously diagnosed OSA or medications or treatments that could affect sleep, breathing, upper airway muscle physiology, or the study outcomes; (v) no use of tobacco products or excessive amounts of alcohol (more than one drink a day); (vi) not involved in structured exercise for ≥1.5 h per week; (vii) no metal implants that are incompatible with MRI; (viii) reported sleep duration >6 h per night and regular nighttime sleep schedules, defined as bedtime between 21:00 h and midnight and wake-time between 4:00 h and 8:00 h on all days of the week. Participants who were eligible based on these criteria also completed an overnight polysomnogram (Grael PSG, Compumedics USA Inc., Charlotte, NC, USA) in the Clinical and Translational Research Unit to further determine eligibility. Participants with OSA were included if (i) the apnea-hypopnea index was ≥20/h; (ii) no polysomnogram finding that would trigger immediate treatment per standard operating protocol in our Sleep Medicine Center (e.g. a single SaO₂ <50%, SaO₂ <70% for >2 min, ECG pause >5 s, or ventricular tachycardia >30 s) and (iii) no other known sleep disorders. Participants in the control group were included if (i) the apnea–hypopnea index (AHI) was <5/h; (ii) the respiratory disturbance index was <5/h; (iii) no sleep disorders. Polysomnograms were performed and scored according to standard criteria set forth by the American Academy of Sleep Medicine (The AASM Manual for the Scoring of Sleep and Associated

https://eje.bioscientifica.com
Events: Rules, Terminology, and Technical Specifications, Version 2.5, 2018). Hypopneas and oxygen desaturations were scored if \( \text{SaO}_2 \) decreased by 3% or more. A total of 50 participants were assessed for eligibility. Eighteen did not meet the inclusion criteria and four were no longer interested in participating after they passed the screening evaluation.

**Body composition analysis and metabolic testing**

A detailed description of the methods is provided in the Supplementary data (see section on supplementary materials given at the end of this article). Total and regional body fat mass, fat-free mass, and muscle mass were determined by using dual-energy X-ray absorptiometry and MRI. In addition, all participants completed a basal metabolic study, a two-stage hyperinsulinemic-euglycemic pancreatic clamp procedure, an i.v. glucose tolerance test, and an oral glucose tolerance test. During the basal metabolic study, infusions of [6,6-\( ^2 \)H]glucose and [U-\( ^13 \)C]palmitate were initiated after participants fasted overnight and blood samples were obtained to determine plasma glucose and palmitate enrichments, and substrate and hormone concentrations. Adipose tissue oxygenation was evaluated by inserting a small fiber optic probe (Oxylite\textsuperscript{	extregistered}, Oxford Optronix Ltd, Abingdon, UK) into s.c. abdominal adipose tissue. The two-stage hyperinsulinemic–euglycemic pancreatic clamp procedure included infusions of octreotide, glucagon, growth hormone, [6,6-\( ^2 \)H]glucose, and [U-\( ^13 \)C]palmitate for 360 min. For the initial 120 min, insulin was infused at 10 mU/m\(^2\) body surface area (BSA) per min (initiated with a two-step priming dose of 40 mU/m\(^2\) BSA/min for 5 min followed by 20 mU/m\(^2\) BSA/min for 5 min); for the remaining 240 min, insulin was infused at 50 mU/m\(^2\) BSA/min (initiated with a two-step priming dose of 200 mU/m\(^2\) BSA/min for 5 min followed by 100 mU/m\(^2\) BSA/min for 5 min). Dextrose, enriched to 2.5% with [6,6-\( ^2 \)H]glucose, was infused at a variable rate to maintain plasma glucose concentration at ~6.0 mM. Blood samples were obtained to determine plasma glucose and palmitate enrichments, and substrate and hormone concentrations. \([^{15}O]H_2O\) and then \([^{18}F]FDG\) were administered and tissue perfusion and glucose uptake rates were determined by dynamic PET scanning of the torso and the thigh. During the intravenous glucose tolerance test, a bolus of 0.55 g glucose per kg body mass was administered intravenously and blood was collected to determine plasma glucose, insulin, and C-peptide concentrations before and at frequent intervals for 10 min afterward. During the oral glucose tolerance test, participants ingested 75 g of glucose and blood samples to determine plasma glucose, insulin, C-peptide, fatty acid, and glucagon concentrations were collected immediately before and every 30 min for 2 h after ingesting the glucose.

**Sample processing and analysis**

Plasma glucose concentration was determined by using the glucose oxidase method (YSI 2300 STAT, YSI Inc, Yellow Springs, OH, USA). Plasma insulin, C-peptide, C-reactive protein, and cortisol concentrations were determined by using an automated immunoassay (Elecsys\textsuperscript{\textregistered}, Roche Diagnostics). Plasma fatty acid concentration during the OGTT was measured by using a spectrophotometric assay (FUJIFILM Wako Diagnostics USA, CA, USA). Plasma glucagon concentration was determined by using enzyme-linked immunosorbent assays (glucagon, Mercodia Inc, NC). Norepinephrine concentration in plasma was determined by chromatography with electrochemical detection (Quest Diagnostics\textsuperscript{\textregistered}, Secaucus, NJ, USA). Plasma glucose and palmitate tracer-to-trace ratios and plasma fatty acid concentrations during the basal study and the hyperinsulinemic–euglycemic clamp were determined by using gas chromatography coupled with mass spectrometry (23, 24). Whole-body palmitate and glucose kinetics, tissue glucose uptake rates, tissue perfusion, hepatic sinusoidal insulin concentration, and insulin kinetics were determined as previously described (23, 24, 25, 26, 27, 28, 29, 30) (see Supplementary data for details).

**Statistical analysis**

Statistical analysis was performed by using Prism 8 (GraphPad Software). All data sets were tested for normality by using the D’Agostino-Pearson omnibus test; skewed data sets were log- or square root-transformed before analysis. Student’s \( t \)-test for unpaired samples and two-way ANOVA with Tukey’s \textit{post hoc} procedure were used to evaluate the differences in outcomes between the control and OSA groups as appropriate. Because age and the proportion of men and women in the control and OSA groups were different, we also performed multivariable regression analysis to adjust the assessment of insulin sensitivity for age and sex. In addition, we evaluated the potential impact of differences in intra-abdominal adipose tissue volume on metabolic outcome measures by adding intra-abdominal adipose tissue volume as an additional independent predictor in the regression model. Insulin secretion rate (i.e. \( \beta \)-cell function) was not adjusted because the computation of insulin secretion rate takes into
account participants' age and sex (28). Values in the text are reported as mean ± s.d. or median (quartiles). Values in tables and figures are reported as noted in the legend. A P-value ≤0.05 was considered significant.

Sample size estimation

The primary study outcome was insulin-stimulated whole-body glucose disposal rate. Based on the inter-individual variability of whole-body insulin-stimulated glucose disposal rate in people with obesity (31), we estimated 8 subjects per group would be needed to detect a 30% difference and 11 subjects per group would be needed to detect a 25% difference in whole-body insulin-stimulated glucose disposal rate with a power of 0.8 and an α-value of 0.05.

Results

Participants' body composition and sleep characteristics

Body mass and total fat mass were not different between the OSA and control groups, but participants with OSA had more intra-abdominal adipose tissue than participants in the control group (Table 1). Habitual sleep schedules, total sleep time, and sleep efficiency were not different between the two groups but the AHI, the oxygen desaturation index, and the respiratory disturbance index were greater in the OSA than in the control group (Table 1). On average, participants with OSA had moderate to severe OSA (AHI >15/h). Peripheral capillary oxygen saturation (SpO₂), while participants were awake, was not different in the two groups but SpO₂, while participants were asleep, was markedly reduced in the OSA compared with the control group (Table 1).

Glucose, insulin, and C-peptide concentrations during the hyperinsulinemic-euglycemic pancreatic clamp

Arterial glucose concentrations during both stages 1 and 2 of the clamp were not different between the OSA and control groups (Table 2). Basal arterial C-peptide concentration was greater in the OSA than the control group, but arterial C-peptide concentration during the clamp (both stages 1 and 2) was not different in the two groups (Table 2). C-peptide concentration during the clamp was markedly less than during basal conditions (Table 2), confirming effective suppression of pancreatic hormone secretion in both groups. Both basal arterial insulin concentration and hepatic sinusoidal insulin concentration were greater in the OSA group compared with the control group (Table 2), but arterial and hepatic sinusoidal insulin concentrations during the clamp (both stages 1 and 2) were not different

Table 1  Participants’ body composition and sleep characteristics. Data are mean ± s.d. or median (quartiles).

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>OSA</th>
</tr>
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<tbody>
<tr>
<td>Total n</td>
<td>13</td>
<td>15</td>
</tr>
<tr>
<td>Males, n</td>
<td>3</td>
<td>7</td>
</tr>
<tr>
<td>Females, n (%)</td>
<td>10 (77%)</td>
<td>8 (53%)</td>
</tr>
<tr>
<td>Age (years)</td>
<td>41 (34, 45)</td>
<td>53 (51, 60)*</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>38 ± 5</td>
<td>38 ± 4</td>
</tr>
<tr>
<td>Body fat (%)</td>
<td>47 ± 5</td>
<td>45 ± 7</td>
</tr>
<tr>
<td>Fat mass (kg)</td>
<td>49 ± 11</td>
<td>49 ± 9</td>
</tr>
<tr>
<td>Fat-free mass (kg)</td>
<td>57 ± 10</td>
<td>59 ± 11</td>
</tr>
<tr>
<td>Intra-abdominal adipose tissue mass (kg)</td>
<td>3.0 ± 1.1</td>
<td>4.6 ± 2.3*</td>
</tr>
<tr>
<td>Subcutaneous abdominal adipose tissue mass (kg)</td>
<td>9.9 ± 2.7</td>
<td>9.3 ± 2.6</td>
</tr>
<tr>
<td>Regular bed time (h)</td>
<td>21:59 (21:12, 22:13)</td>
<td>22:30 (21:34, 23:04)</td>
</tr>
<tr>
<td>Regular wake time (h)</td>
<td>6:49 (5:37, 7:31)</td>
<td>6:30 (6:04, 7:02)</td>
</tr>
<tr>
<td>Total sleep time (min)</td>
<td>414 ± 54</td>
<td>398 ± 74</td>
</tr>
<tr>
<td>Sleep efficiency (%)</td>
<td>84 ± 9</td>
<td>81 ± 10</td>
</tr>
<tr>
<td>Apnea hypopnea index (n/h)</td>
<td>1.0 (1.0, 2.0)</td>
<td>2.6 (19, 46)*</td>
</tr>
<tr>
<td>Respiratory disturbance index (n/h)</td>
<td>2.0 (1.5, 4.0)</td>
<td>33 (29, 68)*</td>
</tr>
<tr>
<td>Oxygen desaturation index (n/h)</td>
<td>1.5 (0.9, 2.0)</td>
<td>26 (19, 47)*</td>
</tr>
<tr>
<td>Capillary oxygen saturation (SpO₂), awake (%)</td>
<td>96 ± 3</td>
<td>94 ± 2</td>
</tr>
<tr>
<td>Nadir capillary oxygen saturation (SpO₂), asleep (%)</td>
<td>90 (90, 92)</td>
<td>81 (73, 86)*</td>
</tr>
</tbody>
</table>

*Statistically significant difference between the OSA and control groups, P < 0.05.
in the two groups. In the control group, arterial insulin concentration during stage 1 of the clamp was greater than basal arterial insulin concentration whereas, in the OSA group, arterial insulin concentration during stage 1 of the clamp was not different from basal arterial insulin concentration (Table 2). Hepatic sinusoidal insulin during stage 1 of the clamp was not different from basal hepatic sinusoidal insulin in the control group whereas in the OSA group hepatic sinusoidal insulin during stage 1 of the clamp was not different from basal hepatic sinusoidal insulin concentration during basal conditions and during the hyperinsulinemic–euglycemic pancreatic clamp procedure and circulating markers of inflammation and adrenergic tone (control: 159 ± 28 mM; OSA: 145 ± 46 mM) were not different between the OSA and control groups, but palmitate appearance rate in plasma during stage 1 (low-dose insulin infusion) of the hyperinsulinemic–euglycemic clamp, when plasma insulin concentration was the same in the two groups, was greater in the OSA than the control group (Fig. 1A). High-dose insulin infusion (stage 2 of the hyperinsulinemic–euglycemic clamp) nearly completely suppressed the palmitate appearance rate in plasma in both the OSA and control groups (Fig. 1A). The differences between groups remained significant after adjusting for age, sex, and intra-abdominal adipose tissue volume (Supplementary Table 1). The dose–response relationship between plasma insulin concentration and palmitate appearance rate in plasma suggests adipose tissue triglyceride lipolysis is less sensitive to insulin in the OSA compared with the control group but still very responsive to increases in plasma insulin in people with OSA (Fig. 1B). The difference in lipolytic rate between the two groups was not due to a difference in adipose tissue oxygenation (50 ± 6 and 51 ± 12 mm Hg in the OSA and control groups, respectively).

Glucose kinetics

Endogenous glucose appearance rate in plasma, an index of hepatic glucose production rate, was inversely related to arterial plasma insulin (Fig. 1C) and hepatic sinusoidal insulin (Fig. 1D) concentrations without a difference between the OSA and control groups. Insulin-stimulated whole-body glucose disposal rate was ~40% less in the OSA than the control group (2302 ± 3665 pmol/min, respectively; P < 0.05) due to markedly reduced glucose uptake in both muscles and subcutaneous adipose tissue (Fig. 1E, G and H). The differences between groups remained significant after adjusting for age, sex, and intra-abdominal adipose tissue volume (Fig. 1F and Supplementary Table 1). The differences in glucose uptake between the OSA and control groups were presumably caused by local tissue factors, because muscle and subcutaneous adipose tissue blood flow during the hyperinsulinemic–euglycemic clamp procedure (muscle: 163 (100; 238) and 167 (114; 293) mL/min/kg tissue; adipose tissue: 11 (8; 24) and 12 (10; 17) mL/min/kg tissue in the OSA and control groups, respectively) and glucose delivery (i.e. product of arterial glucose concentration and blood flow) to muscles and adipose tissue were not different in the OSA and control groups.

Table 2  Plasma glucose, C-peptide, and insulin concentrations during basal conditions and during the hyperinsulinemic–euglycemic pancreatic clamp procedure and circulating markers of inflammation and adrenergic tone. Data are median (quartiles). Stages 1 and 2 refer to low- and high-dose insulin infusion, respectively.

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>OSA</th>
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<tbody>
<tr>
<td>Glucose (mmol/L)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Basal</td>
<td>5.0 (4.8, 5.1)</td>
<td>5.3 (5.0, 5.5)</td>
</tr>
<tr>
<td>Stage 1</td>
<td>6.3 (6.2, 6.9)</td>
<td>6.6 (6.1, 7.2)</td>
</tr>
<tr>
<td>Stage 2</td>
<td>5.9 (5.8, 6.3)</td>
<td>6.1 (5.8, 6.3)</td>
</tr>
<tr>
<td>C-peptide (nmol/L)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Basal</td>
<td>0.83 (0.70, 1.05)</td>
<td>1.22 (1.04, 1.35)*</td>
</tr>
<tr>
<td>Stage 1</td>
<td>0.30 (0.27, 0.35)</td>
<td>0.36 (0.26, 0.57)</td>
</tr>
<tr>
<td>Stage 2</td>
<td>0.13 (0.11, 0.21)</td>
<td>0.19 (0.14, 0.26)</td>
</tr>
<tr>
<td>Arterial insulin (pmol/L)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Basal</td>
<td>64 (51, 91)</td>
<td>104 (86, 164)*</td>
</tr>
<tr>
<td>Stage 1</td>
<td>120 (103, 146)$^1$</td>
<td>125 (89, 140)</td>
</tr>
<tr>
<td>Stage 2</td>
<td>672 (645, 721)$^2$</td>
<td>744 (620, 817)$^2$</td>
</tr>
<tr>
<td>Hepatic sinusoidal insulin (pmol/L)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Basal</td>
<td>279 (208, 294)</td>
<td>401 (347, 498)*</td>
</tr>
<tr>
<td>Stage 1</td>
<td>224 (179, 259)</td>
<td>223 (156, 259)$^1$</td>
</tr>
<tr>
<td>Stage 2</td>
<td>890 (779, 985)$^1$</td>
<td>937 (829, 1044)$^1$</td>
</tr>
<tr>
<td>C-reactive protein (mg/L)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Basal</td>
<td>3.6 (1.8, 4.9)</td>
<td>5.1 (2.3, 8.1)</td>
</tr>
<tr>
<td>Stage 1</td>
<td>207 (139, 310)</td>
<td>223 (155, 324)</td>
</tr>
<tr>
<td>Stage 2</td>
<td>0.86 (0.67, 1.21)</td>
<td>1.12 (0.83, 1.24)</td>
</tr>
</tbody>
</table>

*Statistically significant difference between the OSA and control group values, P ≤ 0.05. $Statistically significant difference between clamp (stage 1 and/or stage 2) and basal values, P ≤ 0.05. $Statistically significant difference between clamp stage 1 and clamp stage 2 values, P ≤ 0.05.

Markers of inflammation and adrenergic tone

The concentrations of C-reactive protein, cortisol, and norepinephrine were assessed during basal conditions and were not different (all P ≥ 0.15) between the OSA and the control groups (Table 2).

Palmitate kinetics

Basal palmitate appearance rate in plasma (Fig. 1A) and basal plasma palmitate concentration (control: 159 ± 28 mM; OSA: 145 ± 46 mM) were not different between the OSA and control groups, but palmitate appearance rate in plasma during stage 1 (low-dose insulin infusion) of the hyperinsulinemic–euglycemic clamp, when plasma insulin concentration was the same in the two groups, was greater in the OSA than the control group (Fig. 1A). High-dose insulin infusion (stage 2 of the hyperinsulinemic–euglycemic clamp) nearly completely suppressed the palmitate appearance rate in plasma in both the OSA and control groups (Fig. 1A). The differences between groups remained significant after adjusting for age, sex, and intra-abdominal adipose tissue volume (Supplementary Table 1). The dose–response relationship between plasma insulin concentration and palmitate appearance rate in plasma suggests adipose tissue triglyceride lipolysis is less sensitive to insulin in the OSA compared with the control group but still very responsive to increases in plasma insulin in people with OSA (Fig. 1B). The difference in lipolytic rate between the two groups was not due to a difference in adipose tissue oxygenation (50 ± 6 and 51 ± 12 mm Hg in the OSA and control groups, respectively).
Glucose tolerance and oral and iv glucose-stimulated insulin secretion rate

During the OGTT, both peak plasma glucose concentration (Fig. 2A) and the glucose concentration area under the curve between 0 and 120 min (1,031 ± 120 vs 901 ± 159 mmol/L × min, P < 0.05) were greater in the OSA than the control group. The difference in the glucose response was due to insulin resistance because insulin secretion and plasma insulin concentration after glucose ingestion were not different or even greater in the OSA than in the control group (Fig. 2B and C). The greater insulin secretion rate in the OSA group was caused by the insulin resistance-mediated increase in plasma glucose, because β-cell function, assessed as the insulin secretion rate at any postprandial glucose concentration was not different between the two groups and tended to be less in the OSA group than the control group (Fig. 2D). Basal plasma fatty acid concentration was not different between the OSA and the control group and glucose ingestion decreasing the plasma fatty acid concentration without a difference between the OSA and the control groups (Fig. 2E). Overall, plasma glucagon concentration tended to be greater in the OSA than in the control group and glucagon concentration decreased after glucose ingestion without a difference in the relative decline between the OSA and the control group (Fig. 2F). Plasma glucose and insulin concentrations and the insulin secretion rate after intravenous glucose injection, which resulted in much greater (about double) plasma glucose concentration than glucose ingestion, were not different between the OSA and the control groups (Fig. 3A, B and C).
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Discussion

We used a combination of sophisticated research techniques to evaluate the effect of OSA on glucose homeostasis. The data from our study suggest OSA is associated with marked peripheral insulin resistance (i.e. impaired insulin action in adipose tissue and muscles) whereas hepatic insulin action and insulin secretion during basal conditions and in response to glucose ingestion are unaffected by OSA. These findings may have important clinical implications because disease progression and the risk of diabetes-related complications vary by T2D subtype (i.e. severe insulin resistance vs insulin deficiency) (32, 33).

The findings also help explain some of the heterogeneity in insulin sensitivity observed in people with obesity (1, 2, 3). Although OSA is very common in people with obesity (prevalence ~50% or even more), it is largely unrecognized and underdiagnosed (4), and it is not customary to perform polysomnograms during participant screening for metabolic research studies to exclude participants with OSA. Therefore, it is likely that a considerable proportion of people with obesity who participate in metabolic research studies have unrecognized OSA.

OSA was associated with impaired insulin-stimulated glucose uptake in both skeletal muscles and adipose tissue. The relative decreases in muscle and adipose tissue glucose uptake in participants with OSA compared to those without was similar. Therefore, OSA did not alter the relative contributions of muscle and adipose tissue glucose uptake to whole-body glucose disposal. Accordingly, impaired muscle glucose uptake was the primary reason for impaired whole-body glucose disposal in people with OSA, because insulin-stimulated glucose uptake is much lower in adipose tissue than in skeletal muscle (both per kg tissue and total).

OSA was also associated with insulin resistance of adipose tissue triglyceride lipolysis. The results from studies conducted in mice suggest OSA causes insulin resistance of glucose metabolism by increasing adipose tissue lipolysis and fatty acid release into the circulation (34). However, we found that the basal fatty acid rate of appearance in plasma and basal and postprandial plasma fatty acid concentrations were not different in the OSA and control groups because the greater insulin secretion rate in people with OSA compensated for the insulin resistance. Therefore, it is unlikely that muscle insulin resistance in people with OSA was caused by fatty acid overload. However, we did not investigate the role of fatty acid overload in muscle insulin resistance in people with OSA.

Figure 2
Metabolic response to glucose ingestion. Plasma glucose (A), insulin (B), fatty acid (C), and glucagon (F) concentrations, insulin secretion rate (C), and the insulin secretion rate in relation to plasma glucose concentration (D) after glucose ingestion in the control (open circles) and OSA (gray circles) groups. Data are means and s.e.m. *OSA vs control group, P ≤ 0.05. ISR, insulin secretion rate; OSA, obstructive sleep apnea.

Figure 3
Plasma glucose (A) and insulin (B) concentrations and insulin secretion rate (C) after intravenous glucose injection in the control (open circles) and OSA (gray circles) groups. Data are means and s.e.m. ISR, insulin secretion rate; OSA, obstructive sleep apnea.
not measure adipose tissue lipolytic activity throughout the day. Nevertheless, it is unlikely that daytime lipolytic activity was increased, because adipose tissue lipolysis is very sensitive to increases in plasma insulin and is nearly completely suppressed at plasma insulin concentrations within the postprandial range (23). Our palmitate kinetic data during stage 2 of the hyperinsulminemic-euglycemic clamp and the fatty acid concentration data during the OGTT confirmed this.

The mechanisms responsible for reduced muscle and adipose tissue insulin action in people with OSA are unknown. OSA is characterized by repetitive upper airway narrowing or collapse, which causes intermittent hypoxia and sleep fragmentation (i.e. short arousals from sleep). Both sleep fragmentation and intermittent hypoxia have been implicated in causing metabolic dysfunction. Carefully-designed laboratory-based studies that applied acoustic and mechanical stimuli to trigger short arousals during sleep but prevented full awakenings, found this type of sleep fragmentation causes a marked reduction in whole-body insulin sensitivity (35, 36). In addition, the adverse effects of OSA on plasma glucose concentration correlate with the degree of night-time hypoxemia (4, 37, 38, 39), which suggests a lack of oxygen also contributes to metabolic dysfunction. However, the results from studies that evaluated the effect of experimentally-induced hypoxia on glucose metabolism in people are equivocal. The reasons for the discrepancy in results are not clear but are most likely related to differences in hypoxia protocols (including duration, severity, and timing) (40). For example, a single 5-h session of experimental intermittent hypoxia during wakefulness decreased glucose tolerance (41) whereas ten nights of experimental continuous night-time hypoxia increased whole-body insulin sensitivity (42). Moreover, it is well-established that hypoxia improves the insulin sensitivity of glucose transport in myocytes (40, 43). We observed no difference in the daytime (shortly upon wakening) arterial oxygen tension between the groups. Moreover, muscle and adipose tissue blood flow, and therefore oxygen delivery to tissues, and adipose tissue oxygenation were not different in the OSA and control groups. These results are consistent with an earlier report on the effect of OSA on adipose tissue perfusion and oxygenation (44); although the results from that study have to be interpreted with caution because the control group was recruited from the general population and the absence of OSA was not confirmed by polysomnography. It is therefore unlikely that hypoxia is causally involved in the pathogenesis of insulin resistance in people with OSA. The relationship between the severity of hypoxemia and metabolic dysfunction often observed in people with OSA (4, 37, 38, 39) is likely a secondary phenomenon of the direct relationship between severity of OSA - which affects the degree of night-time hypoxemia – and metabolic dysfunction. Although this seems to contradict the results from a recent study that concluded the severity of OSA is unrelated to metabolic function (45), it is important to notice that this study only included participants who were at high risk for T2D (assessed on the basis of multiple factors that included lifestyle, family history, medical history) and already had prediabetes or newly diagnosed (during the screening evaluation) diabetes. Moreover, the study did not include a control group without OSA (45). Together, these findings suggest the adverse effects of OSA on metabolic function occur early after the onset of OSA and are not further aggravated by further worsening OSA.

Chronic, low-grade, non-infectious inflammation is considered an important contributor to insulin resistance and metabolic dysfunction (46). However, a causal involvement of inflammation in insulin resistance in people has not been established and many studies report no or only weak inverse relationships between circulating markers of inflammation and insulin sensitivity (1, 46, 47, 48, 49). Consistent with these reports, we found no difference in the plasma concentration of C-reactive protein, a common marker of inflammation, between the OSA and the control group. Chronic low-grade stress has also been proposed as a potential mediator of metabolic function (50), but we found no difference in markers of adrenergic tone between the OSA and the control group. It is possible that other mediators (e.g. circulating exosomes) or cell-intrinsic factors and alterations in signaling pathways induced by epigenetic modification are involved in causing insulin resistance in people with OSA (48, 51).

Although we found both the insulin secretion rate and plasma insulin concentration after glucose ingestion were (or tended to be) greater in the OSA than the control group, the amount of circulating insulin was insufficient to prevent the insulin resistance-mediated increase in postprandial plasma glucose concentration, because \( \beta \)-cell function (the amount of insulin secreted in relation to plasma glucose) was unaffected by OSA. These findings are consistent with the results from studies that evaluated the effect of experimentally induced sleep disruption on glucose homeostasis and found sleep disruption caused marked whole-body insulin resistance without a change in the glucose-stimulated rise in plasma insulin (35, 36).

In summary, the data from our study demonstrate that OSA is a key determinant of insulin sensitivity in people with OSA.
with obesity and underscore the importance of taking OSA status into account when evaluating the metabolic function in people with obesity. These findings also suggest people with OSA may benefit most from the targeted treatment of peripheral insulin resistance and early screening for complications associated with peripheral insulin resistance.

Supplementary materials
This is linked to the online version of the paper at https://doi.org/10.1530/EJE-21-1025.

Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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Author contribution statement
B M, R J G, and Y S J designed the study. H C E K, S v V, C C, B W P, D N R, R L F, R J G, Y S J, and B M contributed to data acquisition, data analysis, and data interpretation. H C E K and B M wrote the first draft of the report. All authors contributed to the revision of the report for crucial intellectual content. B M is the guarantor of this work, had full access to all the data in the study, and assumes full responsibility for the integrity of the data and the accuracy of the data analysis.

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