Subdiaphragmatic vagal deafferentation affects body weight gain and glucose metabolism in obese male Zucker (fa/fa) rats

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Ferrari, Bettina, Myrtha Arnold, Richard D. Carr, Wolfgang Langhans, Giovanni Pacini, Thora B. Bodvarsdottir, and Dorte X. Gram. Subdiaphragmatic vagal deafferentation affects body weight gain and glucose metabolism in obese male Zucker (fa/fa) rats. Am J Physiol Regul Integr Comp Physiol 289: R1027–R1034, 2005. First published May 26, 2005; doi:10.1152/ajpregu.00736.2004.—We investigated the effect of subdiaphragmatic vagal deafferentation (SDA) on food intake, body weight gain, and metabolism in obese (fa/fa) and lean (Fa/?) Zucker rats. Before and after recovery from surgery, food intake and body weight gain were recorded, and plasma glucose and insulin were measured in tail-prick blood samples. After implantation of a jugular vein catheter, an intravenous glucose tolerance test (IVGTT) was performed, followed by minimal modeling to estimate the insulin sensitivity index. Food intake relative to metabolic body weight (g/kg0.75) and daily body weight gain after surgery were lower (P < 0.05) in SDA than in sham obese but not lean rats. Before surgery, plasma glucose and insulin concentrations were lower (P < 0.05) in lean than in obese rats but did not differ between surgical groups within both genotypes. Four weeks after surgery, plasma glucose and insulin were still similar in SDA and sham lean rats but lower (P < 0.05) in SDA than in sham obese rats. IVGTT revealed a downward shift of the plasma insulin profile by SDA in obese but not lean rats, whereas the plasma glucose profile was unaffected. SDA decreased (P < 0.05) area under the curve for insulin but not glucose in obese rats. The insulin sensitivity index was higher in lean than in obese rats but was not affected by SDA in both genotypes. These results suggest that elimination of vagal afferent signals from the upper gut reduces food intake and body weight gain without affecting the insulin sensitivity index measured by minimal modeling in obese Zucker rats.

food intake; insulin sensitivity; minimal modeling; obesity

THE ZUCKER FATTY RAT (fa/fa) rat is homozygous for a mutation in the leptin receptor gene (29). Leptin inhibits the activity of neuropeptide Y and stimulates the expression of proopiomelanocortin and the cocaine- and amphetamine-regulated transcript (CART), resulting in reduced food intake and increased energy expenditure. In the obese Zucker rat, the signal from leptin is not transduced. Neuropeptide Y activity becomes chronically increased, and hardly any CART expression can be found in the arcuate nucleus. This leads to increased food intake and decreased energy expenditure and provides an internal milieu that promotes the accretion of body fat (38). The obese Zucker rat develops severe obesity and pronounced hyperinsulinemia with relatively mildly hyperglycemia (6, 37). Long-lasting hyperinsulinemia is responsible for changes in target tissue sensitivity to the glucose regulatory effect of insulin and may contribute to profound insulin resistance (12, 38). Secondary changes consist of hyperlipemia with elevated concentrations of plasma triglycerides and plasma cholesterol, increased number and size of adipocytes and pancreatic islets, hepatic steatosis, and an increased activity of parasympathetic efferents accompanied by a decreased activity of sympathetic nerves (12, 13, 38).

Vagal afferents provide the major neuroanatomical link between the gut, which digests and absorbs ingested nutrients, and the central nervous system, which ultimately controls energy balance. Several lines of reasoning suggest that gut vagal afferent signals are crucial in the control of food intake and energy balance. One method for examining gut vagal afferent function is treatment with the neurotoxic vanilloid capsaicin, found in red peppers. Systemic capsaicin administration damages poorly myelinated and unmyelinated small-diameter sensory neurons and produces neuronal degeneration of primary sensory afferents and neurons within the nucleus of the solitary tract (32). Capsaicin desensitization improves fasting blood glucose and oral glucose tolerance in normal rodents (11, 16), Zucker diabetic fatty rats, and obese Zucker rats (9). These results raise the question of whether elimination of vagal afferent signaling is the crucial lesion for these metabolic improvements. Subdiaphragmatic vagal deafferentation (SDA), a surgical procedure that eliminates all vagal afferents from the upper gut, including the liver, but leaves more than half of the vagal efferents intact (25), allows for a more specific examination of this question. We therefore investigated the effects of SDA on food intake, body weight gain, insulin sensitivity, and glucose metabolism in male lean and obese Zucker rats.

METHODS

Laboratory animals. Eighteen male genetically obese (fa/fa) Zucker rats and 16 lean littermate controls (Fa/?) (Harlan Netherlands), weighing 135–165 g on arrival, were individually housed in custom-made Macrolon cages (41 × 25 × 31 cm) with grated stainless steel floors. The room was maintained at 21 ± 2°C with a 12:12-h light-dark cycle (light on at 8:00 AM). Rats had free access to water and standard laboratory chow (no. 3433; Provimi Kliba NAFAG, Kaistearugst, Switzerland; metabolizable energy content 12.4 kJ/g), unless noted otherwise; they were weighed and handled...
daily and were adapted to housing conditions for 5 days before the first measurements were made. Obese and lean blood donor rats were purchased at the same time and kept under the same conditions. The Canton of Zurich’s animal use and care committee approved all procedures.

Food intake measurements. Twenty-three-hour spontaneous food intake was measured on five consecutive days in the week before SDA and sham surgery, starting when average body weights of lean and obese rats were 210 ± 2 and 208 ± 5 g (means ± SE), respectively, and on 13 consecutive days starting on day 16 after surgery, when average rates of body weight gain had reached presurgery values. At the end of the 13-day postsurgery measurement period, body weights were 325 ± 4 (sham) and 309 ± 5 g (SDA) for lean as well as 413 ± 4 (sham) and 398 ± 4 g (SDA) for obese rats, respectively. Just before lights out, feeding cups were placed in the cages. Food intake was measured by manually weighing (±0.1 g) the feeding cups after 23 h. Spillage was collected on paper spread beneath the cages and was also measured. Food intake (Table 1) is given as grams per kilogram of metabolic body weight (kg0.75) because of the different body weights of lean and obese rats.

Subdiaphragmatic vagal deafferentation. SDA was performed according to the method of Norgren and Smith (25) when lean rats weighed 251 ± 4 (sham) and 245 ± 2 g (SDA) and obese rats weighed 236 ± 3 (sham) and 246 ± 2 g (SDA), respectively. In a preliminary experiment, SDA reduced body weight gain in obese rats (unpublished results). Therefore, bigger obese rats were chosen for SDA than for sham surgery to achieve approximately the same body weight of both surgical groups at the time of the intravenous glucose tolerance test (IVGTT; see Intravenous glucose tolerance test). The deafferentation procedure consisted of a left afferent vagal rootlet transection at the brain stem level and an ipsilateral subdiaphragmatic vagotomy of the dorsal trunck (34). During sham operations, the left vagus was exposed but left untouched, and a midline laparotomy and exposure of the dorsal subdiaphragmatic trunck was made, leaving the trunck intact. To minimize postsurgery disturbances of intestinal motility in SDA rats (18), all rats received sweetened milk and four or two food pellets starting 2 days before surgery. After surgery, SDA rats received sweetened milk (days 1 and 2 after surgery), sweetened milk and wet mash (day 3 after surgery), and sweetened milk, wet mash, and two to six pellets (days 4–7 after surgery). Sham-operated rats were fed sweetened milk, wet mash, and two to six pellets for the first 3 days after surgery. On day 4, wet mash and pellets were presented.

Milk and water were taken away at 3 and 1 h before surgery, respectively. Temgesic (0.025 mg/rat; Essex Chemie, Luzern, Switzerland) and atropine (0.015–0.025 mg/rat; Sintetica, Mendrisio, Switzerland) were injected subcutaneously at 0.5–1 h before surgery. Inhalation anesthesia was induced and maintained with a mixture of isoflurane, oxygen, and NO2. Body temperature was maintained at 37–38°C throughout surgery with a 39°C water pad. Rats were treated with 80 µl of BorgaI 7.5% subcutaneously (Intervet, Boxmeer, The Netherlands) and 5 ml of saline intraperitoneally during surgery. Postoperatively they received Rimady1 for three consecutive days (5 mg/kg sc; Graub, Bern, Switzerland).

### Table 1. Daily body weight gain and daily food intake/metabolic body weight in lean and obese Zucker rats

<table>
<thead>
<tr>
<th>Zucker Rats</th>
<th>n</th>
<th>Body Weight Gain, g</th>
<th>Food Intake/Metabolic Body Weight, g/kg0.75</th>
</tr>
</thead>
<tbody>
<tr>
<td>Before surgery</td>
<td></td>
<td>After surgery</td>
<td>Before surgery</td>
</tr>
<tr>
<td>Sham lean</td>
<td>8</td>
<td>4.9 ± 0.2</td>
<td>2.9 ± 0.2</td>
</tr>
<tr>
<td>SDA lean</td>
<td>7</td>
<td>5.6 ± 0.4</td>
<td>3.2 ± 0.2</td>
</tr>
<tr>
<td>Sham obese</td>
<td>8</td>
<td>7.6 ± 0.4</td>
<td>6.1 ± 0.2</td>
</tr>
<tr>
<td>SDA obese</td>
<td>10</td>
<td>7.7 ± 0.2</td>
<td>5.1 ± 0.3</td>
</tr>
</tbody>
</table>

Values represent means ± SE for daily body weight gain and daily food intake/metabolic body weight in lean and obese Zucker rats for 5 days before subdiaphragmatic vagal afferentation (SDA) or sham surgery and for 13 days thereafter, starting on day 16 after surgery. *P < 0.05; **P < 0.01; ***P < 0.001 vs. Sham, Fisher’s protected least significant difference (PLSD).

### Functional vagotomy verification.

Previous studies have demonstrated that left vagal afferent rootlet transection combined with dorsal vagal subdiaphragmatic transection blocks the suppression of feeding produced by low (1–6 µg/kg) but not higher (8–16 µg/kg) intraperitoneal doses of CCK-8. To obtain a functional verification of SDA in the present study, we injected PBS vehicle solution (Dulbecco’s without Ca2+ or Mg2+; Invitrogen Life Technologies, Basel, Switzerland) or 4 µg/kg CCK (Bachem, Bubendorf, Switzerland) in PBS (1 µg CCK/150 µl PBS) intraperitoneally just before lights out. The order of drug and vehicle treatments was counterbalanced between two test days, separated by one intervening day. Food intake was measured after 30, 60, and 120 min. The rats were adapted to this procedure by intraperitoneal injections of saline on two consecutive days before the test was carried out.

### Histological vagotomy verification.

For SDA verification, each animal received two intraperitoneal injections (0.5 ml) of Fluoro-Gold (Fluorochrome, Denver, CO) solution (2 mg/ml saline). Three days after injection, rats were anesthetized (see Subdiaphragmatic vagal deafferentation) and perfused with the following two solutions into the left ventricle: 1) 100 ml of saline with heparin (10 U/ml) delivered for 3 min and 2) 400 ml of cold 4% paraformaldehyde dissolved in 0.1 M sodium phosphate buffer delivered over a period of 15 min.

The fixed brain was examined under the microscope to verify that the vagal afferent rootlets were 1) cut on the left side and 2) intact on the right side as they entered the medulla. The brain and brain stem were then removed and postfixsed in 4% paraformaldehyde in 0.1 M sodium phosphate buffer for 1 h and cryoprotected in a solution of 30% sucrose in distilled water for 3 days. The medulla was sectioned into slices of 40 µm. Sections for the Fluoro-Gold analysis were air-dried and examined with a fluorescence microscope. Because Fluoro-Gold is taken up and transported retrogradely in intact neurons but not in neurons with transected axons, a successful dorsal trunk subdiaphragmatic vagotomy was confirmed by 1) the absence of label in the right dorsal motor vagal nucleus, where the cut dorsal subdiafragmatic vagal trunk would project, and 2) the presence of label in the left dorsal motor vagal nucleus, where the intact efferents of the ventral vagal subdiaphragmatic trunk project.

### Baseline blood sampling by tail incision.

In the week before and the 4 weeks after surgery, blood samples were taken by tail incision (Fig. 1). The rats were food deprived for 3 h. Approximately 1 h after dark onset, 150 µl of blood were collected in an EDTA tube (Microvette, Sarstedt, Switzerland). The blood was centrifuged (8 min, 5,000 g) within 15 min, and plasma was stored (−20°C) for later analysis of insulin and glucose.

### Jugular vein catheter implantation.

The jugular vein catheters were assembled as previously described (36). They were exteriorized between the scapulae and terminated in a cap system that was designed to allow easy attachment to a fixed-line catheter extension on the day of experimentation. Catheter implantations were done according to the method described by Steffens (35), with some modifications (22, 36). The distal end of the cannula was implanted into the maxillary vein 1 mm above the junction with the facial and linguofacial vein. The inner end of the cannula was situated 2 mm inside the right atrium, and the cannula was kept in place by two ligatures. After surgery, the
VAGAL DEAFFERENTATION IN ZUCKER RATS

Fig. 1. Timeline of the experiment. The rats' initial body weights were 135–165 g. The first measurements started after an adaptation time of 5 days. IVGTT, intravenous glucose tolerance test.

catheter was filled with heparinized polyvinylpyrrolidone [PVP; 500 U heparin/ml PVP (21 g of PVP in 12 ml of 0.9% NaCl; Sigma, St. Louis, MO)] and flushed daily with saline to ensure patency. The catheters were gas-sterilized with ethylene oxide before use. The rats were food deprived for 6 h and water deprived for 1 h before surgery. Premedication consisted of atropine (0.025 mg/rat) and Temgesic (0.025 mg/rat). 0.5–1 h before anesthesia (see Subdiaphragmatic vagal deafferentation). Post-surgical medication consisted of Boral (80 μl) and Rimadyl (0.5 mg/100 g) over 3 days.

Catheter handling. Six days after jugular vein catheter implantation, the rats were adapted to the experimental procedures. The cap closing the head was replaced by an ~64-cm-long polyethylene tube (Intramedic; Becton-Dickinson, Sparks, MD) filled with sterile saline, allowing the rat to move freely. A syringe was attached to the free end, and 80 μl of blood were aspirated and discarded. The blood sample (170 μl) was taken with a second syringe and immediately transferred to an Eppendorf tube chilled in ice and treated with heparin (25 IU/ml, heparin-Na; B. Braun Medical, Emmenbrücke, Switzerland). The catheter was flushed with 300 μl of saline and 50 μl of heparinized saline (50 μl/ml). A small air bubble was then introduced into the catheter, followed by saline, until the bubble was just above the end of the headset. A second small air bubble was sent 2 cm down the sampling tube to ensure that the first bubble was inside the cannula, to separate the circulating blood from the heparin solution.

Intravenous glucose tolerance test. Exactly 1 wk after implantation of the jugular vein catheters, an IVGTT was performed. Rats were food deprived for 12 h during the light phase. Starting approximately 1 h after dark onset, blood samples (150 μl each) were taken at 0, 3, 6, 9, 12, 15, 20, 30, 40, 50, 60, and 90 min. At time 0, immediately after the second blood sample was drawn, a 50% glucose solution at a dose of 750 mg/kg (440–700 μl) was infused (10 s) into the jugular vein. The blood was centrifuged (8 min, 8,000 rpm) within 15 min after the samples were taken, and plasma was stored for later analysis of glucose, insulin, and lactate. After the last blood sample was taken, 3–3.5 ml of stabilized [ACD-solution (15%); Cantonal Apothecary, Zurich, Switzerland] donor blood was infused to replace the withdrawn blood volume.

Baseline blood sampling via catheters. Rats were food deprived during the light phase for 3 h. Blood was taken 1 h after dark onset: 220 μl of blood were added to 7.6 μl of heparin fluoride (25 IU heparin, 3 mg fluoride/ml blood) for later measurements of plasma glucose and lactate; 1.3 ml of blood was added to 26 μl of EDTA (1.8 mg/ml blood) for later measurements of plasma free fatty acids, triglycerides, and insulin. The samples were placed immediately on ice and centrifuged within 15 min, and the plasma was stored (−20°C). For these measurements, 7.5% trisodium citrate was used instead of heparinized saline to keep the catheters patent.

Plasma analyses. Plasma glucose, lactate, free fatty acids, and triglycerides were determined using standard enzymatic methods adapted for the Cobas Mira autoanalyzer (Roche). Plasma insulin concentration was measured using a rat-specific radioimmunoassay (Linco, St. Charles, MO).

Glucose tolerance and insulin sensitivity. Insulin and glucose data from the IVGTT were analyzed using the minimal model technique. The model assumes first-order nonlinear insulin-controlled kinetics and accounts for the effect of insulin and glucose itself on glucose disappearance following exogenous glucose injection. The analysis provides the parameters $S_I$ (insulin sensitivity index, $10^{-4}$ min$^{-1}$ μU$^{-1}$ml), which is defined as the ability of insulin to enhance glucose disappearance and inhibit glucose production (1), and $S_G$ (glucose effectiveness, min$^{-1}$), representing glucose disappearance from plasma without any change in dynamic insulin (4). The acute insulin response (AIR, μU/ml) was calculated as the mean suprabasal insulin concentration of samples from 3 to 6 min. The disposition index (DI, min$^{-1}$) was obtained by multiplying $S_I$ by AIR. DI is an extension of the concept proposed in humans by Kahn et al. (15) and describes the net insulin effect by including both insulin action and insulin secretion. Finally, the glucose elimination rate after the glucose injection ($K_G$, the glucose tolerance index, %/min) was calculated as the slope for the interval 5–20 min after glucose injection of the logarithmic transformation of the individual plasma glucose values (27). $K_G$ is a combination of insulin-independent ($S_C$) and insulin-dependent processes (DI). The usefulness of the method, originally developed in dogs (3) and largely exploited in humans (1), also has been proved in rodents such as mice (27).

Body fat. Perirenal, mesenterial, and epididymal fat pads were excised and weighed (±0.1 g) at the time of death. The summarized weight of all three fat pads for each animal was taken as an indicator of body fat.

Total liver fat measurements. Total liver fat was analyzed with the Soxhlet method. The livers were dried and homogenized, and petrolether was used as solvent.

RESULTS

Functional verification of complete SDA. Intraperitoneal injection of CCK-8 reduced 30-min food intake after a 4-h food deprivation compared with intraperitoneal vehicle injection in lean (89.5 ± 3.2%) and obese (66.4 ± 3.8%) sham rats but not in lean (10.4 ± 6.6%) and obese (9.3 ± 2.7%) SDA rats ($P < 0.05$).
0.01 for lean and obese). The feeding suppressive effect of CCK had disappeared after 2 h (not shown). With the use of the fluorogold verification procedures, all but one SDA rat were determined to have successful, complete vagal deafferentations.

Food intake. Mean daily food intake relative to metabolic body weight (g/kg^0.75) before and after surgery was consistently greater in obese than in lean Zucker rats. Food intake over five consecutive days before surgery was similar in lean and obese rats designated for SDA and sham surgery, respectively (Table 1). After recovery from surgery, mean daily food intake (g/kg^0.75) during the measurement period of 13 days was lower in SDA than in sham obese rats, but no significant difference was observed between SDA and sham lean rats (Table 1). Absolute daily food intake values for all groups presented a similar picture, i.e., sham and SDA lean rats ate 22.8 ± 0.5 and 21.1 ± 0.5 g (P < 0.05), respectively, whereas sham and SDA obese rats ate 32.4 ± 0.8 and 28.7 ± 0.6 g (P < 0.05), respectively. The pre-to postsurgery differences for food intake (g/kg^0.75) were greater for SDA in obese and lean rats (Table 1).

Body weight gain. Daily body weight gain over five consecutive days before surgery did not differ between lean and obese rats designated for SDA and sham surgery. During the 13 measurement days after recovery from surgery, however, body weight gain was lower in SDA than in sham obese but not in SDA and sham lean rats (Fig. 2). This is not fully reflected by pre-to postsurgery differences within the various groups, which appeared to be bigger in SDA than in sham obese rats, but this difference did not reach statistical significance (Table 1). Nevertheless, body weight of SDA and sham obese rats was similar at the time of the IVGTT [440 ± 9 and 452 ± 10 g (P > 0.05) for SDA and sham, respectively].

Baseline plasma glucose and insulin in tail incision samples. Plasma glucose and insulin values 1 wk before surgery were similar in lean and obese rats designated for SDA or sham surgery. Four weeks after surgery, 3-h fasting plasma glucose and insulin levels were still similar in SDA and sham lean rats but were lower in SDA obese than in sham obese rats. There were no differences between pre- and postsurgery values of glucose and insulin in lean rats. However, postsurgery plasma concentrations were higher than presurgery values in both obese groups for plasma insulin and lower in SDA obese rats for plasma glucose (Table 2).

Plasma metabolites and insulin in catheter blood samples. Basal blood samples after 3 h of fasting were taken 6 wk after surgery (see Fig. 1). Plasma glucose and free fatty acid concentrations showed no group differences. Plasma insulin, lactate, and triglyceride concentrations were not affected by SDA in lean rats but were significantly decreased by SDA in obese rats (Table 3).

Body fat. At the time of death, body fat as estimated by the sum of perirenal, mesenteric, and epididymal fat pad weights was much higher in obese than in lean animals but did not differ between the SDA and sham groups in either lean (absolute: sham 13.3 ± 0.7 g, SDA 12.2 ± 0.5 g, P > 0.05; relative to body weight: sham 3.5 ± 0.2%, SDA 3.4 ± 0.1%, P > 0.05) or obese rats (.absolute: sham 46.4 ± 1.0 g, SDA 46.1 ± 1.4 g, P > 0.05; relative to body weight: sham 8.9 ± 0.2%, SDA 8.9 ± 0.2%, P > 0.05).

Total liver fat. Total liver fat was significantly reduced by SDA in lean (sham 9.1 ± 0.3%, SDA 8.4 ± 0.2%, P < 0.02) and obese rats (sham 36.8 ± 1.7%, SDA 28.3 ± 2.6%, P < 0.03).

Intravenous glucose tolerance test. Metabolic parameters for the four groups are reported in Table 4. Plasma glucose values and first peak AUC for glucose did not differ between SDA and sham in lean or obese rats throughout the IVGTT (Fig. 3). The total AUC for insulin reflects the total release of the hormone related to the specific glucose pattern. However, it may be important to evaluate the first-phase release, i.e., the immediate rise in insulin concentration following the glucose bolus. This is expressed by AIR, the acute insulin response, i.e., the hyperinsulinemia immediately following the glucose bolus, which was not different between SDA and sham lean rats. In obese rats, plasma insulin values were lower in SDA than in sham throughout the IVGTT (overall effect P < 0.01), and SDA decreased the AUC of the first peak (sham 1,777.6 ± 117.2 ng/ml, SDA 895.8 ± 28.6 ng/ml, P = 0.01) (Fig. 4).

The minimal model analysis showed that SDA affected neither insulin sensitivity (S_I) nor glucose effectiveness (S_G) or glucose tolerance (K_G, DI) in both lean and obese Zucker rats (Table 4). S_I and DI were, however, markedly different (P < 0.001) when compared between lean and obese Zucker rats within the same surgical group, i.e., sham or SDA. S_G tended to be lower in obese Zucker rats, showing that there is a general impairment in glucose metabolism (in both the insulin-dependent and insulin-independent mechanisms) in these latter groups.

### Table 2. Plasma glucose and insulin levels 1 wk before SDA or sham surgery and 4 wk thereafter

<table>
<thead>
<tr>
<th>Zucker Rats</th>
<th>Before surgery</th>
<th>After surgery</th>
<th>Before surgery</th>
<th>After surgery</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sham lean</td>
<td>7.1 ± 0.1</td>
<td>6.9 ± 0.2</td>
<td>1.2 ± 0.1</td>
<td>1.3 ± 0.2</td>
</tr>
<tr>
<td>SDA lean</td>
<td>7.1 ± 0.2</td>
<td>6.7 ± 0.1</td>
<td>1.1 ± 0.1</td>
<td>1.2 ± 0.2</td>
</tr>
<tr>
<td>Sham obese</td>
<td>8.2 ± 0.3</td>
<td>8.3 ± 0.2</td>
<td>6.0 ± 0.6d</td>
<td>13.5 ± 1.4</td>
</tr>
<tr>
<td>SDA obese</td>
<td>8.0 ± 0.2c</td>
<td>7.2 ± 0.3d</td>
<td>5.6 ± 0.5b</td>
<td>10.4 ± 0.6a</td>
</tr>
</tbody>
</table>

The samples were taken by tail incision after 3 h of fasting. Values represent means ± SE. *P < 0.01; **P < 0.001 vs. sham. *P < 0.05; **P < 0.01; ***P < 0.001 vs. after SDA surgery (Fisher’s LSD).
Plasma lactate values and first peak AUC for lactate during the IVGTT also were not different in SDA and sham lean rats. In obese rats, however, SDA provoked a change in the plasma lactate profile. Although the overall effect and the first peak AUC for lactate also were not different during the IVGTT, single time point measurements revealed that lactate both increased more initially (P < 0.05 for 15 and 20 min) and returned to baseline faster in SDA obese than in sham obese rats (lower lactate concentration at 45 and 60 min, P < 0.05) (Fig. 5).

**DISCUSSION**

**Food intake and body weight gain.** The present results suggest that SDA decreases food intake and body weight gain and alters metabolism, in particular, in obese Zucker rats. The pre- to postsurgery differences in food intake differed between SDA and sham groups in obese as well as in lean rats, suggesting that SDA had some effect on food intake in the latter group as well. Yet, unlike the significant decrease in food intake and body weight gain in obese rats, this latter finding probably did not account for the different effects of SDA in both genotypes, because in yet another study of ours (unpublished observations), SDA also reduced food intake and body weight gain in obese Zucker rats when performed at an average body weight of 284 g. According to the standard growth curves mentioned above, this body weight translates into a slightly higher age than the lean rats used in the present study. As vagal affenter signals contribute to the control of food intake, SDA may modify satiety or hunger signals from the stomach, the small intestine, or the liver. An SDA-induced change in the central control of food intake or differences in energy expenditure might also contribute to the different food intake of sham and SDA rats. Why this appears to occur primarily in obese rats and less so in lean rats can only be speculated on. Perhaps an effect of SDA on food intake becomes more apparent when energy balance regulation is compromised, as in the obese Zucker rat. In any case, our data suggest that the effect of SDA is strong enough to partially counteract the lack of leptin signaling, i.e., to reduce the degree of obesity, in the obese Zucker rat. Results from a comparable study (unpublished observations) suggest that SDA does not change gastric emptying in lean and obese Zucker rats. Therefore, it appears unlikely that increased gastric fill after a meal inhibited eating in SDA rats. Finally, rats with a genetic deficit in CCK-A receptors are hyperphagic and obese (24). Yet, this does not necessarily contradict the effects of SDA presently observed in obese Zucker rats, because SDA also eliminates feeding stimulatory signals and mechanisms that may require vagal afferents.

The comparison of cumulative metabolizable energy intake over a period of time and body weight changes during this time allows for a rough estimate of energy expenditure (see Ref. 30). The cumulative difference in 13-day postsurgery mean metabolizable energy intake between sham and SDA rats was ~600 kJ for the obese and ~280 kJ for the lean animals. The cumulative difference in body weight gain during this time was ~13 and ~4 g for obese and lean rats, respectively. If this

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**Table 3. Plasma metabolites and insulin in blood samples taken by catheter after 3 h of fasting at 6 wk after SDA or sham surgery**

<table>
<thead>
<tr>
<th>Zucker Rats</th>
<th>n</th>
<th>Lactate, mmol/l</th>
<th>Insulin, ng/ml</th>
<th>Triglycerides, mmol/l</th>
<th>Free Fatty Acids, mmol/l</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sham lean</td>
<td>8</td>
<td>0.8±0.08</td>
<td>1.6±0.17</td>
<td>0.8±0.09</td>
<td>0.4±0.04</td>
</tr>
<tr>
<td>SDA lean</td>
<td>7</td>
<td>0.6±0.04</td>
<td>1.6±0.16</td>
<td>0.9±0.09</td>
<td>0.5±0.05</td>
</tr>
<tr>
<td>Sham obese</td>
<td>8</td>
<td>2.2±0.15</td>
<td>17.1±1.49</td>
<td>2.9±0.31</td>
<td>0.7±0.11</td>
</tr>
<tr>
<td>SDA obese</td>
<td>10</td>
<td>1.4±0.11</td>
<td>12.0±1.22</td>
<td>1.9±0.11</td>
<td>0.9±0.12</td>
</tr>
</tbody>
</table>

Values represent means ± SE. *P < 0.05; **P < 0.01; ***P < 0.001 vs. sham (Fisher’s PLSD).

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**Table 4. Metabolic parameters from IVGTT**

<table>
<thead>
<tr>
<th>Zucker Rats</th>
<th>n</th>
<th>AUC Glucose</th>
<th>Peak Insulin</th>
<th>AUC Insulin</th>
<th>K_G</th>
<th>S_l</th>
<th>S_G</th>
<th>AIR</th>
<th>DI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sham lean</td>
<td>8</td>
<td>13±0.3</td>
<td>100±10</td>
<td>2.9±0.3</td>
<td>3.28±0.15</td>
<td>6.24±1.53</td>
<td>0.053±0.009</td>
<td>93±11</td>
<td>47.0±13.5</td>
</tr>
<tr>
<td>SDA lean</td>
<td>7</td>
<td>13±0.3</td>
<td>98±15</td>
<td>2.3±0.2</td>
<td>2.93±0.21</td>
<td>7.06±1.19</td>
<td>0.052±0.010</td>
<td>90±13</td>
<td>57.9±14.1</td>
</tr>
<tr>
<td>Sham obese</td>
<td>5</td>
<td>16±0.5</td>
<td>978±75</td>
<td>30.4±3.3</td>
<td>3.63±0.31</td>
<td>1.54±0.22</td>
<td>0.037±0.014</td>
<td>897±58</td>
<td>97.0±8.9</td>
</tr>
<tr>
<td>SDA obese</td>
<td>8</td>
<td>16±0.2</td>
<td>821±43</td>
<td>24.4±0.8</td>
<td>4.04±0.25</td>
<td>1.73±0.73</td>
<td>0.043±0.005</td>
<td>763±44</td>
<td>103.6±18.2</td>
</tr>
</tbody>
</table>

Values are means ± SE, determined from intravenous glucose tolerance test. AUC glucose, total area under glucose concentration curve (min·g−1·dl−1); peak insulin, insulin peak following glucose bolus (μU/ml); AUC insulin, total area under insulin concentration curve (min·μU−1·ml−1); K_G, glucose tolerance index (%/min); S_l, insulin sensitivity index (10−4·min−1·μU−1·ml−1); S_G, glucose effectiveness (min−1); AIR, acute insulin response (mean suprabasal insulin concentration from 3 to 6 min after glucose bolus (μU/ml); DI, disposition index, defined as S_l × AIR (10−3·min).
difference in body weight represented solely a decrease in the accretion of adipose tissue, then a food intake difference of \( \sim 520 \) kJ for the obese and \( \sim 160 \) kJ for the lean would be required to account for the difference in body weight (14). Thus, in both obese and lean Zucker rats, SDA appeared to decrease food intake more than required to account for the observed difference in body weight (610 > 520 and 280 > 160 kJ) and, hence, appeared to decrease energy expenditure in addition to energy intake.

Other studies using complete subdiaphragmatic vagotomy (26) or peripheral desensitization with capsaicin (9) in Zucker rats failed to detect an effect on body weight. This discrepancy may be due to differences in the methodology (the complete subdiaphragmatic vagotomy eliminates all vagal efferents in addition to afferents; capsaicin desensitization is not specific for vagal afferents) and/or to the animals’ much higher body weights (400–750 g) at the time of the subdiaphragmatic vagotomy in one study (26) compared with the rats’ body weights at the time of surgery in our study (230–250 g). Thus our data suggest that the effects of vagal deafferentation on body weight gain in obese Zucker rats are only detectable if such manipulations are performed before overt obesity and insulin resistance have developed and if the primary lesion is afferent. Several previous studies with neonatal capsaicin treatment in mice and rats, however, also yielded different results.

**Plasma metabolites and insulin.** Mild hyperglycemia in obese Zucker rats is presumably caused by an increase in hepatic glucose output (12) and a decreased glucose uptake, associated with a defective basal glucose transport (37). The slight decrease of plasma glucose after SDA in obese Zucker rats therefore may be related to a better uptake of circulating glucose into cells or to an enhanced metabolism of glucose.

The marked increase in plasma insulin in the obese rats from the first to the second tail incision is probably related to the characteristic development of insulin resistance in Zucker rats over time. Hyperinsulinemia in obese animals is attributed to a decreased ability of the liver to clear insulin (8) and, more importantly, to increased insulin secretion partially resulting from vagal stimulation (12). The lack of all vagal afferents and of 40–50% of vagal efferents after SDA presumably blocks the stimulatory effect of the vagus on \( \beta \)-cells and, hence, decreases insulin release. In line with this assumption, plasma insulin was lower in SDA than in sham obese rats. It needs to be determined whether this effect is due to the loss of vagal efferent signals that stimulate insulin secretion or to the disruption of afferent neurons that transmit information to the brain, which then reflexively inhibits insulin release via sympathetic efferents to the pancreas, or to a combination of both (5, 16). Hyperinsulinemia correlates with body weight and, in particular, body fat (e.g., Ref. 6). SDA and sham obese rats’ body weights were similar when blood samples were taken, and their body fat did not differ at the time of death. Therefore, a lower body fat mass presumably did not contribute to the lower plasma insulin of SDA obese rats. The observed SDA-induced decrease in plasma insulin in obese Zucker rats is in concordance with recent studies using the capsaicin analog resiniferatoxin in Zucker rats (10, 23) but in contrast to studies with capsaicin-desensitized obese Zucker rats (9). This discrepancy may be due to a role of capsaicin-insensitive afferent nerve fibers, which are cut in SDA, or to the animals’ different body weights at the time of the manipulation in the two studies, in that desensitization at a lower body weight (i.e., at an earlier age) in the present study and in the study with resiniferatoxin blunted development of hyperinsulinemia.

The increased plasma lactate levels of obese compared with lean Zucker rats may be due to a change in adipocyte metab-
olism. Small fat cells from lean rats convert about 5–15% of the glucose taken up by lactate, whereas larger fat cells from fatter rats convert 40–50% of the glucose to lactate (7). In addition, a decreased efficiency of lactate removal by muscle of obese Zucker rats may contribute to the increase in basal plasma lactate (31). After 3 h of fasting, plasma lactate levels were decreased in SDA obese rats, which may contribute to an increase in glucose utilization by muscle and a reduction of insulin resistance, because basal lactate levels are inversely related to insulin sensitivity (21). All in all, SDA obese rats may have lower plasma lactate levels because of smaller fat cells, conversion of less glucose to lactate, and a better removal of lactate by increased glycogen synthesis. This idea should be examined in future studies.

Plasma triglycerides in obese Zucker rats were higher than in lean littermates. Expansion of the fat mass in obesity increases fatty acid release, which leads to higher plasma free fatty acid concentrations and may contribute to insulin resistance in the glucose metabolic pathway and concomitant hyperinsulinemia through overstimulation of the β-cells (2, 19). Even if there is insulin resistance, the effect of insulin on lipid metabolism does not seem to be diminished (13, 33). The cumulative effects of free fatty acids, which stimulate gluconeogenesis in the liver and inhibit glucose uptake in insulin-sensitive tissues, and hyperinsulinemia, which has an antilipolytic effect and increases triglyceride synthesis, all contribute to alterations in fat metabolism that favor the accretion of fat. After 3 h of food deprivation, plasma triglycerides in SDA obese rats were decreased, in line with a possible switch in fat metabolism toward fat utilization.

Hepatic steatosis in obese Zucker rats is a result of markedly elevated fatty acid and triglyceride synthesis and reduced fatty acid oxidation (8), stimulated by chronic hyperinsulinemia (28). SDA markedly lowered liver fat content in obese and lean rats, whereas inactivation of the capsaicin-sensitive nerves did not reduce the accumulation of intracellular fat in the liver (9). This discrepancy could again be due to the above-mentioned methodological differences of these two techniques. The observed reduction in circulating insulin might contribute to the effect of SDA on liver fat, because it would increase fatty acid oxidation and decrease fatty acid synthesis and, hence, reduce the amount of stored liver fat.

Whole body glucose tolerance did not appear to be improved after IVGTT in SDA obese Zucker rats. Desensitization of sensory nerves with capsaicin (9, 11, 16) or with the potent capsaicin analog resiniferatoxin (10, 23), however, resulted in an improved oral glucose tolerance. It is well known that oral glucose elicits a greater insulin response than parenteral insulin because of incretin effects. It is possible that improvement of oral glucose tolerance by capsaicin or resiniferatoxin relies on this effect more than on the direct glucose-induced insulin secretion. This could explain why we did not find an improved glucose tolerance in an IVGTT. Future studies should address this possibility. An alternative and not mutually exclusive possibility is that the obese SDA rats did not loose intramyocellular fat, which is thought to be an important mediator of insulin resistance. Despite the unaltered glucose tolerance, the glucose-stimulated insulin release was considerably lower in SDA obese than in sham obese rats. However, this did not affect insulin sensitivity. The disposition index did not differ between sham and SDA rats within lean and obese Zucker rats, suggesting that possible changes in insulin sensitivity between surgical groups were compensated by an adaptation of insulin secretion. It is interesting to note that the marked reduction of S1 in obese Zucker rats was accompanied by a substantial increase in insulin secretion, making the disposition index much higher than in the lean animals. This indicates that the obese animals are able to compensate for the increased insulin resistance by augmenting hormone secretion, thus maintaining their glucose tolerance (Kc).

In contrast to basal lactate levels, the capacity for acute lactate generation following oral (20) or intravenous glucose administration is reduced with obesity. The change in plasma lactate profiles during the IVGTT in the SDA obese Zucker rats in our study was characterized by a higher initial peak and an enhanced subsequent removal of circulating lactate. Increased insulin sensitivity allows for a higher rate of glucose uptake into cells and might thus increase the early lactate peak. Thereafter, a faster removal of lactate by increased glycogen synthesis in insulin-sensitive tissues may accelerate the decline of the curve to baseline. Thus the observed changes in plasma lactate concentration during the IVGTT in SDA obese rats appear to be consistent with the assumption that SDA improved insulin sensitivity in these animals.

In conclusion, these data show that eliminating the vagal afferent signals from the proximal gut to the central nervous system by means of SDA decreases food intake, body weight gain, and insulin release in obese Zucker rats. Some effects of SDA on food intake and body weight gain also are present in lean Zucker rats. Although some metabolic findings suggest improved insulin sensitivity after SDA in the obese Zucker rats, the insulin sensitivity index was unchanged. The underlying mechanisms of the observed changes need be determined.

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GRANTS

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