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ORIGINAL PAPER

Vagotomy diminishes obesity in cafeteria rats by decreasing cholinergic potentiation of insulin release

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Abstract Herein, we investigated whether subdiaphragmatic vagotomy has benefits on obesity, body glucose homeostasis, and insulin secretion in cafeteria (CAF)-obese rats. *Wistar* rats were fed a standard or CAF diet for 12 weeks. Subsequently, CAF rats were randomly submitted to truncal vagotomy (CAF Vag) or sham operation (CAF Sham). CAF Sham rats were hyperphagic, obese, and presented metabolic disturbances, including hyperinsulinemia, glucose intolerance, insulin resistance, hyperglycemia, and hypertriglyceridemia. Twelve weeks after vagotomy, CAF Vag rats presented reductions in body weight and perigonadal fat stores. Vagotomy did not modify glucose tolerance but normalized fed glycemia, insulinemia, and insulin sensitivity. Isolated islets from CAF Sham rats secreted more insulin in response to the cholinergic agent, carbachol, and when intracellular cyclic adenine monophosphate (cAMP) is enhanced by forskolin or 3-isobutyl-1-methylxanthine. Vagotomy decreased glucose-induced insulin release due to a reduction in the cholinergic action on β-cells. This effect also normalized islet secretion in response to cAMP. Therefore, vagotomy in rats fed on a CAF-style diet effectively decreases adiposity and restores insulin sensitivity. These effects were mainly associated with the lack of cholinergic action on the endocrine pancreas, which decreases insulinemia and may gradually reduce fat storage and improve insulin sensitivity.

Keywords Truncal vagotomy · Cafeteria diet · Glucose homeostasis · Insulin secretion · Obesity

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Introduction

Obesity is rapidly expanding worldwide and predisposes to several diseases, including hypertension, cardiovascular diseases, cancer, and type 2 diabetes (T2D) [16]. Although several factors may contribute to the development of the obesity [32], evidence suggests that an autonomic nervous system (ANS) dysfunction contributes to increased adiposity and maintain this syndrome [8, 9, 31].

The ANS regulates several aspects of energy homeostasis through both parasympathetic and sympathetic branches [21]. The parasympathetic nervous system (PNS) transmits peripheral signals to the central nervous system (CNS), regulating food intake, through the afferent fibers in the vagus nerves [25]. Efferent vagal fibers, which comprise less than 20 % of the vagus nerve fibers, indirectly control energy homeostasis by

regulating gastric emptying and accommodation, hepatic glucose and lipid metabolism, and insulin secretion [15, 18, 21, 22].

Hyperinsulinemia is a common finding in the early stages of obesity development [13, 26]. Pancreatic islets are richly innervated by the PNS and SNS, which increase and decrease, respectively, insulin secretion [1]. Since truncal vagotomy in morbid obese patients [17], and in hypothalamic [3] and genetic obese rodents [26], normalized insulin plasma levels, it has been suggested that, in obesity, an unbalance in ANS action, with increase in PNS activity, but reduction in SNS action, contributes to the etiology of this syndrome [27, 29].

Several experimental rodent models are used in basic research to understand the pathophysiology of obesity. Among these, the model that most accurately resembles the intake of the highly palatability foods that are prevalent in the Western society and represents the actual human obesogenic condition is the cafeteria (CAF) diet [28]. The consumption of a CAF diet in rodents promotes obesity, hyperinsulinemia, glucose intolerance, and insulin resistance [2, 28, 34]. Despite the involvement of ANS dysfunction, more specifically PNS hyperactivity, in the development of hypothalamic, genetic and high-fat diet obesity has been often investigated [3, 19, 26, 31]; no studies have reported the contribution of the PNS on CAF diet obesity. Therefore, we herein investigate the effects of subdiaphragmatic vagotomy on obesity, body glucose homeostasis, and insulin secretion in CAF-obese rats.

Materials and methods

Experimental groups

All experiments were approved by the University's Committee for Ethics in Animal Experimentation (CEEAAP/UNIOESTE no. 8709). Eight-week-old male *Wistar* rats were randomly divided into two groups: control (CTL, $n = 10$), which received standard rodent chow (Biobase®, Águas Frias, SC, BRA) consisting of 3.8 kcal/g (70 % carbohydrate, 20 % protein and 10 % fat) and filtered water ad libitum, or the Cafeteria (CAF, $n = 16$) group, which received a cafeteria diet. Table 1 presents the nutritional composition of foods offered to the animals in the standard chow and cafeteria diet.

After 12 weeks of CAF diet consumption, CAF rats were submitted to subdiaphragmatic truncal vagotomy (CAF Vag group, $n = 08$) or a sham operation (CAF Sham, $n = 08$). For this procedure, 12-h fasted rats were anesthetized with a mixture of ketamine and xylazine (90 and 9 mg/kg via *i.p.*, respectively; Vetbrands®, Paulínia, SP, BRA). Subsequently, the stomach and esophagus were exteriorized from the peritoneal cavity, and both dorsal and subdiafragmatic vagal trunk was separated from the esophagus and cut off. Sham-operation rats underwent the same procedures, but the vagus nerve was kept intact. At the end of the experimental period, to confirm subdiaphragmatic vagotomy, stomach food retention from all groups of rats was evaluated by the ratio between the stomach weight per body weight (BW) [3].

Table 1 Nutritional composition of the diets given to the rats

Cafeteria diet composition (%)	
Cheetos Balls snack (Cheetos, Pepsico, Brazil)	11
Bacon snack (Troféu, Santa Helena, Brazil)	13
Cookie cornstarch (Zadimel, Brazil)	10
Chocolate cake (Renata, Selmi, Brazil)	10
Coca-Cola (Coca-Cola, Brazil)	1
Guaraná (Antarctica, AmBev, Brazil)	1
Italian salami (Sadia, Brazil)	11
Mixed sausage (Sadia, Brazil)	9
Nutrella bread (Nutrella, Brazil)	8
Chocolate wafer (Bauducco, Brazil)	13
Mortadella (Frimesa, Brazil)	5
Marshmallow (Fini, Brazil)	8
Macronutrients contents (%)	

	Standard diet	Cafeteria diet
Carbohydrate	70	49
Lipids	10	24
Proteins	20	22
kcal/g	3.8	5.4

ipGTT

After 12 weeks of subdiaphragmatic vagal denervation, rats were submitted to 8 h fasting and a blood sample was taken from the tail tip to obtain fasting glucose (time 0), using a glucose analyzer (Abbott®, Optium Xceed, Alameda, CA). Subsequently, all groups of rats received an *i.p.* injection of 2 g/kg BW glucose, and blood glucose was also analyzed

after 15, 30, 60, 120, and 180 min of glucose administration. Additional blood samples were collected at 0 and 30 min of the test to measure insulin by radioimmunoassay (RIA).

Obesity and general nutritional parameters

Body weight was measured weekly for 23 weeks. During the last week, four rats per group were maintained in individual metabolic cages for 5 days, for measurement of 12 h food and fluid intake, and amount of urine and feces excreted. After 12 weeks of subdiaphragmatic vagotomy, the final BW and nasoanal length were measured to calculate the Lee index [ratio of BW (g)/nasoanal length (cm) × 1000] [5]. Subsequently, all groups of rats were euthanized by decapitation, and total blood samples were collected to measure glucose (as above mentioned), insulin by RIA, total cholesterol (CHOL), and triglycerides (TG) using standard commercial kits (Merck®, Germany and Boehringer® Mannhein, Germany). Retroperitoneal and perigonadal fat pads were also removed and weighed.

HOMA-IR

Tissue insulin sensitivity was evaluated by the previously validated [6] homeostasis model of assessment (HOMA) using the HOMA index of insulin resistance [(HOMA-IR) = fasting insulin (μU/mL) × fasting glucose (mM)/22.5] [23].

Pancreatic islet isolation and static insulin secretion

Islets were isolated by collagenase (Sigma-Aldrich Chemicals, St Louis, MO, USA) digestion of the exocrine pancreas. For static incubations, four islets from each group were incubated for 30 min at 37 °C, with 0.5-mL Krebs–bicarbonate (KBR) buffer with the following composition: 115 mM NaCl, 5 mM KCl, 2.56 mM $CaCl_2$, 1 mM $MgCl_2$, 10 mM $NaHCO_3$, 15 mM HEPES, supplemented with 5.6 mM glucose, 3 g BSA/L, pH 7.4, and aired with a mixture of 95 % O_2/5 % CO_2. This medium was discarded and the islets were incubated for a further 1 h with 1-mL fresh KBR buffer containing 11.1 mM glucose without or with 100 μM carbachol (Cch), 10 μM forskolin, or 1 mM 3-isobutyl-1-methylxanthine (IBMX). Aliquots of the supernatant were collected at the end of the incubation period and kept at −20 °C for posterior insulin measurement by RIA.

Statistical analysis

Results are presented as means ± SEM for the number of determinations (n) indicated. Statistical analyses were carried out using one-way analysis of variance (ANOVA) followed by the Tukey posttest ($P < 0.05$). Tests were performed using GraphPad Prism® version 5.00 for Windows (San Diego, CA, USA).

Results

General nutritional and obesity features

Body weight was measured weekly in the CAF and CTL groups, as illustrated in Fig. 1a. CAF diet rats were significantly heavier from the fourth week after beginning the diet treatment, when compared to CTL rats ($P < 0.05$; Fig. 1a). The total BW gain, as judged by the area under the growth curve (AUC), before the subdiaphragmatic vagotomy, was higher in the CAF groups, when compared with CTL rats ($P < 0.01$; Fig. 1b). After 2 weeks of vagotomy, the BW in CAF Vag rats was lower than that observed for CAF Sham rats ($P < 0.03$), but similar to that of the CTL group (Fig. 1a). The AUC of total BW gain during 11 weeks after vagal denervation in CAF Vag group was also similar to that registered for CTL rats (Fig. 1b and Table 2). In addition, CAF Sham rats presented a higher final BW and adiposity, as demonstrated by the 48 and 53 % increases in retroperitoneal and perigonadal fat stores, respectively, and by the greater Lee index, when compared with the CTL group ($P < 0.001$, $P < 0.03$, and $P < 0.001$, respectively; Table 2). CAF Sham rats were also hyperphagic, consuming 32 % more food in 12 h, in comparison with CTL rats ($P < 0.05$; Table 2). The CAF-style diet decreased feces produced in 12 h, enhancing the ratio between the food consumed per residue excreted ($P < 0.0001$ and $P < 0.001$; Table 2). After 12 weeks of subdiaphragmatic vagotomy, CAF Vag rats presented reductions of 9 % in the Lee index and 19 % in perigonadal fat pad weight,

when compared with CAF Sham rats ($P < 0.0001$ and $P < 0.05$). Although the CAF Vag group presented only a partial reduction in food consumption, without alteration in total feces excreted in 12 h, a significant lower ratio between food intake per feces produced was observed in the CAF Vag compared with the CAF Sham group ($P < 0.05$; Table 2). Furthermore, the CAF Vag rats presented a higher stomach weight per BW ratio, when compared to the CAF Sham rats ($P < 0.001$; Table 2), which confirmed the bilateral subdiaphragmatic vagotomy. Fluid intake and urine excretion in 12 h were similar in all groups of rats (Table 2).

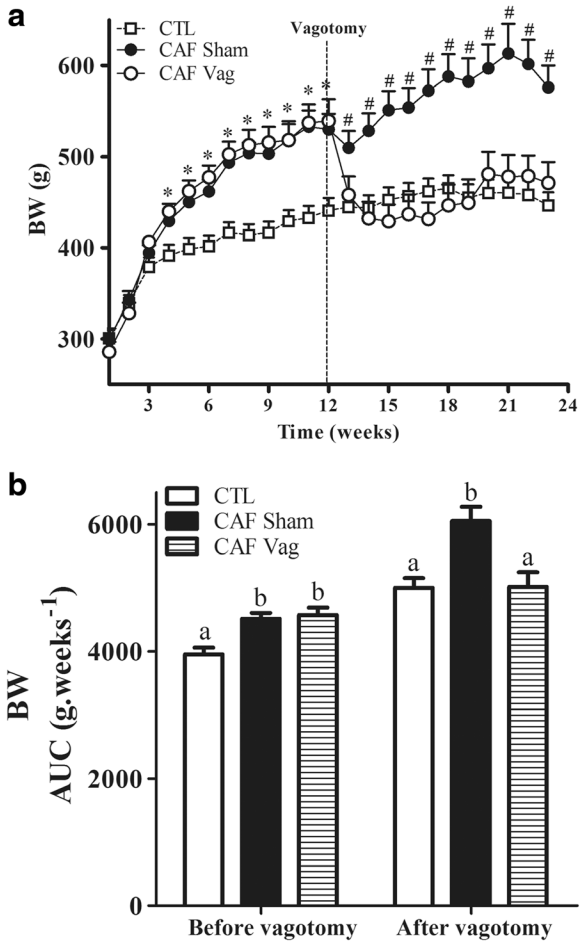

Fig. 1 **a** Body weight of CTL, CAF Sham, and CAF Vag rats recorded over 23 weeks. **b** Total BW before and after subdiaphragmatic vagotomy, expressed by the area under curve (AUC). *Indicates a significant statistical difference between CAF Sham and CAF Vag groups from CTL. #CAF Sham is different from CAF Vag and CTL. *Different letters over the bars* represent significant differences. Data are means ± SEM ($n = 6$–10). One-way ANOVA followed by the Tukey posttest, $P < 0.05$

Glucose Homeostasis

The CAF-style diet increased glycemia and insulinemia in both fasting and fed conditions in CAF Sham, when compared with CTL rats ($P < 0.0001$ and $P < 0.005$; Fig. 2a, b). Although, after 12 weeks of subdiaphragmatic vagotomy, fasted CAF Vag rats also displayed higher blood glucose ($P < 0.05$; Fig. 2a), this glycemia was associated with a normal insulinemia (Fig. 2b). In addition, normalizations of the glucose and insulin plasma concentrations under fed conditions were observed in the CAF Vag rats (Fig. 2a, b). The CAF Sham rats also presented insulin resistance, since HOMA-IR was 5.6- and 2.8-fold higher in the fasted and fed conditions, respectively, than observed in CTL rats ($P < 0.001$ and $P < 0.05$; Fig. 2c). Vagotomy normalized insulin sensitivity in CAF Vag rats in both conditions, when compared with CAF Sham rats ($P < 0.05$; Fig. 2c).

To analyze body glucose control, an intraperitoneal glucose tolerance test (ipGTT) was performed at 12 weeks after vagotomy and sham operations. After glucose administration, glycemia reached maximum levels at 15 min in all groups of rats (Fig. 2d). CAF Sham rats presented persistent hyperglycemia from 30 to 120 min of the test, when compared with CTL rats ($P < 0.05$; Fig. 2d). The total glycemia during the ipGTT was 36 % higher in the CAF Sham compared with CTL rats ($P < 0.005$; Fig. 2e). This effect was accompanied by a higher insulin secretion in response to glucose administration, since at 30 min of the test, insulinemia was 86 % higher in CAF Sham rats in comparison with the CTL group ($P < 0.004$; Fig. 2f). CAF Vag rats also presented higher blood glucose at 30 and 120 min of the test ($P < 0.01$ and $P < 0.05$; Fig. 2f), but total glycemia during ipGTT presented intermediary values compared to those of the CAF Sham and CTL rats (Fig. 2e). In contrast, the CAF Vag rats presented normal insulinemia at 30 min of the ipGTT (Fig. 2f).

Furthermore, CAF Sham rats were hypertriglyceridemic under fasting (188 ± 24 mg/dL) and fed conditions (244 ± 32 mg/dL), when compared with CTL (97 ± 9 and 117 ± 6 mg/dL, respectively; $P < 0.001$). After 12 weeks of

Table 2 General features of CTL, CAF Sham, and CAF Vag rats

	CTL	CAF Sham	CAF Vag
Food intake in 12 h (g)	19 ± 0.7^{a}	25 ± 1^{b}	22 ± 2^{ab}
Feces excreted in 12 h (g)	4.0 ± 0.5^{a}	0.8 ± 0.7^{b}	1.3 ± 0.06^{b}
Food intake/excreted feces ratio	4.9 ± 0.5^{a}	32 ± 4.5^{b}	9.3 ± 1.1^{c}
Hydrous intake in 12 h (mL)	13 ± 1.7	9.5 ± 0.5	14 ± 2.2
Urine excreted in 12 h (mL)	12 ± 1.7	9.5 ± 0.6	9.3 ± 1.1
Lee index	321 ± 2^{a}	350 ± 2^{b}	314 ± 6^{a}
Retroperitoneal fat pads (% BW)	2.5 ± 0.2^{a}	3.7 ± 0.1^{b}	3.7 ± 0.2^{b}
Perigonadal fat pads (% BW)	1.7 ± 0.2^{a}	2.7 ± 0.1^{b}	2.1 ± 0.1^{a}
Stomach weight/BW ratio × 100	0.4 ± 0.02^{a}	0.3 ± 0.01^{a}	0.5 ± 0.04^{b}

Data are means ± SEM ($n = 4$–9). Different letters indicate significant difference. One-way ANOVA followed by Tukey posttest ($P < 0.05$)

vagotomy, CAF Vag presented plasma TG concentrations (108 ± 14 and 117 ± 15 mg/dL, for fasting and fed conditions, respectively) that were similar to those of CTL rats. No alterations in total CHOL

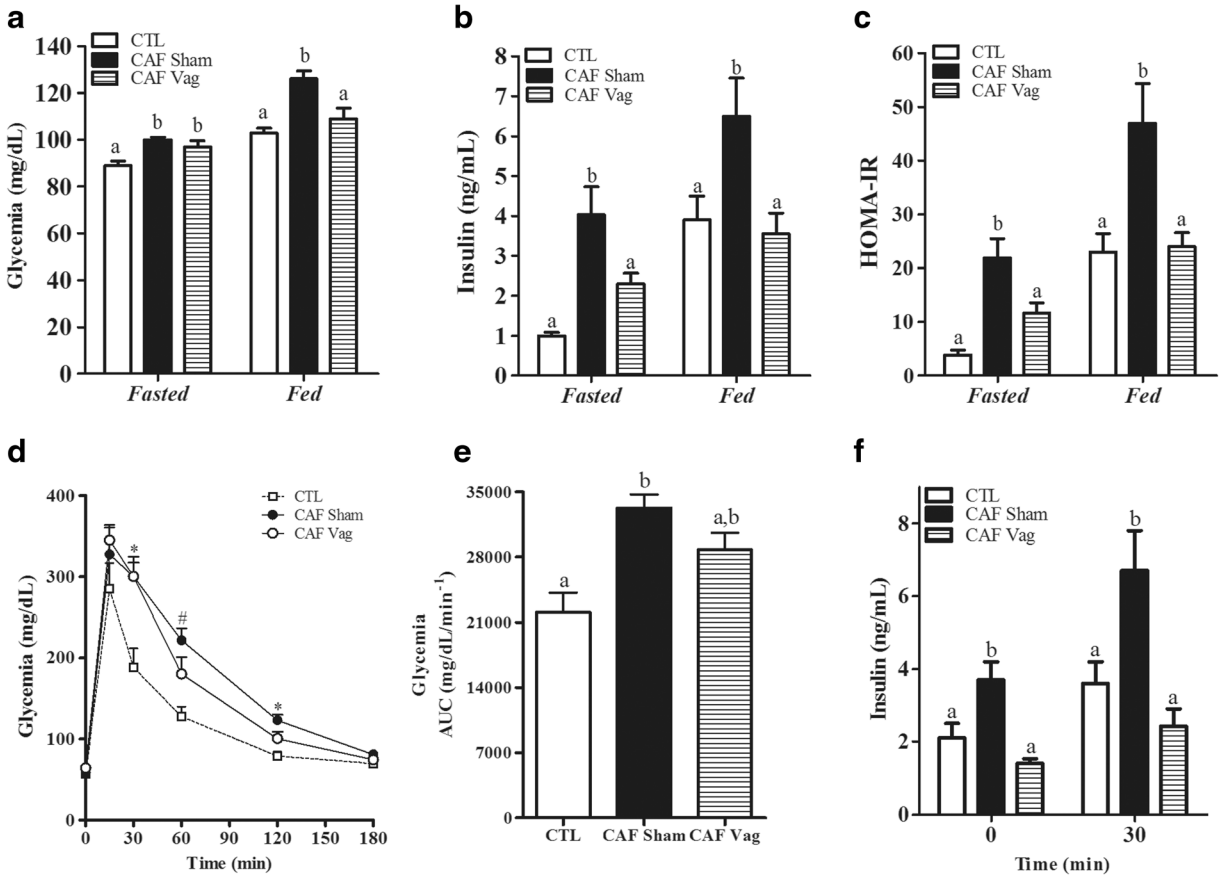

Fig. 2 **a** Plasma glucose and **b** insulin concentrations, and **c** insulin sensitivity, measured by the HOMA-IR in fasted and fed CTL, CAF Sham, and CAF Vag rats. Changes in blood glucose (**d**) and total glycemia (**e**) during the ipGTT. **f** Insulinemia at 0 and 30 min of the ipGTT. Data are means ± SEM ($n = 6$–10). *CAF Sham and CAF Vag are different from CTL. #CAF Sham is different from the CTL group. *Different letters over the bars* indicate significant differences. One-way ANOVA followed by the Tukey posttest, $P < 0.05$

plasma levels were observed in the fed and fasted state, between all experimental groups (data not shown).

Islet insulin secretion in response to glucose and potentiating agents

Figure 3 shows insulin secretion in islets isolated from CAF rats submitted, or not, to subdiaphragmatic vagal denervation. The release of insulin at a stimulatory glucose concentration (11.1 mM) did not differ between CAF Sham and CTL islets. However, CAF Sham islets secreted more insulin when stimulated by Cch, a cholinergic agonist ($P < 0.0001$), as well as in response to intracellular cyclic adenosine monophosphate (cAMP) elevation by forskolin, an adenylate cyclase activator, or IBMX, a phosphodiesterase inhibitor ($P < 0.001$ and $P < 0.05$, respectively; Fig. 3). Conversely, isolated islets from CAF Vag rats secreted less insulin in response to glucose than islets from the CAF Sham group ($P < 0.05$). This effect was accompanied by a reduction in the activation of the cholinergic pathway, since the insulin release in response to Cch by CAF Vag islets was approximately 71 and 56 % lower than in the CAF Sham and CTL groups, respectively. In addition, CAF Vag islets presented reduced insulin secretion in response to forskolin, when compared with CAF Sham islets ($P < 0.0001$). When islets were stimulated by IBMX, a partial reduction in insulin secretion was observed, since insulin release by CAF Vag islets was similar to that of CAF Sham and CTL islets (Fig. 3).

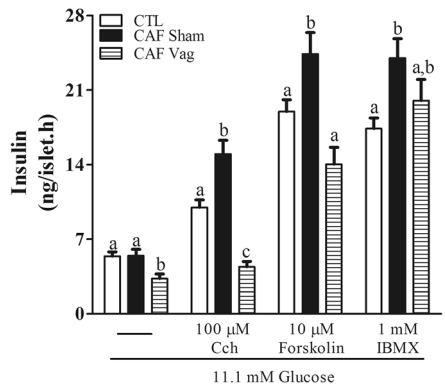

Fig. 3 Insulin secretion in response to glucose without or with potentiating agents in islets isolated from CTL, CAF Sham, and CAF Vag rats. Groups of four islets were incubated for 1 h in the presence of 11.1 mM glucose alone or with 100 μM Cch, 10 μM forskolin, or 1 mM IBMX. Data are means ± SEM obtained from 10 to 16 groups of islets in two independent experiments. *Different letters over the bars* indicate significant differences. One-way ANOVA followed by the Tukey posttest, $P < 0.05$

Discussion

In this study, we confirm previous observations that the CAF-style diet effectively induces metabolic damages that resemble a human "bad style" diet, since rats that consumed the CAF diet were obese, hyperphagic, hyperglycemic, hypertriglyceridemic, and insulin resistant. For the first time, we demonstrated that subdiaphragmatic vagotomy, after installation of obesity, is a good strategy for reducing adiposity and ameliorating glucose homeostasis in the CAF diet.

Although the pathophysiology of obesity is complex and not completely understood, several investigations using obese rodents or humans have demonstrated that the vagus nerve may contribute to obesity development and maintenance [3, 4, 17, 19, 30, 31]. An increased PNS action, associated with reduced SNS activity, is implicated in fat deposition in hypothalamic, genetic, and high-fat diet-induced obesity [27, 29, 31]. Accordingly, truncal vagotomy has been shown to be effective in promoting weight loss [3, 4, 17, 19, 31]. Here, we demonstrate that vagotomized CAF rats presented decreased BW, Lee index and perigonadal fat stores, in association with only a partial reduction in food consumption, since 12-h food intake in CAF Vag rats was similar to that of CAF Sham and CTL rats.

The involvement of the PNS in adiposity regulation also comprises afferent vagus fibers, since *Sprague-Dawley* rats presented decreased visceral fat deposition after 11 months of truncal vagal de-afferentation [31]. Afferent vagal fibers are also involved in the transmission of satiety signals from the gut to the CNS [25]. Studies indicate that these vagal afferents are altered in obesity, which contributes to hyperphagia [10, 12]. Consistent with these findings, truncal vagotomy has been shown to reduce food consumption in rodents [19, 26] and humans [17]. Conversely, high-fat diet rats did not demonstrate any alteration in food intake after vagotomy or vagal de-afferentiation [31], similarly to our observations for food consumption in CAF Vag rats.

In agreement with previous observations [2, 28, 34], we found that the CAF-style diet induced a disruption in glucose homeostasis. The vagus nerve is also involved in body glucose control by regulation of insulin action [14, 18] and pancreatic islet insulin secretion [15].

Vagotomy has been shown to restore glucose tolerance in hypothalamic obesity [3, 19]; however, benefits of vagotomy on body glucose control under high-caloric regimens have not been reported to date [31]. The mechanism of action by which PNS contributes to insulin action in peripheral tissues is not completely known. Studies indicate that hepatic PNS action in the postprandial state regulates glucose output in the liver and also produces a factor (denominated hepatic insulin-sensitizing substance) that enhances insulin peripheral actions, especially in skeletal muscle [14, 21]. As such, an impaired PNS action may contribute to glucose intolerance and progression to T2D; it is possible that the vagus nerve hypertonia in obesity downregulates the hepatic actions of the PNS, since higher acetylcholinesterase activity was reported in the liver of obese rodents [20, 24]. The above observations may suggest that the partial amelioration of glucose tolerance in CAF Vag rats may be due to the lack of action of PNS in insulin target tissues. Therefore, the better fed glycemia, tryglyceridemia, and insulinemia in the CAF Vag group may be associated with the lack of cholinergic action upon endocrine pancreas.

Several lines of investigation demonstrated that, in hypothalamic and genetic obesity, enhancements in the PNS activity in the pancreas contribute to hyperinsulinemia [19, 26], which may increase adiposity. Hyperinsulinemia can also time-dependently downregulate the insulin pathway in peripheral tissues [35]. Therefore, these observations indicate that the effects of PNS hyperactivity on endocrine pancreatic function may contribute to obesity onset and maintenance.

Our experiments in isolated pancreatic islets demonstrated that despite presenting hyperinsulinemia, the CAF Sham group did not alter insulin secretion in response to glucose. In fact, although hyperinsulinemia was often been observed in CAF rodents [2, 34], conflicting data regarding insulin secretion have been reported, showing reductions in hormone release by female CAF islets after 4 months of diet ingestion [34], and increases in insulin secretion in islets from male rats that consumed a CAF diet per 10 months [2]. In addition, we herein observed that CAF Sham islets hypersecreted insulin in response to the activation of cholinergic and cAMP pathways, indicating an increased effect of the PNS on the endocrine pancreas, resulting in increased insulin release to compensate the lower hormone peripheral action. Increased cAMP-induced insulin secretion in CAF Sham islets may also be associated with the activity of the PNS, since cholinergic activity in T2D has also been linked to the cAMP pathway activation [11], although acetylcholine acts mainly in the pancreatic β-cells through activation of the muscarinic type 3/phospholipase C (PLC) pathway, enhancing intracellular Ca^{2+} concentrations and PKC activation [7, 15].

Subdiaphragmatic vagotomy in CAF rats decreased insulin secretion in response to glucose and normalized it in response to cAMP. Furthermore, the PLC pathway also participates in glucose-induced insulin secretion [33]. Therefore, in CAF Vag rats, the lack of PNS action on the endocrine pancreas, as confirmed by the lower insulin release in response to the Cch in this group, may gradually normalize insulinemia, which restores insulin peripheral sensitivity. The normalization of blood TG levels in CAF Vag rats may also be due to the lack of hepatic PNS action, since vagal action in the liver is involved in modulation of the insulin-induced hepatic lipogenesis [22].

In summary, the CAF-style diet in rats efficiently induced obesity and metabolic disturbances, characterizing a prediabetic condition. For the first time, we demonstrated that truncal vagotomy in CAF rats, an experimental model that closely resembles the human "obesogenic" diet, decreases adiposity and restores insulin sensitivity. These effects were due to the lack of cholinergic action on the endocrine pancreas, which normalizes insulinemia and may gradually decrease fat storage and restore the action of insulin.

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Compliance with ethical standards

Conflict of interest All contributing authors declare that they have no conflicts of interest.

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