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

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ABSTRACT

We investigated the effect of subdiaphragmatic vagal deafferentation (SDA) on food intake, body weight gain, and metabolism in obese and lean Zucker rats. Prior to 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 IVGTT was performed followed by minimal modeling to estimate the insulin sensitivity index. Food intake relative to metabolic body weight (g/kg^{0.75}) and daily body weight gain after surgery were lower in SDA obese than in SHAM obese Zucker rats (Food intake SHAM: 67.5 ± 1.2, SDA: 60.8 ± 1.1 g/kg^{0.75}, mean ± SE, p < 0.05; Body weight gain SHAM: 6.1 ± 0.2, SDA, 5.1 ± 0.3 g, mean ± SE, p < 0.01), but not in lean (Food intake SHAM: 55.1 ± 0.8, SDA: 53.4 ± 1.0 g/kg^{0.75}, ns; Body weight gain SHAM, 2.9 ± 0.2, SDA, 3.2 ± 0.2, ns) rats. Prior to surgery, plasma glucose and insulin concentrations were lower in lean than in obese rats, but did not differ between surgical groups within both genotypes. Four weeks after surgery, plasma glucose (SHAM, 6.9 ± 0.2, SDA, 6.7 ± 0.1 mmol/L, ns) and insulin (SHAM, 1.3 ± 0.2, SDA, 1.2 ± 0.2 ng/ml, ns) were still similar in SDA and SHAM lean rats, but significantly lower in SDA than in SHAM obese rats (glucose: SHAM, 8.3 ± 0.2, SDA, 7.2 ± 0.3, p < 0.05; insulin: SHAM, 13.5 ± 1.4, SDA, 10.4 ± 0.6, p < 0.01). The IVGTT revealed a significant downward shift of the plasma insulin profile by SDA in obese, but not in lean animals, while the plasma glucose profile was not affected. SDA decreased the area under the curve for insulin, but not glucose, in obese Zucker rats. The insulin sensitivity index was higher in lean than in obese rats, but was not affected by SDA in both genotypes (lean: SHAM, 6.24 ± 1.53, SDA, 7.06 ± 1.19, ns; obese: SHAM, 1.54 ±
0.22, SDA, 1.73 ± 0.30, ns). These results suggest that the 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 Zucker obese rats.
The Zucker fatty (fa/\textit{fa}) rat is homozygous for a mutation in the leptin receptor gene (29). Leptin inhibits the activity of neuropeptide Y (NPY), and stimulates the expression of proopiomelanocortin (POMC) 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. NPY 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 mild 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 that 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, The Netherlands), weighing 135-165g on arrival, were individually housed in
custom-made Macrolon cages (41x25x31cm) 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 a.m.). Rats had
free access to water and standard laboratory chow (No. 3433, Provimi Kliba NAFAG,
Kaiseraugst, Switzerland, metabolizable energy content: 12.4 kJ/g), unless noted
otherwise; they were weighed and handled daily, and were adapted to housing conditions
for five days before the first measurements. 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 prior to SDA and SHAM surgery, starting when average body weights of lean and obese rats were 210 ± 2 and 208 ± 5 g (mean ± SE), respectively, and on 13 consecutive days starting on day 16 after surgery, when average rates of body weight gain had reached pre-surgical values. At the end of the 13 day post-surgery measurement period, body weights were 325 ± 4 (SHAM) and 309 ± 5 (SDA) for lean as well as 413 ± 4 (SHAM) and 398 ± 4 (SDA) for obese rats, respectively. Just before lights out, feeding cups were placed in the cages. Food intake was measured by manually weighing (±0.1g) the feeding cups after 23 hours. Spillage was collected on paper spread beneath the cages and was also measured. Food intake (Table 1) is given as g/kg metabolic body weight (=kg^{0.75}) because of the different body weights of lean and obese rats.

Subdiaphragmatic vagal deafferentation (SDA)

SDA was performed according to the method of Norgren and Smith (25) when lean rats weighed 251 ± 4 (SHAM) and 245 ± 2 (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 below).
The deafferentation procedure consisted of a left afferent vagal rootlet transection at the brain stem level and a ipsilateral subdiaphragmatic vagotomy of the dorsal trunk (34). During SHAM operations, the left vagus was exposed but left untouched, and a midline laparotomy and exposure of the dorsal subdiaphragmatic trunk was made, leaving the trunk intact. To minimize post-surgery disturbances of intestinal motility in SDA rats (18), all rats received sweetened milk and 4 or 2 food-pellets starting two days prior to surgery. After surgery, SDA rats received sweetened milk (on days 1 and 2 after surgery), sweetened milk and wet mash (on day 3 after surgery), and sweetened milk wet mash and 2-6 pellets (days 4-7 after surgery). SHAM operated rats were fed sweetened milk, wet mash and 2-6 pellets for the first three days after surgery. On day four, wet mash and pellets were presented.

Milk and water were taken away at 3 and 1 hours prior to surgery, respectively. Temgesic (0.025 mg/rat, Essex Chemie, Luzern, Switzerland) and atropine (0.015-0.025 mg/rat, Sintetica, Mendrisio, Switzerland) were injected s.c. at 0.5-1 hour before surgery. Inhalation anesthesia was induced and maintained with a mixture of isofluran, oxygen and NO₂. Body temperature was maintained at 37-38°C throughout surgery with a 39°C water pad. Rats were treated with 80µl Borgal 7.5% s.c. (Intervet, The Netherlands), 5ml saline i.p. during surgery and postoperatively for three consecutive days with Rimadyl s.c. (5mg/kg, Gräub AG, Bern, Switzerland).

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) i.p. doses of cholecystokinin (CCK-8). To obtain a functional verification of SDA in the present study, 4µg/kg CCK (Bachem, Bubendorf, Switzerland) in PBS (1µg CCK/150µl PBS) or PBS (Dulbecos w/o Ca, Mg; Invitrogen life technologies, Basel, Switzerland) vehicle solution were injected i.p. 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 minutes. The rats were adapted to this procedure by i.p. injections of saline on two consecutive days prior to the test.

Histological vagotomy verification

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

The fixed brain was examined under the microscope to verify that the vagal afferent rootlets were a) cut on the left side and b) intact on the right side as they entered the medulla. The brain and brain stem were then removed and postfixed in 4% paraformaldehyde in 0.1M sodium phosphate buffer for one hour and cryoprotected in a solution of 30% sucrose in distilled water for three days. The medulla was sectioned into slices of 40µm. Sections for the fluorogold analysis were air dried and examined with a fluorescence microscope. Because fluorogold 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 a) the absence of label in the right dorsal motor vagal nucleus, where the cut dorsal subdiaphragmatic vagal trunk would project, and b) 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 four weeks after surgery blood samples were taken by tail incision. The rats were food-deprived for three hours. Approximately one hour after dark onset, 150µl of blood were collected in an EDTA tube (Microvette, Sarstedt, Switzerland). The blood was centrifuged (8min, 5000g) within 15 minutes and plasma 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 which 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 1mm above the junction with the facial and linguofacial vein. The inner end of the cannula was situated 2mm inside the right atrium and the cannula kept in place by two ligatures. After surgery, the catheter was filled with heparinized polyvinylpyrrolidone (500U heparin/ml PVP (21g PVP in 12ml 0.9%NaCl, Sigma, St.
Louis, MO)), and flushed daily with saline to ensure patency. The catheters were gas-
sterilized with ethylenoxid before use. The rats were food-deprived for six hours and 
water-deprived for one hour before surgery. Premedication consisted of atropine 
(0.025mg/rat) and Temgesic (0.025mg/rat) 0.5-1 hour prior to anesthesia (see above). 
Postsurgical medication consisted of Borgal (80µl) and Rimadyl (0.5mg/100g) over three 
days.

Catheter Handling

Six days after jugular vein catheter implantation, the rats were adapted to the 
experimental procedures. The cap closing the headset was replaced by a ~64cm 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 80µl of 
blood were aspirated and discarded. The blood sample (170µl) was taken with a second 
syringe and immediately transferred to an Eppendorf tube chilled in ice and treated with 
heparin (25IU/ml, Heparin-Na, B. Braun Medical AG, Emmenbrücke, Switzerland). The 
catheter was flushed with 300µl of saline and 50µl heparinized saline (50IU/ml). Then, a 
small air bubble was 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 2cm down the 
sampling tube to ensure that the first bubble was inside the cannula, in order to separate 
the circulating blood from the heparin solution.
Intravenous Glucose Tolerance Test (IVGTT)

Exactly one week after implantation of the jugular vein catheters, an IVGTT was performed. Rats were food deprived for 12 hours during the light phase. Starting approximately one hour after dark onset, blood samples (150µl each) were taken at -15, 0, 3, 6, 9, 12, 15, 20, 30, 40, 50, 60 and 90min. At time point 0, immediately after the second blood sample, a 50% glucose solution at a dose of 750mg/kg (440-700µl) was infused (10 seconds) into the jugular vein. The blood was centrifuged (8min, 8000rpm) within 15 minutes after taking the samples and plasma was stored for later analysis of glucose, insulin and lactate. After the last blood sample, 3-3.5ml 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 hours. Blood was taken one hour after dark onset: 220µl blood were added to 7.6µl heparin-fluoride (25IU heparin, 3mg fluoride/ml blood) for later measurements of plasma glucose and lactate; 1.3ml blood were added to 26µl EDTA (1.8mg/ml blood) for later measurements of plasma free fatty acids, triglycerides and insulin. The samples were placed immediately on ice, centrifuged within 15 minutes and plasma 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 by standard enzymatic methods adapted for the Cobas Mira autoanalyzer (Roche). Plasma insulin concentration was measured by a rat specific radioimmunoassay (Linco, St. Charles, MO).

Glucose Tolerance and Insulin Sensitivity

Insulin and glucose data from the IVGTT were analyzed with the minimal model technique. The model assumes first-order non-linear insulin controlled kinetic 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, $10^{-4}\text{min}^{-1}/[\mu\text{U/ml}]$), which is defined as the ability of insulin to enhance glucose disappearance and inhibit glucose production (1) and $S_g$ (glucose effectiveness, $\text{min}^{-1}$), representing glucose disappearance from plasma without any change in dynamic insulin (4). The acute insulin response (AIR, $\mu\text{U/ml}$) was calculated as mean suprabasal insulin concentration of samples from 3 to 6 min. The disposition index, DI ($\text{min}^{-1}$) was obtained by multiplying $S_i$ times 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, $\%\text{min}^{-1}$) 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 ($S_g$) and insulin-dependent
processes (DI). The usefulness of the method, originally developed in dogs (3) and largely exploited in humans (1), has been proved also in rodents such as mice (27).

Body fat

Perirenal, mesenterial, and epididymal fat pads were excised and weighed (± 0.1g) at the time of sacrifice. 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.

Statistical analyses

Differences between group means were analyzed by separate two-way repeated or factorial measures ANOVA, followed by a Fisher’s PLSD test when appropriate. $P<0.05$ was considered statistically significant. For the IVGTT, the incremental changes over time for glucose, insulin and lactate were determined by subtracting baseline values from each time point. The areas under the curves (AUC) were determined using the trapezoidal method (30). For comparison of the IVGTT results, a two-way repeated measure ANOVA (overall effect) was performed followed by single time point comparisons if needed. All data are presented as mean ± SEM.
RESULTS

Functional verification of complete SDA

Intraperitoneal injection of CCK-8 reduced 30 minute food intake after a four hour food deprivation compared to i.p. 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<0.001 for lean and obese). The feeding suppressive effect of CCK had disappeared after two hours (not shown). With 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}) prior to and after surgery was consistently greater in obese than in lean Zucker rats. Food intake over five consecutive days prior to 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 g 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 post-surgery 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 prior to surgery did not differ between lean or 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 (Table 1). This is not fully reflected by pre-to-post surgery differences within the various groups, which appeared to be lower 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 one week prior to surgery were similar in lean and obese rats designated for SDA or SHAM surgery. Four weeks after surgery, three hour 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 post-surgery values of glucose and insulin in lean rats. However, post-surgery plasma concentrations were higher than pre-surgery 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 hours of fasting were taken six weeks after surgery (Figure 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, but were significantly decreased by SDA in the obese rats (Table 3).

**Body fat**

At the time of sacrifice, 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, neither in lean (absolute: SHAM 13.3±0.7g, SDA 12.2±0.5g, p > 0.05, relative to body weight: SHAM 3.5 ± 0.2 %, SDA 3.4 ± 01, p > 0.05) nor in obese (absolute: SHAM 46.4±1.0g, SDA 46.1±1.4g, p > 0.05, relative: SHAM 8.9 ± 0.2, SDA 8.9 ± 0.2, p > 0.05) rats.

**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 (SHAM 36.8±1.7, SDA 28.3±2.6%, p<0.03) rats.

**Intravenous Glucose Tolerance Test (IVGTT)**

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 (Figure 2). The total AUC for insulin reflects the total release of the hormone related to the specific glucose pattern. It may however 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 1177.6±117.2 ng/ml, SDA 895.8±28.6 ng/ml, p=0.01).

The minimal model analysis showed that SDA affected neither insulin sensitivity (SI), nor glucose effectiveness (SG), nor glucose tolerance (KG, DI) both in lean and obese Zucker rats (Table 4). SI 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. SG tended to be lower in obese Zucker rats, showing that there is a general impairment in glucose metabolism (both in the insulin-dependent and insulin-independent mechanisms) in these latter groups.

Plasma lactate values and first peak AUC for lactate during the IVGTT were also not different in SDA and SHAM lean rats. In obese rats however, SDA provoked a change in the plasma lactate profile. While the overall effect and the first peak AUC for lactate were also not different during the IVGTT, single time point measurements revealed that lactate both increased more initially (p<0.05 for 15 and 20 minutes) and returned to baseline faster in SDA obese than in SHAM obese rats (lower lactate concentration at 45 and 60 minutes, p<0.05) (Figure 4).

**DISCUSSION**

**Food intake and body weight gain**

The present results suggest that subdiaphragmatic vagal deafferentation (SDA) decreases food intake and body weight gain and alters metabolism in particular in obese Zucker rats.
rats. The pre- to post-surgery differences in food intake differed between SDA and SHAM 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 was not observed in a similarly designed experiment that is not reported in detail. In this experiment, mean daily food intake for 11 post-surgery days was $59.4 \pm 2.1$ and $56.5 \pm 0.6 \text{ g/kg}^{0.75}$ (mean ± SEM, p>0.05) for SHAM (n=3) and SDA (n=5) lean rats, with pre- to post-surgical differences of $-15.3 \pm 2.5$ and $17.9 \pm 1.4 \text{ g/kg}^{0.75}$ (p>0.05). The corresponding values for SHAM (n=5) and SDA (n=6) obese rats were $66.9 \pm 1.4$ and $60.2 \pm 1.2 \text{ g/kg}^{0.75}$ (p<0.01), with pre- to post-surgical differences of $-29.2 \pm 2.6$ and $-36.5 \pm 1.8 \text{ g/kg}^{0.75}$ (p<0.05), respectively.

The similar body weights of lean and obese rats on the day of surgery suggest that obese rats were somewhat younger. Unfortunately we do not know the rats’ exact age at the time of surgery, but standard growth curves for lean and obese Zucker rats (courtesy of Harlan) suggest that all rats were approximately 8 weeks old and that the age difference between lean and obese rats was presumably less than a week. This age difference did probably not account for the different effects of SDA in both genotypes because in yet another study of ours (Ferrari et al., unpublished), 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 afferent 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 and less so in lean rats, can only be speculated upon. 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 rats. Results from a comparable study (Ferrari et al., unpublished) 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 here observed effects of SDA 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 post-surgery 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 difference in body weight represented solely a decrease in the accretion of adipose tissue, then a food intake difference of ~520 kJ for the obese and ~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 employing 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, capsaizin desensitization is not specific for vagal afferents) and/or to the animals’ much higher body weight (400-750 g) at the time of the subdiaphragmatic vagotomy in one study (26) compared to the rats’ body weight 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 (slow-down of growth rate (17), no effect on growth rate (11; 16).

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 may therefore 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 due 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 β-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 sacrifice. Therefore, a lower body fat mass did presumably 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 analogue 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 weight 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 in comparison to lean Zucker rats
may be due to a change in adipocyte metabolism. Small fat cells from lean rats convert
about 5-15% of the glucose taken up to 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 hours 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, as 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, converting 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 that stimulate gluconeogenesis in the liver and inhibit glucose uptake in insulin-sensitive tissues, and hyperinsulinemia that has an antilipolytic effect and increases triglyceride synthesis, all contribute to alterations in fat metabolism that favor the accretion of fat. After 3 hours of food deprivation, plasma triglycerides in SDA obese rats were decreased, in line with a possible switch in fat metabolism towards 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 following IVGTT in SDA obese Zucker rats. Desensitization of sensory nerves with capsaicin (9; 11; 16) or with the potent capsaicin-analogue 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 due to 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 lose 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. This however 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 $S_I$ 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 ($K_G$).

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 are also 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.
Acknowledgements:

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FIGURE CAPTIONS

FIGURE 1: Timeline of the experiment. The rats’ initial body weight was 135-165g. The first measurements started after an adaptation time of five days.

FIGURE 2: Cumulative body weight gain in SHAM lean (white rhombus, n=8), SDA lean (black rhombus, n=7), SHAM obese (white triangle, n=8) and SDA obese (black triangle, n=10) rats after SHAM or SDA surgery and over 13 days thereafter.

FIGURE 3: Plasma glucose profiles in SHAM lean (white rhombus, n=8), SDA lean (black rhombus, n=7), SHAM obese (white triangle, n=5) and SDA obese (black triangle, n=8) rats before and after intravenous administration of a 50% glucose solution at a dose of 750mg/kg (IVGTT). Data represent means ± SEM. There were no group differences.

FIGURE 4: Plasma insulin profiles in SHAM lean (white rhombus, n=8), SDA lean (black rhombus, n=7), SHAM obese (white triangle, n=5) and SDA obese (black triangle, n=8) rats before and after intravenous administration of a 50% glucose solution at a dose of 750mg/kg (IVGTT). Data represent means ± SEM. SDA caused a downward shift of the plasma insulin profiles during the IVGTT in obese (p=0.01) but not in lean Zucker rats.

FIGURE 5: Plasma lactate profiles in SHAM lean (white rhombus, n=8), SDA lean (black rhombus, n=7), SHAM obese (white triangle, n=5) and SDA obese (black triangle,
n=8) rats before and after intravenous administration of a 50% glucose solution at a dose of 750mg/kg (IVGTT). Data represent means ± SEM. SDA did not affect the AUC, but altered plasma lactate profiles (p<0.01) in obese rats. Plasma level profiles of lean rats were not affected by SDA.
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TABLE 1: Daily body weight gain (g) and daily food intake/metabolic body weight (g/kg^{0.75}) in lean and obese Zucker rats for 5 days prior to SDA or SHAM surgery and for 13 days thereafter, starting on day 16 after surgery.

<table>
<thead>
<tr>
<th></th>
<th>Body weight gain</th>
<th></th>
<th>Food intake/Metabolic body weight (g/kg^{0.75})</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>before surgery</td>
<td>after surgery</td>
<td>delta before-after</td>
</tr>
<tr>
<td>SHAM lean (n=8)</td>
<td>4.9 ± 0.2</td>
<td>2.9 ± 0.2</td>
<td>-2.0 ± 0.2</td>
</tr>
<tr>
<td>SDA lean (n=7)</td>
<td>5.6 ± 0.4</td>
<td>3.2 ± 0.2</td>
<td>-2.4 ± 0.5</td>
</tr>
<tr>
<td>SHAM obese (n=8)</td>
<td>7.6 ± 0.4</td>
<td>6.1 ± 0.2</td>
<td>-1.5 ± 0.4</td>
</tr>
<tr>
<td>SDA obese (n=10)</td>
<td>7.7 ± 0.2</td>
<td>5.1 ± 0.3*</td>
<td>-2.6 ± 0.4</td>
</tr>
</tbody>
</table>

Data represent means ± SEM. *p<0.05, **p<0.01, ***p<0.001 vs. SHAM (Fisher’s PLSD).
TABLE 2: Plasma glucose and insulin levels 1 week prior to SDA or SHAM surgery and 4 weeks thereafter.

<table>
<thead>
<tr>
<th></th>
<th>Plasma glucose (mmol/L)</th>
<th></th>
<th>Plasma insulin (ng/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>before surgery</td>
<td>after surgery</td>
<td>before surgery</td>
</tr>
<tr>
<td>SHAM lean (n=8)</td>
<td>7.1 ± 0.1</td>
<td>6.9 ± 0.2</td>
<td>1.2 ± 0.1</td>
</tr>
<tr>
<td>SDA lean (n=7)</td>
<td>7.1 ± 0.2</td>
<td>6.7 ± 0.1</td>
<td>1.1 ± 0.1</td>
</tr>
<tr>
<td>SHAM obese (n=8)</td>
<td>8.2 ± 0.3</td>
<td>8.3 ± 0.2</td>
<td>6.0 ± 0.6°°°</td>
</tr>
<tr>
<td>SDA obese (n=10)</td>
<td>8.0 ± 0.2°°</td>
<td>7.2 ± 0.3**</td>
<td>5.6 ± 0.5°°°</td>
</tr>
</tbody>
</table>

The samples were taken by tail incision after a 3 hours of fasting. Data represent means ± SEM. **p<0.01, ***p<0.001 vs. SHAM, °p<0.05, °°p<0.01, °°°p<0.001 vs after SDA surgery (Fisher's PLSD).
TABLE 3: Plasma metabolites and insulin in blood samples taken by catheter after 3 hours of fasting at 6 weeks after SDA or SHAM surgery in lean and obese Zucker rats.

<table>
<thead>
<tr>
<th></th>
<th>Lactate</th>
<th>Insulin</th>
<th>Triglycerides</th>
<th>Free Fatty Acids</th>
</tr>
</thead>
<tbody>
<tr>
<td>SHAM lean (n=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 (n=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 (n=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 (n=10)</td>
<td>1.4±0.1***</td>
<td>12.0±1.2*</td>
<td>1.9±0.11**</td>
<td>0.9±0.12</td>
</tr>
</tbody>
</table>

Data represent means ± SEM. *p<0.05, **p<0.01, ***p<0.001 vs. SHAM (Fisher’s PLSD).
Table 4. Metabolic parameters from IVGTT for the four groups of rats.

<table>
<thead>
<tr>
<th>Group</th>
<th>AUC Gluc</th>
<th>Peak Ins</th>
<th>AUC Ins</th>
<th>$K_g$</th>
<th>$S_i$</th>
<th>$S_g$</th>
<th>AIR</th>
<th>DI</th>
</tr>
</thead>
<tbody>
<tr>
<td>SHAM lean (n=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 (n=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 (n=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 (n=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±1.73</td>
<td>0.043±0.005</td>
<td>763±44</td>
<td>103.6±18.2</td>
</tr>
</tbody>
</table>

AUC Gluc (min g/dl) total area under glucose concentration; Peak Ins (µU/ml) insulin peak following glucose bolus; AUC Ins (min U/l) total area under insulin concentration; $K_g$ (%min⁻¹) glucose tolerance index; $S_i$ (10⁻⁴ min⁻¹[µU/ml]) insulin sensitivity index; $S_g$ (min⁻¹) glucose effectiveness; AIR (µU/ml) acute insulin response (mean suprabasal insulin concentration from 3 to 6 min after glucose bolus; DI (10⁻³min⁻¹) disposition index, defined as $S_i \times$ AIR.
Deafferentation

Baseline plasma glucose and insulin (Tail incision)
3 hours fasting

Baseline plasma glucose and insulin (Tail incision)
3 hours fasting

Baseline plasma metabolites and insulin
3 hours fasting

Deafferentation

Catheter implantation

IVGTT (750mg/kg)
12 hours fasting

Perfusion

Food intake measurement

Food intake measurement

recovery

5 days
16 days
13 days
1 week
3 days
2 days
3-8 days

FIGURE 1: Ferrari et al.
FIGURE 2: Ferrari et al.
FIGURE 3: Ferrari et al.

Graph showing glucose levels (mmol/l) over time (min) from -20 to 100 minutes.
FIGURE 4: Ferrari et al.

The graph shows the insulin levels (ng/ml) over time (min). The x-axis represents time in minutes, ranging from -20 to 100, and the y-axis represents insulin levels in ng/ml, ranging from 0 to 50. There are multiple data points and error bars indicating the variability of the insulin levels over time.
FIGURE 5: Ferrari et al.