Articles in PresS. Am J Physiol Heart Circ Physiol (March 15, 2013). doi:10.1152/ajpheart.00827.2012

AORTOCAVAL FISTULA DELAYS GASTRIC EMPTYING OF LIQUID TEST MEAL IN AWAKE RATS

Moisés T. B. Silva[1], Raimundo C. Palheta Jr.[2], Francisca G. V. Oliveira[1], Juliana B. M. de Lima[3], José Antunes-Rodrigues[3], Ricardo B. Oliveira[3], Pedro J. C. Magalhães[1], Armênio A. Santos[1*]

[1]Department of Physiology and Pharmacology, School of Medicine, Federal University of Ceará, Fortaleza, CE, Brazil.

[2]School of Veterinary Medicine, Federal University of Vale do São Francisco, Petrolina, PE, Brazil

[3]School of Medicine of Ribeirão Preto, University of São Paulo, Ribeirão Preto, SP, Brazil.

[*]Corresponding author

Prof. Armenio Aguiar dos Santos
Departamento de Fisiologia e Farmacologia
Faculdade de Medicina,
Universidade Federal do Ceará
R. Cel. Nunes de Melo 1127, Rodolfo Teófilo
60430-270, Fortaleza, CE, Brasil
Phone: +55 85 3366 8345; Fax: +55 85 3366 8333;
E-mail: meno@ufc.br

ABSTRACT

Arteriovenous (AV) anastomoses disrupt cardiovascular and renal homeostasis, eliciting hemodynamic adjustments, resetting the humoral pattern, and inducing cardiac hypertrophy. Because acute circulatory imbalance alters gut motor behavior, we studied the effects of AV fistula placement on the gastric emptying (GE) of a liquid meal in awake rats. After laparotomy, we created an aortocaval fistula (ACF) by aorta and cava wall puncture with a 21-, 23-, or 26-gauge needle. The ACF was not created in the control group, which underwent sham surgery. After 12, 24, or 48 h, mean arterial pressure (MAP), heart rate (HR), and central venous pressure (CVP) were continuously recorded, and cardiac output (CO) was estimated by thermal dilution. The rats were then gavage-fed a test meal (i.e., phenol red in glucose solution), and fractional dye retention was determined 10, 20, or 30 min later. The effect of prior bleeding on ACF-induced GE delay, the role of neuroautonomic pathways, and changes in plasma hormone levels (i.e., angiotensin II, arginine vasopressin, atrial natriuretic peptide, corticosterone, and oxytocin) were evaluated. Compared with the sham group, ACF rats exhibited arterial hypotension, higher ($p < 0.05$) HR, CVP, and CO values, and increased ($p < 0.05$) gastric dye retention, a phenomenon prevented by bilateral subdiaphragmatic vagotomy and hexamethonium treatment. Pirenzepine also impaired the occurrence of gastric delay in virtue of ACF. In addition to causing hyperkinetic circulation, ACF placement delayed the GE of liquid in awake rats, an effect that likely involves a parasympathetic pathway.

Keywords: Arteriovenous Fistula; Gastrointestinal Motility; Hyperkinetic Circulation; Intestinal Transit; Natriuretic Hormone

INTRODUCTION

Hyperkinetic circulation is a systemic condition that may originate from an arteriovenous (AV) fistula, either as a congenital malformation or acquired after invasive procedures (e.g., percutaneous renal biopsy), surgeries (e.g., nephrectomy), or trauma (e.g., stab or gunshot wounds) (24,11). Large AV fistulas are considered a simple and reliable model to elicit congestive heart failure (13). Laboratory animals with an aortocaval fistula (ACF) present hemodynamic and neurohumoral changes that resemble those seen in patients with heart failure, including cardiac hypertrophy (22). These animals have high levels of cardiac output and mean circulatory filling pressure, in addition to tachycardia, low resistance to blood flow in circulatory vessels, and decreased mean arterial pressure (MAP) (17).

As a consequence of inducing such changes, the placement of an AV fistula activates complex neurohumoral mechanisms that may restore hemodynamic homeostasis. During the decompensated phase, the exacerbated activity of vasoconstrictors and sodium-retaining agents (e.g., the renin-angiotensin system, arginine vasopressin [AVP], oxytocin [OT], the sympathetic nervous system) overwhelm the vasodilatory and natriuretic influence of atrial natriuretic peptide (ANP), bradykinin, and nitric oxide (NO), leading to avid salt retention. For compensation to occur, the effects of natriuretic substances must prevail over the effects of opposing systems, increasing the renal excretion of fluid and electrolytes (39,38).

In addition to their vasoactive and diuretic properties, these agents alter the activity of gut smooth muscle and may enhance gastric tonus (16). Moreover, we have shown that blood volume redistribution modifies gastrointestinal motor behavior (40,14). Taken together, our hypothesis is that the opening of an AV fistula impels the circulation to a hyperkinetic status, which affects the gut motor behavior in virtue of hemodynamic adjustments and multiple neurohumoral changes. Thus, this study was designed to verify in awake rats (i) if the hemodynamic changes elicited by an ACF alter the gastric emptying rate and upper

gastrointestinal transit of a liquid test meal; (ii) if the autonomic nervous system exerts a role on such phenomenon and (iii) if the establishment of ACF acutely alters the plasma levels of AVP, ANG, OT, ANP, and corticosterone.

MATERIALS AND METHODS

All of the procedures were performed in accordance with the ethical principles for the care and use of laboratory animals of the Brazilian Society for Laboratory Animal Science after approval by the local ethics committee (protocol no. 46/07). Male Wistar rats (230-280 g) were obtained from colonies raised by the Federal University of Ceará and maintained in a temperature-controlled room on a 12 h/12 h light/dark cycle. They were isolated in Bollman's cages and fasted for 18 h with free access to an oral rehydration solution (ORS; 75 mM Na^+, 65 mM Cl^-, 20 mM K^+, 75 mM glucose, and 10 mM citrate) to clear the stomach of food residue while maintaining normovolemia and euglycemia.

Surgical procedures

After overnight fasting, the rats were anesthetized with tribromoethanol (250 $mg.kg^{-1}$, i.p.). After laparotomy, they were subjected to ACF placement (ACF group) or a sham procedure with no ACF placement (sham group) as previously reported (13). Briefly, the abdominal aorta and inferior vena cava were exposed and dissected together. Using a vascular clamp, the vessels were occluded together below the renal artery, briefly stopping distal blood flow. A disposable needle was then used to puncture the aorta and advanced until it perforated the opposite wall to reach the vena cava lumen. After removal of the needle, the vascular holes were sealed with cyano-acrylate glue. Prompt observation of the pulsatile flow of oxygenated blood in the vein was considered confirmation of a successful AV shunt. Separate subgroups of ACF rats were formed according to the extent of the AV shunt created by 26-gauge (fine), 23-gauge (intermediate), and 21-gauge (wide) needles, termed the 26G, 23G, and 21G groups,

respectively. A similar procedure was performed in sham rats, which did not receive an aortocaval puncture.

Three stainless-steel wires (0.203 mm outer diameter; Teflon-coated; A. M. Systems, Everett, WA, USA) were affixed to the chest muscles and hip muscle of the left paw and then exteriorized at the interscapular region. After connecting them to a bioamplifier (ML132 BioAmp) coupled to a data acquisition system (PowerLab/8SP, ADInstruments®,Australia), an electrocardiographic signal could be derived to continuously record heart activity. The femoral and common carotid arteries and jugular vein were then cannulated with polyethylene-50 (PE-50) thermocouple and PE-90 catheters, respectively. The distal ends of the catheters were subcutaneously exteriorized and fixed at the interscapular region. Continuous monitoring of MAP (in mmHg), central venous pressure (CVP; in cmH_2O), and heart rate (HR; in $beats.min^{-1}$) was performed by connecting the catheters to pressure transducers coupled to a digital system. Cardiac output (CO; in $mL.min^{-1}$) was estimated using the thermal dilution method (6). Systemic vascular resistance (SVR) was calculated as $SVR = MAP.CO^{-1}$, and stroke volume (SV) was calculated as $SV = CO.HR^{-1}$. After surgery, the rats were subjected to 12, 24, or 48 h of fasting but with free access to the ORS. Hemodynamic monitoring was performed just before the gut motility tests.

Gastric emptying assessment

A dye dilution technique, previously adapted by us (34), was used to evaluate the GE of a liquid (1.5 mL) test meal (0.5 $mg.mL^{-1}$ of phenol red in 5% glucose solution). After 10, 20, or 30 min of the meal gavage, the rats, respectively named 10, 20, and 30-min postprandial subgroups, were sacrificed by an intravenous (i.v.) thiopental overdose.

After laparotomy, the gut was divided into consecutive segments: stomach and small intestine. Each segment volume was calculated by submerging it in a graduated cylinder with 100 mL of 0.1 N NaOH. After homogenization, the proteins in each segment were precipitated

with 0.5 mL of 20% trichloroacetic acid. After centrifugation, 3 mL of the supernatant was added to 4 mL of 0.5 N NaOH, and the samples were read by a spectrophotometer at 560 nm to construct dilution curves by plotting the dye concentrations against optic densities. The value of fractional gastric dye recovery was estimated from the following equation:

$$\text{Gastric dye retention (\%)} = 1 - \left[\frac{\text{amount of phenol red recovered in stomach}}{\text{total amount of phenol red recovered from all segments}} \right] \times 100$$

To exclude the possible influence of gastric acid secretion on the effects of ACF on gut motor behavior, a separate group of rats was subjected to a similar protocol (i.e., laparotomy either followed or not by ACF placement with a 21G needle). After 24 h, the rats received an i.v. injection (0.1 $mL.kg^{-1}$) of omeprazole (20 $mg.kg^{-1}$). After basal hemodynamic monitoring, the rats were gavage fed the test meal and sacrificed 20 min later for gastric dye recovery analysis as described above.

To verify the influence of blood volume on the effects of ACF on gut motor behavior, we studied a group of rats either previously subjected or not to ACF placement with a 21G needle. After a period of basal hemodynamic monitoring, they underwent acute hypovolemia by bleeding (15 $mL.kg^{-1}$) via the femoral artery. They were then gavage fed the test meal and sacrificed 20 min later for gastric dye recovery analysis as described above.

The role of the autonomic nervous system on the phenomena studied herein was assessed in separate groups of rats that were previously subjected to tribromoethanol anesthesia (250 $mg.kg^{-1}$, i.p.) and laparotomy, followed or not by bilateral subdiaphragmatic vagotomy (19) or celiac ganglionectomy + splanchnicectomy (12). Two days later, they were anesthetized again and subjected or not (sham) to ACF (21G) placement. Twenty-four hours after the second surgery, the rats were subjected to basal hemodynamic monitoring followed by gavage feeding with the test meal and sacrificed 20 min later for gastric dye recovery analysis.

To verify the involvement of nicotinic neural pathways in the effects of ACF on gut motor behavior, a separate group of rats was subjected to laparotomy under anesthesia followed or not by ACF (21G) placement. After 24 h, they were subjected to hemodynamic monitoring and pretreated with the ganglioplegic compound hexamethonium (10 $mg.kg^{-1}$, i.v.). They were then gavage fed with the test meal and sacrificed 20 min later for gastric dye recovery analysis as described above. To investigate the role of cholinergic pathways, a separate group of rats was subjected to laparotomy under anesthesia followed or not by ACF (21G). After 24 h, they were subjected to hemodynamic monitoring and pretreatment with pirenzepine (7 $mg.kg^{-1}$, i.p), a muscarinic antagonist more selective for M_1 receptors. They were then gavage fed with the test meal and sacrificed 20 min later for gastric dye recovery analysis as described above.

Small intestine transit assessment

To assess the effect of ACF on small intestine transit, another group of rats underwent a different protocol. After tribromoethanol anesthesia and laparotomy, the wall of the stomach fundus was cut to insert a silicone cannula (0.3 mm outer diameter; Silastic, Dow Corning, Midland, MI, USA) into the gut. The cannula was advanced into the duodenal lumen 1 cm distal to the pylorus. It was fixed to the stomach wall using a purse-string suture, with its free end subcutaneously exteriorized and fixed at the dorsal region. Three days later, the rats were anesthetized again and subjected to laparotomy, followed or not (sham) by ACF (21G) placement. After 24 h, the rats were subjected to basal hemodynamic monitoring followed by gavage feeding with the test meal (1.0 mL) via the duodenal cannula and sacrificed 20 min later. After gut exeresis, the stomach and first 1 cm of the duodenum that contained the tip of the cannula comprised segment 1. The remaining gut was carefully stretched and removed. Obstructive ligatures were performed to obtain five consecutive segments of the small intestine (approximately 20 cm long). Each segment was homogenized, and its dye content was

determined spectrophotometrically as described above. Fractional marker retention was calculated for each gut segment as the ratio between the amount obtained in it and the sum of the amounts of all of the segments, including the gastro-duodenal segment. The value obtained for each segment was then multiplied by the respective number of segments and summed to calculate the geometric center of the marker distribution throughout the gut as previously reported (31).

Plasma hormone analysis

For plasma hormone measurements, a separate group of rats was decapitated 24 h after ACF placement, and blood was collected in chilled tubes that contained heparin (for AVP and OT) or peptidase inhibitors (for ANG II and ANP). Plasma was obtained after centrifugation (20 min, 3000 rotations per minute, 4°C) and stored at -20°C until specific extraction and the radioimmunoassay procedures. The specific antibodies for the corticosterone, ANG II, AVP, and OT radioimmunoassays were obtained from Peninsula (ANG II, T4007; AVP, T4561; OT, T4084; San Carlos, CA, USA). The antibody for ANP was generously donated by Dr. Jolanta Gutkowska (Hotel Dieu, University of Montreal, Montreal, Quebec, Canada). Radioimmunoassay sensitivity and intra- and interassay variation coefficients were 0.5 $pg.mL^{-1}$ and 14.9-27.1% for ANG II, 0.7 $pg.mL^{-1}$ and 4.8-10.0% for ANP, 0.8 $pg.mL^{-1}$ and 7.7-11.9% for AVP, 0.9 $pg.mL^{-1}$ and 7.0-12.6% for OT, and 0.4 $\mu g.dL^{-1}$ and 5.1-8.4% for corticosterone (18).

Blood gas analysis

To verify a putative effect of blood pH and gases parameters in the ACF effects on gut motor behavior, subsets of sham and 21G ACF rats were anesthetized with tribromoethanol (2.5 $g.kg^{-1}$, i.p.). Next, a polyethylene cannula (PE-50, Intramedic Clay Adams, Franklin Lakes, NJ, USA) was inserted into the carotid artery, allowing for the collection of a 3 mL blood sample. A blood gas and electrolyte automatic analyzer (Cobas b 121, Roche) was used for measurement of pH, P_{CO2}, P_{O2}, base excess, O_2 saturation, HCO_3^- concentration and hematocrit (36).

Statistical analysis

The hemodynamic data that were recorded throughout the studies were pooled as mean MAP, CVP, and HR values into consecutive 10-min intervals: basal (i.e., the first 40 min of monitoring, which included the pharmacological treatments) and postprandial (i.e., up to 30 min, just after gavage of the test meal). Each subgroup consisted of 6-9 rats. All of the data are expressed as mean ± SEM, with the exception of the gastrointestinal transit index values, which are presented as medians and interquartile ranges. Differences in gastric retention values between groups were assessed by one-way analysis of variance (ANOVA), followed by Tukey's multiple comparison test compared with the control group. Hemodynamic intra-group data differences between the basal period and successive intervals were compared using one-way repeated-measures ANOVA, followed by the Bonferroni test when appropriate. Values of $p < 0.05$ were considered statistically significant. For the analysis of plasma hormone levels, we used the unpaired Student's *t*-test.

RESULTS

In the present study, the following hemodynamic parameters were monitored: MAP, CVP, HR, CO, SV and SVR. During the basal monitoring period, they varied spontaneously, but no difference ($p > 0.05$, ANOVA) was found between the baseline mean values recorded throughout the 40 min observation interval, either in control or ACF rats.

The impact of ACF placement on hemodynamic indices in awake rats is shown in Fig. 1. Compared with the basal mean values of their respective sham group, the 23G and 21G ACF rats had higher CVP, CO, HR, and SV levels ($p < 0.05$, ANOVA and Tukey's test) and lower MAP and SVR levels ($p < 0.05$, ANOVA and Tukey's test). Compared with the mean values in the respective sham rats, 26G ACF placement did not significantly alter MAP, CVP, or HR levels but increased CO levels ($p < 0.05$, ANOVA and Tukey's test; Fig. 1A, B, and D). In other hand, their

220 respective MAP and SVR values, the 21G and 23G ACF rats showed lower ($p < 0.05$) levels in
221 comparison with those of 26G ACF subset. On the other hand, both 21G and 23G ACF rats
222 showed higher ($p < 0.05$) values of CVP and HR in comparison with the respective levels of 26G
223 ACF subset. Thus, we used a 21G needle to further study the mechanisms that underlie ACF-
224 induced GE delay. Fig. 2 shows that 21G ACF placement significantly increased CVP, HR, CO, and
225 SV levels but decreased MAP and SVR levels, which were manifested as soon as 12 h and
226 persisted for at least 48 h.

227 Fig. 3 shows that ACF placement delayed the GE of a liquid test meal in awake rats.
228 Compared with the values in the respective sham rats, no significant difference in fractional
229 gastric dye recovery was observed in the 21G ACF group, but it was enhanced at both 24 hand
230 at 48 h ($P < 0.05$, ANOVA and Tukey's test; Fig. 3A). In rats studied 24 h after AV shunt or sham
231 surgery, ACF placement consistently increased fractional gastric dye recovery compared with
232 their respective control values at different postprandial time intervals (i.e., after 10, 20, and 30
233 min of meal gavage; $p < 0.05$, ANOVA and Tukey's test; Fig. 3B). Moreover, gastric retention
234 caused by ACF placement depended on the dimensions of the AV shunt. When analyzing all of
235 the data from ACF rats handled with 21G needles and studied 20 min postprandially, a strong (r
236 = 0.96) and significant ($p < 0.001$) positive correlation was found between the degree of the AV
237 shunt and respective fractional gastric dye recovery value (Fig. 04).

238 Gastric retention caused by ACF placement appeared to be unrelated to a putative
239 effect of hyperdynamic circulation on gastric acid secretion. Omeprazole pretreatment did not
240 alter the increase in gastric dye recovery values ($p < 0.05$, ANOVA and Tukey's test) elicited by
241 ACF placement ($33.5 \pm 2.5\%$ *vs.* $51.4 \pm 4.5\%$ in sham and ACF rats, respectively). In contrast,
242 acute bleeding prevented the GE delay in ACF rats (Fig. 05).

243 Fig. 6B shows that bilateral subdiaphragmatic vagotomy prevented gastric retention in
244 ACF rats. Compared with the gastric recovery values in respective sham rats, ACF placement

enhanced ($p < 0.05$) gastric retention in rats previously subjected to coeliac ganglionectomy + splanchnicectomy (32.8 ± 6.7% *vs.* 54.0 ± 4.0%, respectively; Fig. 6A). Hexamethonium and pirenzepine treatment increased gastric recovery values ($p < 0.05$) compared with vehicle-treated sham rats (53.5 ± 4.6% and 52.1 ± 3.4% *vs.* 37.1 ± 2.5%, respectively) but prevented the ACF-induced GE delay of the liquid test meal (53.5 ± 4.6% *vs.* 48.7 ± 2.9% and 52.1 ± 3.4% *vs.* 48.0 ± 3.5% respectively; Fig. 6C and 6D).

The plasma levels of ANP, ANG II, and corticosterone are shown in Fig. 6. In comparison with their respective values of sham group, the establishment of an AV shunt by creating an ACF significantly decreased the plasma levels of ANG II (46.8 ± 12.2 $\rho g.mL^{-1}$ *vs.* 114.4 ± 15.1 $\rho g.mL^{-1}$; $p < 0.01$, unpaired Student's t-test), while it significantly increased corticosterone (19.2 ± 2.3 $\mu g.dL^{-1}$ *vs.* 8.7 ± 1.9 $\mu g.dL^{-1}$; $p < 0.05$, unpaired Student's t-test) and ANP levels (72.9 ± 5.0 $\rho g.mL^{-1}$ *vs.* 44.2 ± 5.9 $\rho g.mL^{-1}$). On the other hand, ACF establishment did not affect plasma values of OT (2.6 ± 0.5 $\rho g.mL^{-1}$ *vs.* 3.4 ± 0.3 $\rho g.mL^{-1}$) or AVP (1.3 ± 0.3 $\rho g.mL^{-1}$ *vs.* 1.4 ± 0.1 $\rho g.mL^{-1}$).

In contrast, ACF placement did not alter the small intestine transit of the liquid test meal. No significant changes in marker progression in the gut were found between the 21G ACF and sham rats studied 20 min postprandially, reflected by the median values of the meal's geometric center (3.3 [2.7-3.4] *vs.* 3.4 [0.3-4.0], respectively). No significant changes were observed in the blood pH and gas analysis between sham and 21G ACF rats [Supplementary material].

DISCUSSION

The present study showed that ACF placement delayed the GE of a liquid test meal in awake rats. The phenomenon occurred during the decompensated phase of blood volume

redistribution, unbalancing cardiovascular function, inducing arterial hypotension and tachycardia, and increasing CVP and CO levels.

The vascular puncture technique that is used to create an ACF elicits hyperkinetic status that clearly depends on the extent of the AV shunt. The ACF placement with a 26G needle did not change MAP, CVP, or HR levels. In contrast, these indices changed when the vessels were punctured with wider bevels. Under such conditions, the hemodynamic changes were reliable and noticeable 24 h after surgery. However, some of the effects faded after 48 h, especially the increase in SV, because of the influence of homeostatic factors. Thus, we used a 21G needle and allowed a 24-h interval for the full expression of the hyperkinetic state.

Although the difference in gastric retention between the sham and ACF rats studied 12 h after surgery was not statistically significant, ACF placement delayed GE at 24 h, a phenomenon that persisted for at least 48 h. The gastric dye recovery values in the sham group studied at 12 h were significantly elevated, likely because anesthesia and laparotomy caused gastroparesis (4,5). Such an hypothesis seems plausible because gastric dye recovery decreased at 24 h and plateaued thereafter. According to Coimbra *et al*. (1996) (8), the paralytic ileus is a short-lived phenomenon in rats because their gut motor behavior pattern returns to normal with 12 to 24 h.

The gut motility assessment was performed using a dye dilution technique, a simple and reliable method (27). Nonetheless, the phenol red dye used as a marker is a pH-dependent reagent, which may have biased the present analysis if one considers that AV placement eventually increases gastric acid secretion, thus inhibiting GE via duodenal chemical stimulation (9). However, we may exclude the possibility of such bias because both vehicle- and omeprazole-pretreated ACF rats had similar gastric dye recovery values.

The present work provides data that further strengthen the idea of a functional connection between cardiovascular and gastrointestinal systems in the context of blood

volume homeostasis. Such a concept was first advanced by Sjövall et al. (1983) (33), in which passive postural maneuvers were found to alter gut permeability in healthy volunteers. Orthostasis maximized intestinal salt and water absorption, whereas tilting favored secretion. Consistent with such observations, we found that acute hypervolemia, caused by saline or blood transfusion increased gastric tonus in dogs and rats; a response preventable by bleeding (7,32). Moreover, mechanical stretch of the right atrium by a balloon catheter increased, in a volume dependent way, the gastric retention in awake rats, phenomenon also preventable by bleeding (25). In the present study, a clearly positive relationship was found between CO values and the amount of dye recovered from the stomach in ACF rats, and bleeding hindered the ACF-induced GE delay.

A sympathetic influence on the present results appears to be unlikely because disruption of sympathetic input to the gut by splanchnicectomy + celiac ganglionectomy did not prevent gastric retention caused by ACF placement. In rats previously subjected to bilateral subdiaphragmatic vagotomy, gastric dye recovery values in the sham and ACF subgroups were similar. Thus, parasympathetic innervation appears to be involved in ACF-induced GE delay. Such involvement seems to depend on cholinergic pathways since pretreatment with pirenzepine, an antagonist of M_1 muscarinic receptors, was able to prevent the decrease in gastric emptying induced by ACF. Further neuroautonomic involvement was investigated by blocking nicotinic receptors with hexamethonium. Pretreatment with the ganglion-blocking quaternary ammonium compound in sham rats increased gastric retention compared with the vehicle-untreated sham group. Further supporting our present findings, a previous study showed that hexamethonium delayed GE (15). Notably, however, ACF placement failed to further promote gastric retention in hexamethonium-treated rats. Thus, neuronal parasympathetic synapses appear to be involved in ACF-induced GE delay.

The role of vagus nerves in the present phenomenon may involve two mechanisms: (*i*) inhibition of the parasympathetic excitatory input to the stomach that sustains gastric motor tonus or (*ii*) an increase in the descending inhibitory drive to the stomach via a vago-vagal reflex that increases gastric compliance (“receptive relaxation”) (3).

Although further studies are necessary to identify the actual physiological mechanisms responsible for the present GE delay caused by ACF placement, the present study generated other important data, especially with regard to the ability of ACF placement to alter the plasma levels of circulating hormones, with the exception of oxytocin and AVP. Confirming previous reports both in animals (1) and humans (20) under hyperkinetic conditions, the ACF rats in the present study exhibited higher plasma ANP levels. This likely has a cardiac origin as a part of compensatory vasodilator responses elicited by the creation of such a large AV fistula. In fact, the ACF group exhibited an increase in CVP concomitant with a decrease in SVR. Nevertheless, ANP may also influence gut motor behavior through a direct action because of its inhibitory effects on smooth muscle contractility (21). Moreover, the stomach and colon also appear to contain the highest amount of ANP messenger RNA (41).

Conversely, other compensatory responses involve vasoconstrictor pathways, including activation of the renin-angiotensin-aldosterone system and AVP release. The AV fistula opening appears to increase ANG II and AVP blood levels because of sustained arterial hypotension via baroreceptor activation in the arterial circulation (2). However, in the present ACF rats, blood levels of AVP remained unaltered, whereas blood levels of ANG II decreased. These results clearly conflict with previous findings obtained from animals that were studied at least 1 week after opening the AV fistula (30). This apparent discrepancy may be understood if one considers that the present data were obtained acutely (i.e., only 24 h after ACF placement) during a stage prior to full activation of the renin-angiotensin-aldosterone system. Nevertheless, the low ANG

342 II level observed in the present study is consistent with ACF-induced GE delay. In fact, ANG II is
343 considered to have predominantly stimulatory actions on small intestine motility (35).

344 Another important finding was the increase in the blood levels of corticosterone in ACF
345 rats studied at 24 h, which may be putatively involved in ACF-induced GE delay since
346 laparotomy increases the secretion of corticosterone, thus stressful conditions may delay GE in
347 laboratory animals (29). However, it should be taken into account the specific temporal
348 patterns of gastric retention observed in the present study (i.e., a gradual decrease 12 h after
349 surgery in sham rats while a steady elevation in the ACF group even 48 h later). Moreover,
350 corticosterone, even when administered systemically, does not change GE rate in mice and
351 dogs (28,23). Thus, corticosterone seems not to be directly responsible for the ACF-induced GE
352 delay.

353 Considering the complex process that modulates the gastroduodenal flow of liquid
354 meals in awake mammals (26), the ACF-induced GE delay observed in the present study may
355 have resulted from increased gastric relaxation, decreased antral contractility, or enhanced
356 pyloric or duodenal resistance (10). Because an isotonic liquid was used as the test meal, such
357 an effect is unlikely to be mediated through the enhanced intestinal inhibition of GE (i.e., via
358 the “duodenal brake”). Moreover, no difference was found in the marker progression of
359 intestinal transit in sham and ACF rats. Thus, we consider that ACF placement increased gastric
360 dye recovery by inhibiting the tonus of the proximal stomach.

361 As a fact, ACF establishment delays the GE of a liquid test meal in awake rats and,
362 therefore, its respective inflow to the small intestine, which, in turn, postpones the absorption
363 of fluids and electrolytes by the enteric epithelium. Thus, it is conceivable to cogitate that the
364 hyperkinetic circulation elicited by ACF placement alters the gut motor and permeability
365 behavior, lessening the blood volume overload, at least acutely. Moreover, the present GE

delay may be associated with the gut dysmotility complains (i.e., bloating and dyspepsia) reported by patients with heart failure syndrome due to AV fistula (37).

In conclusion, the placement of a large AV infrarenal shunt (i.e., ACF) induced a hyperkinetic circulation and elicited the GE delay of a liquid test meal in awake rats, phenomena that depended on an intact and functional parasympathetic nerve drive to the gut.

ACKNOWLEDGEMENTS

This work was part of an M.Sc. dissertation on Pharmacology presented by Dr. Silva to the Department of Physiology and Pharmacology, Federal University of Ceará. The authors would like to thank Mr. Willy Okoba for revising the manuscript and Mr. Haroldo Pinheiro for his helpful technical assistance.

GRANTS & DISCLOSURES

CAPES, CNPq, FAPESP, and FUNCAP supported this study. No conflicts of interest, financial or otherwise, are declared by the authors.

AUTHOR CONTRIBUTION

MTBS, RCP Jr, FHL, TAM, TNFG, and FGVO performed the experiments. MTBS and RCP Jr discussed the results and wrote the paper. PJCM and AAS designed the experiments, discussed the results, and revised the paper.

REFERENCES

1. **Abassi ZA, Brodsky S, Karram T, Dobkin I, Winaver J, Hoffman A**. Temporal changes in natriuretic and antinatriuretic systems after closure of a large arteriovenous fistula. *Cardiovasc Res*. 51: 567-576, 2001.
2. **Abassi Z, Goltsman I, Karram T, Winaver J, Hoffman A**. Aortocaval fistula in rat: a unique model of volume-overload congestive heart failure and cardiac hypertrophy. *J Biomed Biotechnol*. 2011: 729497, 2011.
3. **Azpiroz F, Malagelada JR**. Importance of vagal input in maintaining gastric tone in the dog. *J Physiol*. 384: 511-524, 1987.
4. **Barquist E, Zinner M, Rivier J, Taché Y**. Abdominal surgery-induced delayed gastric emptying in rats: role of CRF and sensory neurons. *Am J Physiol*. 262: G616-G620, 1992.
5. **Barquist E, Bonaz B, Martinez V, Rivier J, Zinner MJ, Taché Y**. Neuronal pathways involved in abdominal surgery-induced gastric ileus in rats. *Am J Physiol*. 270: R888-R894, 1996.
6. **Cabrales P, Acero C, Intaglietta M, Tsai AG**. Measurement of the cardiac output in small animals by thermodilution. *Microvasc Res.* 66: 77-82, 2003.
7. **Capelo LR, Cavalcante DM, Leitão IA, Filho GC, da Silva EA**. Modifications of gastric compliance in dogs related to changes of extracellular fluid volume: a possible physiological role. *Braz J Med Biol Res*. 16: 73-76, 1983.
8. **Coimbra CR, Plourde V**. Abdominal surgery-induced inhibition of gastric emptying is mediated in part by interleukin-1β. *Am J Physiol*. 270: R556-R560, 1996.
9. **De Rosalmeida MC, Saraiva LD, da Graça JR, Ivo BB, da Nóbrega MV, Gondim FA, Rola FH, dos Santos AA**. Sildenafil, a phosphodiesterase-5 inhibitor, delays gastric emptying and gastrointestinal transit of liquid in awake rats. *Dig Dis Sci*. 48: 2064-2068, 2003.
10. **Dooley CP, Valenzuela JE**. Antropyloroduodenal activity during gastric emptying of liquid meals in humans. *Am J Physiol*. 255: G93-G98, 1988.

11. **Durakoglugil ME, Kaya MG, Boyaci B, Cengel A**. High output heart failure 8 months after an acquired arteriovenous fistula. *Jpn Heart J*. 44: 805-809, 2003.
12. **Fujita S, Donovan CM**. Celiac-superior mesenteric ganglionectomy, but not vagotomy, suppresses the sympathoadrenal response to insulin-induced hypoglycemia. *Diabetes* 54: 3258-3264, 2005.
13. **Garcia R, Diebold S**. Simple, rapid, and effective method of producing aortocaval shunts in the rat. *Cardiovasc Res*. 24: 430-432, 1990.
14. **Gondim FA, Oliveira GR, Graca JR, Cavalcante DI, Souza MA, Santos AA, Rola FH**. Variations in gastric emptying of liquid elicited by acute blood volume changes in awake rats. *Braz J Med Biol Res*. 31: 967-973, 1998.
15. **Goodall P**. The effect of hexamethonium and atropine on gastric emptying of hyperosmolar glucose solution. *Br J Surg*. 57: 857, 1970.
16. **Gower WR Jr, Premaratne S, McCuen RW, Arimura A, McAfee Q, Schubert ML**. Gastric atrial natriuretic peptide regulates endocrine secretion in antrum and fundus of human and rat stomach. *Am J Physiol* 284: G638-G645, 2003.
17. **Guo L, Tabrizchi R**. Haemodynamic effects of vasoactive agents following chronic state of high cardiac output in anaesthetized rats. *Eur J Pharmacol*. 586: 266-274, 2008.
18. **Haanwinckel MA, Elias LK, Favaretto AL, Gutkowska J, McCann SM, Antunes-Rodrigues J.** Oxytocin mediates atrial natriuretic peptide release and natriuresis after volume expansion in the rat. *Proc Natl Acad Sci U S A*. 92: 7902-7906, 1995.
19. **Hansen MK, Krueger JM**. Subdiaphragmatic vagotomy blocks the sleep- and fever-promoting effects of interleukin-1β. *Am J Physiol*. 273: R1246-R1253, 1997.
20. **Iwashima Y, Horio T, Takami Y, Inenaga T, Nishikimi T, Takishita S, Kawano Y**. Effects of the creation of arteriovenous fistula for hemodialysis on cardiac function and natriuretic peptide levels in CRF. *Am J Kidney Dis*. 40: 974-982, 2002.

21. **Li CH, Pan LH, Li CY, Zhu CL, Xu WX**. Localization of ANP-synthesizing cells in rat stomach. *World J Gastroenterol*. 12: 5674-5679, 2006.

22. **Liu Z, Hilbelink DR, Crockett WB, Gerdes AM**. Regional changes in hemodynamics and cardiac myocyte size in rats with aortocaval fistulas: 1. Developing and established hypertrophy. *Circ Res*. 69: 52-58, 1991.

23. **Ohtani T, Mano T, Hikoso S, Sakata Y, Nishio M, Takeda Y, Otsu K, Miwa T, Masuyama T, Hori M, Yamamoto K**. Cardiac steroidogenesis and glucocorticoid in the development of cardiac hypertrophy during the progression to heart failure. *J Hypertens*. 27: 1074-1083, 2009.

24. **Okamoto M, Hashimoto M, Akita T, Sueda T, Karakawa S, Ohishi Y, Hamanaka N**. Congestive heart failure caused by aortocaval fistula after nephrectomy. *Intern Med*. 40: 1113-1116, 2001.

25. **Palheta RC Jr, Silva MT, Barbosa HL, Pinheiro AD, Cardoso KV, Graça JR, Magalhães PJ, Oliveira RB, Santos AA.** Atrial stretch delays gastric emptying of liquids in awake rats. *Life Sci*. 24: 1-10, 2013 [in press].

26. **Read NW, Houghton LA**. Physiology of gastric emptying and pathophysiology of gastroparesis. *Gastroenterol Clin North Am*. 18: 359-373, 1989.

27. **Reynell PC, Spray GH**. A technique for the simultaneous measurement of absorption and transit in the gastro-intestinal tract of the rat. *J Physiol (London)*. 131: 452-462, 1956.

28. **Roesch DM, Blackburn-Munro RE, Verbalis JG**. Mineralocorticoid treatment attenuates activation of oxytocinergic and vasopressinergic neurons by icv ANG II. *Am J Physiol.* 280: R1853-R864, 2001.

29. **Ruginsk SG, Oliveira FR, Margatho LO, Vivas L, Elias LL, Antunes-Rodrigues J.** Glucocorticoid modulation of neuronal activity and hormone secretion induced by blood volume expansion. *Exp Neurol*. 206: 192-200, 2007.

30. **Ruzicka M, Yuan B, Harmsen E, Leenen FH**. The renin-angiotensin system and volume overload-induced cardiac hypertrophy in rats: effects of angiotensin converting enzyme inhibitor versus angiotensin II receptor blocker. *Circulation*. 87: 921-930, 1993.

31. **Sallam HS, Oliveira HM, Gan HT, Herndon DN, Chen JD**. Ghrelin improves burn-induced delayed gastrointestinal transit in rats. *Am J Physiol* 292: R253-R257, 2007.

32. **Santos AA, Xavier-Neto J, Santiago Júnior AT, Souza MA, Martins AS, Alzamora F, Rola FH**. Acute volaemic changes modify the gastroduodenal resistance to the flow of saline in anaesthetized dogs. *Acta Physiol Scand*. 143: 261-269, 1991.

33. **Sjovall H, Brunsson I, Jodal M, Lundgren O**. The effect of vagal nerve stimulation on net fluid transport in the small intestine of the cat. *Acta Physiol Scand*. 117: 351-357, 1983.

34. **Souza MA, Souza MH, Palheta RC Jr, Cruz PR, Medeiros BA, Rola FH, Magalhães PJ, Troncon LE, Santos AA**. Evaluation of gastrointestinal motility in awake rats: a learning exercise for undergraduate biomedical students. *Adv Physiol Educ*. 33: 343-348, 2009.

35. **Spak E, Casselbrant A, Olbers T, Lönroth H, Fändriks L**. Angiotensin II-induced contractions in human jejunal wall musculature *in vitro*. *Acta Physiol (Oxf).* 193: 181-190, 2008.

36. **Takada SH, Sampaio CA, Allemandi W, Ito PH, Takase LF, Nogueira MI**. A modified rat model of neonatal anoxia: Development and evaluation by pulseoximetry, arterial gasometry and Fos immunoreactivity. *J Neurosci Methods*. 198 :62-69, 2011.

37. **Vauthey JN, Tomczak RJ, Helmberger T, Gertsch P, Forsmark C, Caridi J, Reed A, Langham MR Jr, Lauwers GY, Goffette P, Lerut J**. The arterioportal fistula syndrome: clinicopathologic features, diagnosis, and therapy. *Gastroenterology*. 113: 1390-401, 1997.

38. **Winaver J, Hoffman A, Burnett JC Jr, Haramati A**. Hormonal determinants of sodium excretion in rats with experimental high-output heart failure. *Am J Physiol*. 254: R776-R784, 1988.

39. **Wu CL, Hung CR, Chang FY, Pau KY, Wang JL, Wang PS**. Involvement of cholecystokinin receptor in the inhibition of gastric emptying by oxytocin in male rats. *Pflugers Arch*. 445: 187-193, 2002.

40. **Xavier-Neto J, dos Santos AA, Rola FH**. Acute hypervolaemia increases gastroduodenal resistance to the flow of liquid in the rat. *Gut*. 31: 1006-1010, 1990.

41. **Xu DY, Liu L, Cai YL, Li XL, Qiu ZX, Jin Z, Xu WX.** Natriuretic peptide-dependent cGMP signal pathway potentiated the relaxation of gastric smooth muscle in streptozotocin-induced diabetic rats. *Dig Dis Sci*. 55: 589-595, 2010.

493 Figures

494 Fig. 1. Effects of aortocaval fistula (ACF) and its respective sham surgery (control, □) on basal
495 hemodynamic values in awake rats. The fistula was created by vascular puncture with a 26-
496 gauge (26G, ■), 23-gauge (23G, ▣), or 21-gauge (21G, ■) needle. Twenty-four hours after
497 surgery, the animals were subjected to continuous hemodynamic monitoring for 40 min. (a)
498 Mean arterial pressure (MAP, in mmHg). (b) Mean central venous pressure (CVP, in cmH_2O). (c)
499 Cardiac output (CO, in $mL.min^{-1}$). (d) Heart rate (HR, in $beats.min^{-1}$). (e) Systemic vascular
500 resistance (SVR, in $dynes.s.cm^{-5}$). (f) Systolic volume (SV, in $mL.beats^{-1}$). Each subgroup
501 consisted of 6-9 rats. The data (mean ± SEM) are expressed as bars and vertical lines. *$p < 0.05$,
502 *vs.* their respective controls and between ACF-groups values (ANOVA followed by Tukey's test).
503
504 Fig. 2. Comparison of basal hemodynamic values between rats previously subjected to
505 aortocaval fistula (ACF group, black bars) or not (sham or control group, white bars). The fistula
506 was created by vascular puncture with a 21-gauge needle. Twelve, 24, or 48 h after surgery, the
507 rats were subjected to continuous hemodynamic monitoring for 40 min. (A) Mean arterial
508 pressure (MAP, in mmHg). (B) Mean central venous pressure (CVP, in cmH_2O). (C) Cardiac
509 output (CO, in $mL.min^{-1}$). (D) Heart rate (HR, in $beats.min^{-1}$). (E) Systemic vascular resistance
510 (SVR, in $dynes.s.cm^{-5}$). (F) Systolic volume (SV, in $mL.beats^{-1}$). Each subgroup consisted of 6-9
511 rats. The data (mean ± SEM) are expressed as bars and vertical lines. *$p < 0.05$, *vs*. respective
512 sham (unpaired Student's *t* test).
513
514 Fig. 3. Effects of aortocaval fistula (ACF) on fractional gastric retention of a liquid meal in awake
515 rats. The fistula was created via vascular puncture with a 21-gauge needle. After a 40 min
516 interval of basal hemodynamic monitoring, the rats were gavage-fed (1.5 mL) the test meal
517 (phenol red in glucose solution) and sacrificed 10, 20, or 30 min later to determine gastric dye

recovery by spectrophotometry. (A) Gastric dye recovery 20 min after feeding. The studies were performed 12, 24, or 48 h after surgery. (B) Gastric dye recovery 10, 20, or 30 min after feeding. The studies were performed 24 h after surgery. The data are expressed as mean GR values ± SEM (○, controls; ●, ACF rats). Each subgroup consisted of 6-9 rats. *$p < 0.05$, *vs*. respective sham subgroup (unpaired Student's *t* test).

Fig. 4. Correlation between cardiac output values ($mL.min^{-1}$) and respective fractional gastric dye recovery values in awake rats previously subjected to aortocaval fistula (black, ACF group; white, sham or control group). The fistula was created by a vascular puncture with a 21-gauge needle. Twenty-hours after surgery or sham-operation, the rats were gavage-fed (1.5 mL) the test meal (phenol red in glucose solution) and sacrificed 20 min later to determine gastric dye recovery by spectrophotometry. Each data-point indicates one individual. A strong ($r = 0.952$) and significant ($p < 0.001$) positive correlation was found between gastric retention and cardiac output values in ACF rats.

Fig. 5. Effect of bleeding on aortocaval fistula (ACF)-induced gastric emptying delay of liquid in awake rats. After laparotomy, the rats were randomly subjected (black bars) or not (i.e., sham-operation; white bars) to ACF placement by vascular puncture with a 21-gauge needle and studied 24 h later. After a 40 min interval of basal hemodynamic monitoring, both the ACF and sham-operation rats were bled ($15 mL.kg^{-1}$) and gavage-fed (1.5 mL) with the test meal (phenol red in glucose solution). After 20 min later they were sacrificed to determine the gastric dye recovery by spectrophotometry. The data (mean ± SEM) are expressed as bars and vertical lines. Each subgroup consisted of 6-9 rats. *, $p < 0.05$, *vs*. respective sham subgroup and $^{\#}p, <$ 0.05, ACF rats *vs*. ACF + bleeding (unpaired Student's *t* test).

Fig. 6. Effects of neuroautonomic extrinsic denervation on aortocaval fistula (ACF)-induced gastric emptying delay of liquid in awake rats. After laparotomy, the rats were randomly subjected (black bars) or not (i.e., sham-operation; white bars) to ACF placement by vascular puncture with a 21-gauge needle and studied 24 h later. After a 40 min interval of basal hemodynamic monitoring, both the ACF and sham-operation rats were gavage-fed (1.5 mL) the test meal (phenol red in glucose solution) and sacrificed 20 min later to determine gastric dye recovery spectrophotometrically. (A) Rats that underwent laparotomy and subjected or not (control) 5 days earlier to coeliac ganglionectomy + splanchnicectomy. (B) Rats that underwent laparotomy and subjected or not (control) 5 days earlier to bilateral subdiaphragmatic vagotomy. (C) Rats that underwent laparotomy and subjected or not (control) to pretreatment (30-min earlier) with hexamethonium. (D) Rats that underwent laparotomy and subjected or not (control) to pretreatment (30-min earlier) with pirenzepine. Each subgroup consisted of 6-9 rats. The data (mean $\pm$ SEM) are expressed as bars and vertical lines. *, $p < 0.05$, *vs.* respective sham-operated rats. $^{\#}$, $p < 0.05$, subdiaphragmatic vagotomy – ACF *vs.* ACF rats. $^{+}$, $p < 0.05$, hexamethonium + sham-operated and pirenzepine + sham-operated *vs.* sham-operated (unpaired Student's *t*-test).

Fig. 7. Comparison between plasma levels of (A) atrial natriuretic peptide (ANP), (B) angiotensin II (ANG II), and (C) corticosterone in rats subjected to aortocaval fistula and sham-operated rats. The data are expressed as scatter plots (mean values). Each data-point indicates one individual. *, $p < 0.05$, **, $p < 0.01$, *vs.* respective sham group (unpaired Student's *t*-test).

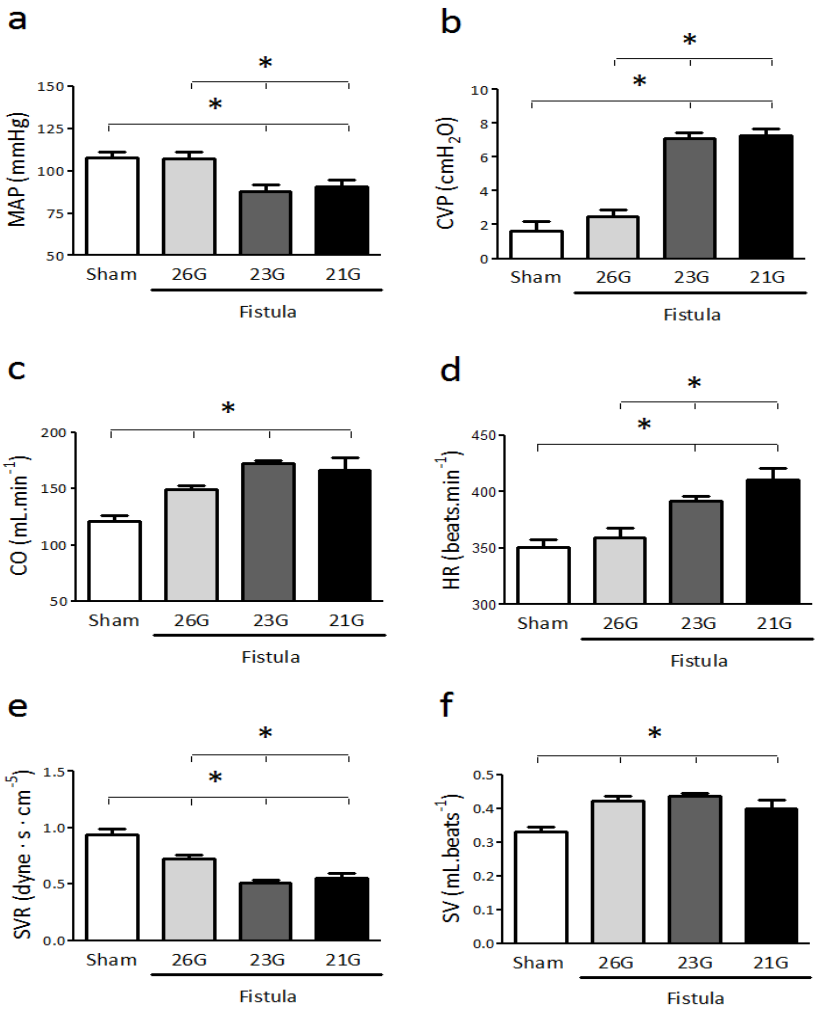

Fig. 1. Effects of aortocaval fistula (ACF) and its respective sham surgery (control, ☐) on basal hemodynamic values in awake rats. The fistula was created by vascular puncture with a 26-gauge (26G, ■), 23-gauge (23G, ▣), or 21-gauge (21G, ■) needle. Twenty-four hours after surgery, the animals were subjected to continuous hemodynamic monitoring for 40 min. (a) Mean arterial pressure (MAP, in mmHg). (b) Mean central venous pressure (CVP, in cmH_2O). (c) Cardiac output (CO, in $mL.min^{-1}$). (d) Heart rate (HR, in $beats.min^{-1}$). (e) Systemic vascular resistance (SVR, in $dynes.s.cm^{-5}$). (f) Systolic volume (SV, in $mL.beats^{-1}$). Each subgroup consisted of 6-9 rats. The data (mean ± SEM) are expressed as bars and vertical lines. *$p < 0.05$, *vs.* their respective controls and between ACF-groups values (ANOVA followed by Tukey's test).

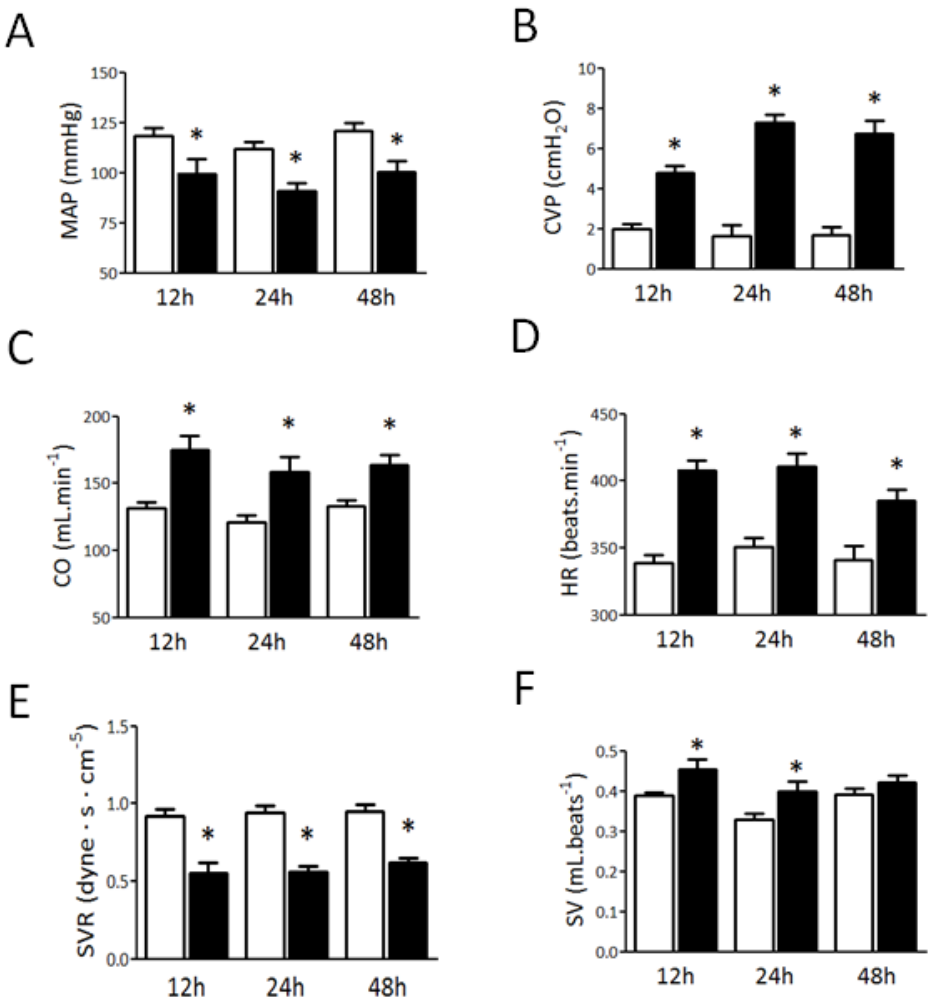

Fig. 2. Comparison of basal hemodynamic values between rats previously subjected to aortocaval fistula (ACF group, black bars) or not (sham or control group, white bars). The fistula was created by vascular puncture with a 21-gauge needle. Twelve, 24, or 48 h after surgery, the rats were subjected to continuous hemodynamic monitoring for 40 min. (A) Mean arterial pressure (MAP, in mmHg). (B) Mean central venous pressure (CVP, in cmH_2O). (C) Cardiac output (CO, in $mL.min^{-1}$). (D) Heart rate (HR, in $beats.min^{-1}$). (E) Systemic vascular resistance (SVR, in $dynes.s.cm^{-5}$). (F) Systolic volume (SV, in $mL.beats^{-1}$). Each subgroup consisted of 6-9 rats. The data (mean ± SEM) are expressed as bars and vertical lines. *$p < 0.05$, *vs*. respective sham (unpaired Student's *t* test).

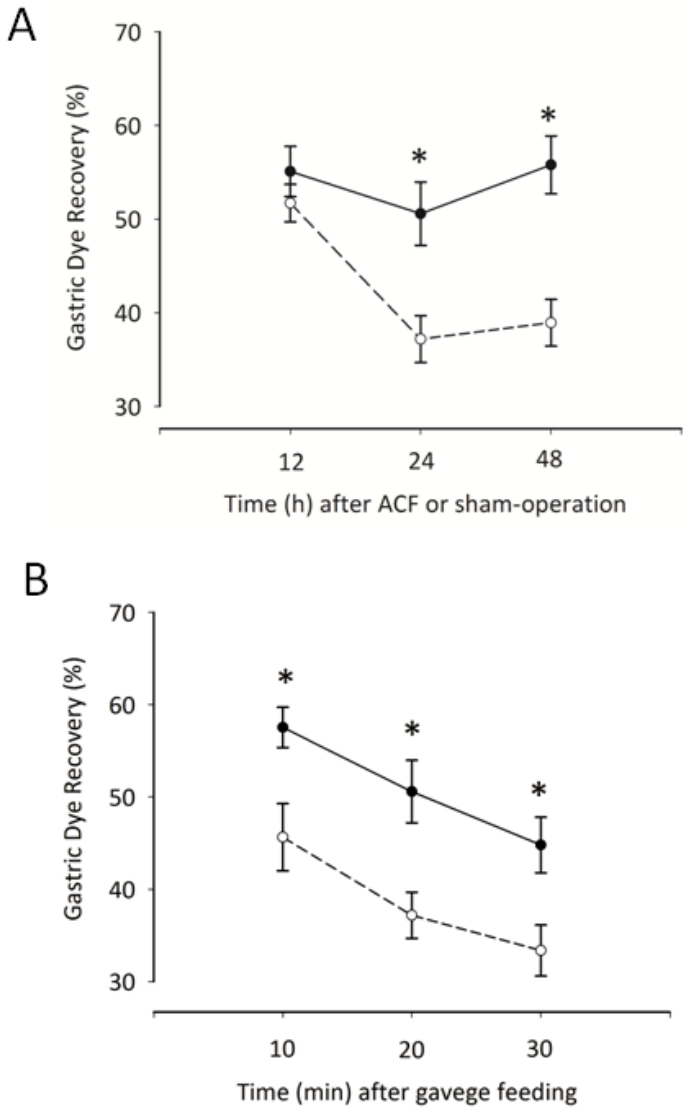

Fig. 3. Effects of aortocaval fistula (ACF) on fractional gastric retention of a liquid meal in awake rats. The fistula was created via vascular puncture with a 21-gauge needle. After a 40 min interval of basal hemodynamic monitoring, the rats were gavage-fed (1.5 mL) the test meal (phenol red in glucose solution) and sacrificed 10, 20, or 30 min later to determine gastric dye recovery by spectrophotometry. (A) Gastric dye recovery 20 min after feeding. The studies were performed 12, 24, or 48 h after surgery. (B) Gastric dye recovery 10, 20, or 30 min after feeding. The studies were performed 24 h after surgery. The data are expressed as mean GR values ± SEM (○, controls; ●, ACF rats). Each subgroup consisted of 6-9 rats. *$p < 0.05$, *vs.* respective sham subgroup (unpaired Student's *t* test).

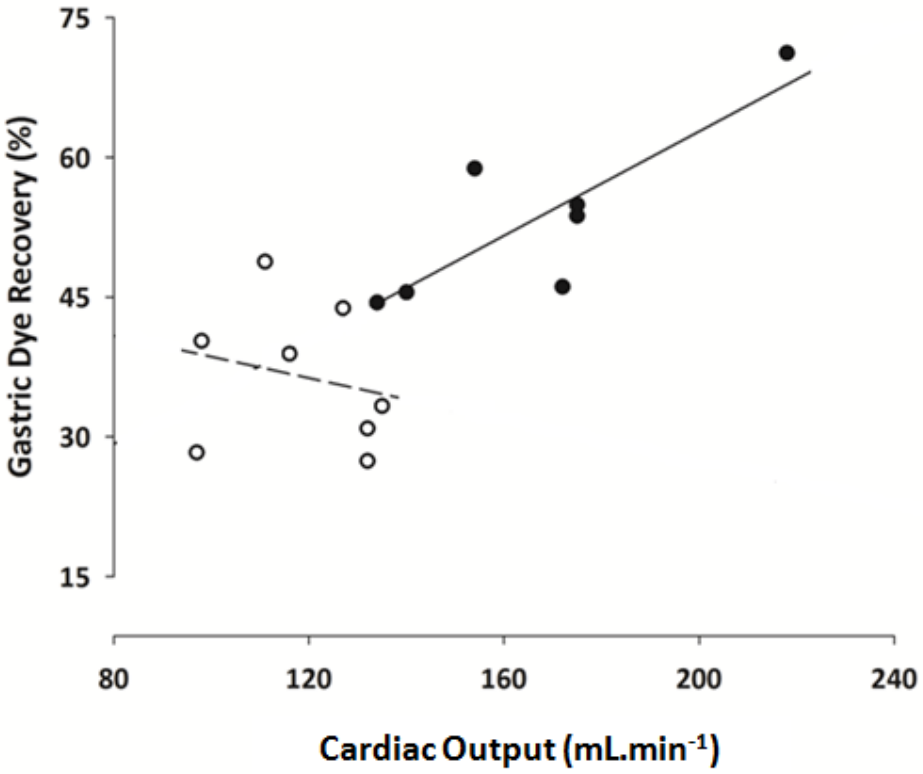

Fig. 4. Correlation between cardiac output values (mL.min^{-1}) and respective fractional gastric dye recovery values in awake rats previously subjected to aortocaval fistula (black, ACF group; white, sham or control group). The fistula was created by a vascular puncture with a 21-gauge needle. Twenty-hours after surgery or sham-operation, the rats were gavage-fed (1.5 mL) the test meal (phenol red in glucose solution) and sacrificed 20 min later to determine gastric dye recovery by spectrophotometry. Each data-point indicates one individual. A strong (r = 0.952) and significant ($p < 0.001$) positive correlation was found between gastric retention and cardiac output values in ACF rats.

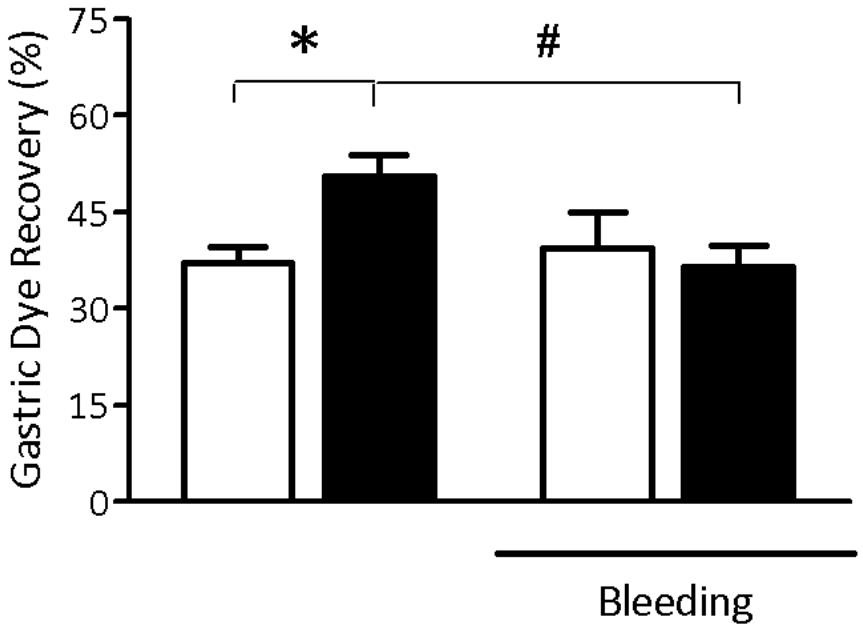

Fig. 5. Effect of bleeding on aortocaval fistula (ACF)-induced gastric emptying delay of liquid in awake rats. After laparotomy, the rats were randomly subjected (black bars) or not (i.e., sham-operation; white bars) to ACF placement by vascular puncture with a 21-gauge needle and studied 24 h later. After a 40 min interval of basal hemodynamic monitoring, both the ACF and sham-operation rats were bled (15 $mL.kg^{-1}$) and gavage-fed (1.5 mL) with the test meal (phenol red in glucose solution). After 20 min later they were sacrificed to determine the gastric dye recovery by spectrophotometry. The data (mean $\pm$ SEM) are expressed as bars and vertical lines. Each subgroup consisted of 6-9 rats. *, $p < 0.05$, *vs*. respective sham subgroup and $^{\#}$ p, $<$ 0.05, ACF rats *vs*. ACF + bleeding (unpaired Student's *t* test).

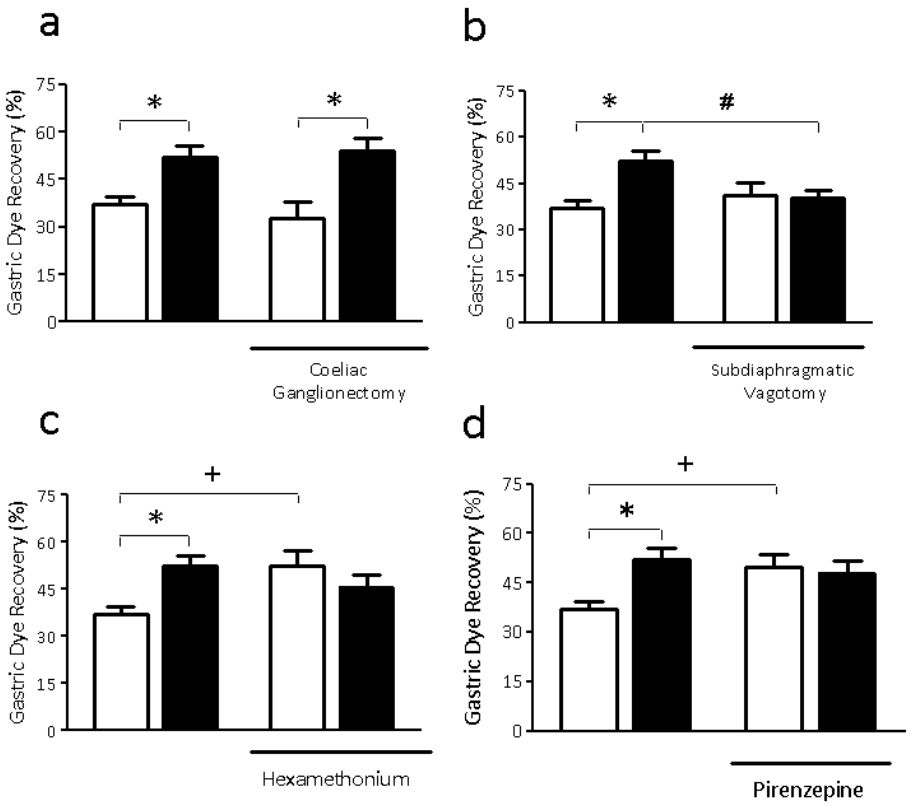

Fig. 6. Effects of neuroautonomic extrinsic denervation on aortocaval fistula (ACF)-induced gastric emptying delay of liquid in awake rats. After laparotomy, the rats were randomly subjected (black bars) or not (i.e., sham-operation; white bars) to ACF placement by vascular puncture with a 21-gauge needle and studied 24 h later. After a 40 min interval of basal hemodynamic monitoring, both the ACF and sham-operation rats were gavage-fed (1.5 mL) the test meal (phenol red in glucose solution) and sacrificed 20 min later to determine gastric dye recovery spectrophotometrically. (A) Rats that underwent laparotomy and subjected or not (control) 5 days earlier to coeliac ganglionectomy + splanchnicectomy. (B) Rats that underwent laparotomy and subjected or not (control) 5 days earlier to bilateral subdiaphragmatic vagotomy. (C) Rats that underwent laparotomy and subjected or not (control) to pretreatment (30-min earlier) with hexamethonium. (D) Rats that underwent laparotomy and subjected or not (control) to pretreatment (30-min earlier) with pirenzepine. Each subgroup consisted of 6-9 rats. The data (mean ± SEM) are expressed as bars and vertical lines. *, $p < 0.05$, *vs.* respective sham-operated rats. $^{\#}$, $p < 0.05$, subdiaphragmatic vagotomy – ACF *vs.* ACF rats. $^{+}$, $p < 0.05$, hexamethonium + sham-operated and pirenzepine + sham-operated *vs.* sham-operated (unpaired Student's *t*-test).

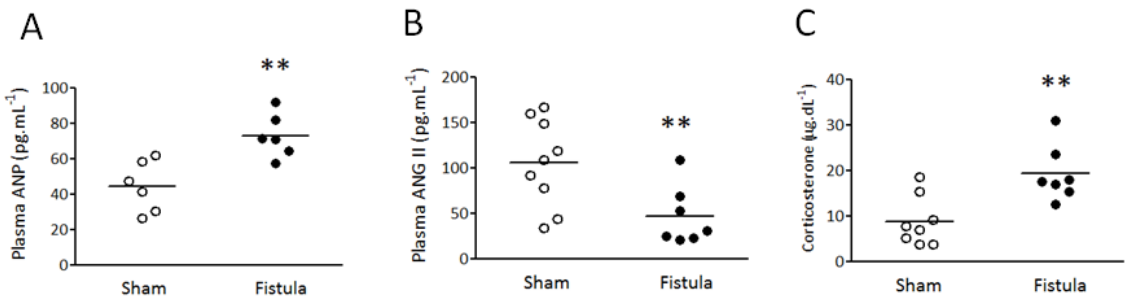

Fig. 7. Comparison between plasma levels of (A) atrial natriuretic peptide (ANP), (B) angiotensin II (ANG II), and (C) corticosterone in rats subjected to aortocaval fistula and sham-operated rats. The data are expressed as scatter plots (mean values). Each data-point indicates one individual. *, $p < 0.05$, **, $p < 0.01$, *vs.* respective sham group (unpaired Student's *t*-test).

"AORTOCAVAL FISTULA DELAYS GASTRIC EMPTYING OF LIQUID TEST MEAL IN AWAKE RATS"

Supplementary Material H-00827-2012

Table 01. Arterial gasometrical analysis obtained from awake rats previously submitted to sham surgery or aortocaval fistula (ACF) placement. Values are expressed as mean±SEM.

	Sham (n=8)	ACF (n=8)
pH	7.39±0.02	7.38±0.01@
Base Excess ($mmol.L^{-1}$)	1.22±0.57	2.00±0.71@
[HCO_3^-] ($mmol.L^{-1}$)	25.43±0.55	26.23±0.65@
PCO_2 (mmHg)	45.45±2.71	48.03±3.12@
PO_2 (mmHg)	62.61±4.95	61.31±4.86@
$SatO_2$ (%)	77.10±3.62	78.66±2.81@
Hct (%)	37.93±0.55	40.28±1.08@

@, $P > 0.05$ after unpaired Student's "t" test.