

Acute vagal stimulation attenuates cardiac metabolic response to β -adrenergic stress

Claudio Vimercati¹, Khaled Qanud¹, Itamar Ilsar², Gianfranco Mitacchione¹, Roberto Sarnari¹, Daniella Mania¹, Ryan Faulk¹, William C. Stanley³, Hani N. Sabbah⁴ and Fabio A. Recchia^{1,5}

¹Department of Physiology, New York Medical College, Valhalla, NY, USA

²BioControl Medical Ltd, Yehud, Israel

³University of Maryland School of Medicine, Baltimore, MD, USA

⁴Henry Ford Hospital, Detroit, MI, USA

⁵Gruppo Intini-SMA Laboratory of Experimental Cardiology, Institute of Life Sciences, Scuola Superiore Sant'Anna, Pisa, Italy

Key points

- Whereas the effects of catecholamines on myocardial metabolism are well characterized, the potential role of the parasympathetic system is generally considered minor or absent.
- We tested the hypothesis that acute stimulation of the right vagus nerve alters the balance between cardiac free fatty acid and carbohydrate oxidation and opposes the metabolic effects of beta-adrenergic stimulation.
- Using a clinical-type selective stimulator of the vagal efferent fibers in dogs, we found that vagal stimulation did not significantly affect baseline cardiac performance, haemodynamics and myocardial metabolism.
- During dobutamine stress, vagal stimulation attenuated the increase in left ventricular mechanical performance, cardiac oxygen consumption and myocardial glucose oxidation, while free fatty acid oxidation was affected only at low catecholamine dose.
- Our results elucidate a previously unexplored parasympathetic function, indicating that selective vagal efferent stimulation antagonizes the effects of beta-adrenergic activation on myocardial metabolism.

Abstract The effects of vagal stimulation (VS) on cardiac energy substrate metabolism are unknown. We tested the hypothesis that acute VS alters the balance between free fatty acid (FFA) and carbohydrate oxidation and opposes the metabolic effects of β -adrenergic stimulation. A clinical-type selective stimulator of the vagal efferent fibres was connected to the intact right vagus in chronically instrumented dogs. VS was set to reduce heart rate by 30 beats min^{-1} , and the confounding effects of bradycardia were then eliminated by pacing the heart at 165 beats min^{-1} . [³H]Oleate and [¹⁴C]glucose were infused to measure FFA and glucose oxidation. The heart was subjected to β -adrenergic stress by infusing dobutamine at 5, 10 and 15 $\mu\text{g kg}^{-1} \text{min}^{-1}$ before and during VS. VS did not significantly affect baseline cardiac performance, haemodynamics or myocardial metabolism. However, at peak dobutamine stress, VS attenuated the increase in left ventricular pressure–diameter area from 235.9 ± 72.8 to $167.3 \pm 55.8\%$, and in cardiac oxygen consumption from 173.9 ± 23.3 to $127.89 \pm 6.2\%$ (both $P < 0.05$), and thus mechanical efficiency was not enhanced. The increase in glucose oxidation fell from 289.3 ± 55.5 to $131.1 \pm 20.9\%$ ($P < 0.05$), while FFA oxidation was not increased by β -adrenergic stress and fell below baseline during VS only at the lowest dose of dobutamine. The functional and in part the metabolic changes were reversed by 0.1 mg kg^{-1} atropine i.v. Our data show that acute right VS does not affect baseline cardiac metabolism, but attenuates myocardial oxygen consumption and glucose

H. N. Sabbah and F. A. Recchia contributed equally to the work.

oxidation in response to adrenergic stress, thus functioning as a cardio-selective antagonist to β -adrenergic activation.

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Corresponding author F. A. Recchia: Department of Physiology, Temple University School of Medicine, Philadelphia, PA 19140, USA. Email: fabio.recchia@temple.edu

Abbreviations FFA, free fatty acid; LV, left ventricle/left ventricular; PDA, pressure–diameter area; VS, vagal stimulation.

Introduction

Cardiac metabolism is finely controlled by a complex network of intracellular feedback mechanisms and neuro-hormonal signals (Drake-Holland, 1983; Stanley *et al.* 2005). Among the extracellular signals, catecholamines released by sympathetic nerve endings exert a profound effect on myocardial energy turnover. Elegant quantitative studies performed in isolated heart preparations with controlled substrate delivery have shown that acute increases in contractile performance induced by β -adrenergic agonists stimulate myocardial glucose utilization to meet the higher metabolic demand (Goodwin *et al.* 1998; Doenst & Taegtmeier, 1999). Because sympathetic system activation notoriously causes cardiac oxygen wasting (Ohgoshi *et al.* 1991), the selective reliance on glucose and not on the less efficient substrate free fatty acids (FFAs) (Korvald *et al.* 2000) during adrenergic stress might serve to limit oxygen consumption, while maintaining an adequate mechanical response. Conversely, carbohydrate oxidation is impaired in the chronically denervated heart, probably due to a fall in active pyruvate dehydrogenase (Van Der Vusse *et al.* 1998).

Whereas the effects of catecholamines on myocardial metabolism are well characterized, the potential role of the parasympathetic system is generally considered minor or absent, despite the fact that muscarinic receptors in the heart are more abundant than β -adrenoreceptors (Böhm *et al.* 1990). Nonetheless, classic *in vivo* studies found that electrical stimulation of the vagal efferents can reduce cardiac contractility (DeGeest *et al.* 1964) mainly by inhibiting noradrenaline release at presynaptic level (Daggett *et al.* 1967; Henning *et al.* 1990; Xenopoulos & Applegate, 1994) and can oppose the inotropic action of dobutamine (Hare *et al.* 1995). This negative inotropic action is mediated by nitric oxide (Hare *et al.* 1995), a molecule that is also implicated in the control of energy substrate utilization (Young & Leighton, 1998; Recchia *et al.* 2002; Lei *et al.* 2005), yet virtually no information is available on the effects of vagal output on the balance between FFA and carbohydrate oxidation, which is an important determinant of chemical conversion efficiency (Lopaschuk *et al.* 2010). A few studies have assessed myocardial oxygen consumption during vagal electrical stimulation in resting hearts and reported no

significant changes (Daggett *et al.* 1967; Downing *et al.* 1977). However, the effects of vagal stimulation (VS) on oxygen use and energy substrate metabolism during concurrent β -adrenergic receptor stimulation remain unexplored.

Interest in cardiac parasympathetic innervation has recently been revitalized by experimental and clinical evidence of a beneficial effect of cervical right VS on failing hearts (Li *et al.* 2004; Zhang *et al.* 2009; De Ferrari *et al.* 2011). This approach is very appealing, as implantable vagal stimulators allow a selective therapeutic action on the target organ, thus avoiding systemic and untoward effects of pharmacological agents. The underlying mechanisms are likely to be numerous, but they remain largely unknown. It will be important first to characterize in more detail the consequences of super-physiological firing of vagal efferents on normal heart in the intact organism. In fact, one important limitation of previous studies was their invasive approach based on vagus severing and/or open chest preparations. In the present study, we used an electrical stimulator designed to generate unidirectional impulses along the efferent fibres of the intact nerve, which allowed us to test the effects of acute right VS on cardiac performance and energy substrate metabolism in dogs with chronically implanted probes and catheters. Our hypothesis was that vagal activation alters the balance between FFA and carbohydrate oxidation and opposes the metabolic effects of β -adrenergic stimulation, i.e. attenuates the oxygen demand, while preserving and possibly enhancing mechanical efficiency.

Methods

Surgical instrumentation

The surgical and experimental protocols were approved by the Institutional Animal Care and Use Committee of the New York Medical College and conform to the *Guide for the Care and Use of Laboratory Animals* published by the National Institutes of Health.

Fifteen adult male mongrel dogs (body weight 23–27 kg, purchased from Hodgins Kennels, Inc., Howell, MI, USA) were sedated with acepromazine maleate (1 mg kg⁻¹ i.m.), anaesthetized with propofol (4 mg kg⁻¹ i.v.) and ventilated

with room air. An adequate level of gas anaesthesia was maintained by 1.5% isoflurane and monitored by checking somatic reflexes (corneal and toe pinch) and visceral reflexes [unexpected changes in heart rate (HR) and blood pressure], while arterial oxygen content was measured by pulse oximetry. The entire surgical procedure was carried out under aseptic conditions. A thoracotomy was performed in the left fifth intercostal space. One catheter was placed in the descending thoracic aorta, and a second catheter was placed in the coronary sinus with the tip leading away from the right atrium. A solid-state pressure gauge (P6.5; Konigsberg Instruments, Pasadena, CA, USA) was inserted into the left ventricle (LV) through the apex. A Doppler flow transducer (Craig Hartley, Houston, TX, USA) was placed around the left circumflex coronary artery, and a pair of 3 MHz piezoelectric crystals was fixed on opposing endocardial surfaces at the base of the LV. A human, screw-type, unipolar myocardial pacing lead was fixed in the LV wall. Wires and catheters were run subcutaneously to the intrascapular region, the chest was closed in layers and the pneumothorax was reduced. After surgery, dogs underwent analgesic therapy for 3 days (fentanyl, $75 \mu\text{g kg}^{-1}$ intradermal) and antibiotic therapy for 10 days (amoxicillin, $16 \text{ mg kg}^{-1} \text{ day}^{-1}$ I.M.). They responded very well to this therapeutic regimen, and therefore post-surgical fever and weight loss were only transient. Ten days was sufficient for full recovery, which was indicated by normal ambulation, appetite, and faecal and urine output. Once this phase was completed, the dogs were trained to lie quietly on the laboratory table. Rectal temperature was monitored daily for the entire duration of the protocol and, if found to be higher than 39°C , amoxicillin (16 mg kg^{-1}) or cefazolin (40 mg kg^{-1}) was given I.M. We have used these methods in previous studies (Recchia *et al.* 2002; Osorio *et al.* 2002).

Protocol

The experiments were conducted in 11 dogs lightly anaesthetized with propofol (rapid bolus of 4 mg kg^{-1} I.V., followed by $0.2 \text{ mg kg}^{-1} \text{ min}^{-1}$ I.V.) and in spontaneous respiration. Immediately after the propofol bolus, when the level of anaesthesia was deeper, a neck area along

the right cervical line was infiltrated with 2% lidocaine. A 3 cm longitudinal cervical incision was then made and the right carotid sheath and right carotid nerve were rapidly exposed. The vagal nerve stimulator lead (CardioFit Stimulation Lead; BioControl Medical Ltd, Yehud, Israel) was then placed around the vagus nerve and secured by tightening the pre-existing tightening strings. The lead was then connected to the stimulator (CardioFit-X external stimulator, Model 5510; BioControl Medical Ltd), and the wound was closed around the lead using 2–0 silk suture. This procedure typically lasted 5 min. The experimental protocol is schematically depicted in Fig. 1. The isotopic tracers [$9,10\text{-}^3\text{H}$]oleate and [$\text{U-}^{14}\text{C}$]glucose were continuously infused for the duration of the experiment through a peripheral vein following a protocol previously established by us (Recchia *et al.* 2002). A 6-French, custom-made balloon catheter was introduced into a peripheral vein of a posterior leg and advanced to the inferior vena cava. After recording of systemic haemodynamics, coronary blood flow and LV diameter at spontaneous HR, occlusions of the inferior vena cava, separated by 5 min intervals, were performed by inflating the balloon to obtain end-systolic pressure–diameter curves. Once the baseline haemodynamics were re-established, the heart was paced at $165 \text{ beats min}^{-1}$ via the LV leads, haemodynamics were recorded, and paired blood samples were taken from the aortic and the coronary sinus catheters. Cardiac pacing was used to eliminate the confounding effect of bradycardia on myocardial metabolism that could have affected the comparison between measurements obtained before and after VS. The heart was then subjected to β -adrenergic stress with dobutamine at 5, 10 and $15 \mu\text{g kg}^{-1} \text{ min}^{-1}$ I.V. Haemodynamics were recorded and paired blood samples were taken from aorta and coronary sinus after each dose of dobutamine, once the effects had reached steady state (usually 5 min). Dobutamine infusion and pacing were stopped to re-establish baseline conditions (re-baseline) and record haemodynamics and pressure–diameter curves with vena cava occlusion. After completion of this step, the vagal stimulator was activated to reduce the spontaneous HR by $30 \text{ beats min}^{-1}$. VS was delivered in asynchronous mode, using an asymmetric,

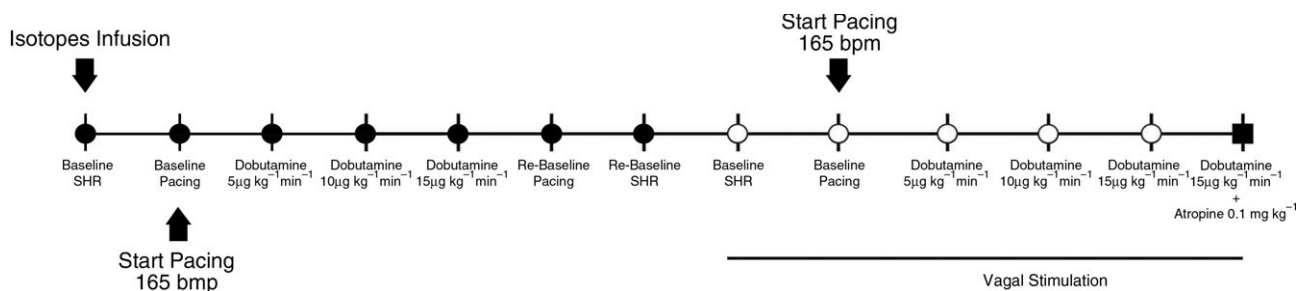


Figure 1. Experimental protocol

biphasic, charge-balanced waveform, with a pulse width of 0.5 ms. In each animal, several current settings and number of pulses were first tested for a few seconds to find the best combination for the desired HR reduction. The average current used was 4.8 mA, with an average of 2.1 pulses per stimulation cycle. Haemodynamics and pressure–diameter curves were recorded again, cardiac pacing was then restarted and the heart was again subjected to β -adrenergic stress as described above. Finally, during infusion of the highest dose of dobutamine, 0.1 mg kg⁻¹ atropine was administered as a bolus i.v. and the last haemodynamics and blood samples were taken at the peak of haemodynamic changes, which occurred after ~1 min. Beyond that peak, haemodynamics became very unstable, and the experiment was considered concluded.

To rule out possible tachyphylaxis due to repeated dobutamine infusions, four additional dogs were subjected to the same protocol described above: the first series of infusions was followed by re-baseline and then by the second series of infusions, but in the absence of VS.

At the end of the protocol, the dogs were killed via an overdose of sodium pentobarbitone (100 mg kg⁻¹ i.v.)

Haemodynamic and mechanoenergetic measurements

The aortic catheter was attached to a P23ID strain-gauge transducer for measurement of aortic pressure. LV pressure was measured using the solid-state pressure gauge. Blood flow in the left circumflex coronary artery was measured with a pulsed Doppler flowmeter (model 100; Triton Technology, San Diego, CA, USA). LV diameter was measured by connecting the implanted piezoelectric crystals to an ultrasonic dimension gauge. All signals were also digitally stored on an AT-type computer via an analog–digital interface (National Instruments, Austin, TX, USA) at a sampling rate of 250 Hz. Digitized data were analysed off-line by custom-made software. The parameters were determined during one respiratory cycle and comprised HR, mean aortic pressure, LV end-diastolic, peak systolic and end-systolic pressure, mean blood flow in the left circumflex coronary artery, the maximum and minimum of the first derivative of LV pressure, and end-diastolic and end-systolic LV internal diameters. The percentage shortening of the short axis diameter was calculated as the ratio of the difference between end-diastolic and end-systolic diameter (stroke dimension) to end-diastolic diameter (Saavedra *et al.* 2002; Post *et al.* 2003). The slope of the LV end-systolic pressure–diameter relationship (Ees) was calculated using the first 7–10 beats during inferior vena cava occlusion before any HR increase (Senzaki *et al.* 2000; Saavedra *et al.* 2002; Post *et al.* 2003). Effective arterial elastance

(Ea), an index of LV afterload (Senzaki *et al.* 2000; Post *et al.* 2003), was calculated as the ratio of LV end-systolic pressure to stroke dimensions. An index of stroke work was determined by calculating the area of the LV pressure–diameter loop (pressure–diameter area, PDA) during a cardiac cycle (Senzaki *et al.* 2000; Saavedra *et al.* 2002; Post *et al.* 2003). The difference between end-diastolic and end-systolic LV diameter was used as a surrogate of stroke volume and multiplied by HR to obtain an index of cardiac output.

Blood gases were measured using a blood gas analyser (Instrumentation Laboratory, Bedford, MA, USA) and oxygen concentration was measured using a haemoglobin analyser (CO-Oximeter; Instrumentation Laboratory). LV myocardial oxygen consumption ($M\dot{V}_{O_2}$) was calculated by multiplying the arterial–coronary sinus difference in oxygen content by total mean coronary blood flow, assumed to be double the mean blood flow in the left circumflex coronary artery (Recchia *et al.* 2002; Osorio *et al.* 2002).

Cardiac energy substrate metabolism

Total cardiac substrate concentrations were measured in arterial and coronary sinus blood samples, as previously described (Recchia *et al.* 2002). In brief, FFA concentration was determined spectrophotometrically in plasma. Glucose was measured in blood deproteinized with ice-cold 1 M perchloric acid (1:2 v/v) using spectrophotometric enzymatic assays. The concentrations of labelled metabolites were determined in arterial and coronary sinus blood samples. In particular, [³H]oleate activity was measured in plasma, whereas [¹⁴C]glucose activity was determined in blood deproteinized with ice-cold 1 M perchloric acid (1:2 v/v). ³H₂O and ¹⁴CO₂ activities were also measured in plasma and whole blood, respectively, and utilized to calculate the rate of FFA and glucose oxidation. Lactate concentrations were measured with the same cartridges utilized for blood gas analysis (Instrumentation Laboratory). The net chemical uptake of lactate by the heart was calculated based on arterial–coronary sinus difference and coronary blood flow and used as an index of oxidation, assuming that in the normo-oxygenated healthy heart, the fraction of lactate output remains constant.

Statistical analysis

All data are given as mean \pm SEM. Data were compared using one-way ANOVA for repeated measurements or by two-way ANOVA followed by Dunnett or Tukey tests (SigmaStat 2.03; Systat Software Inc., Chicago, IL, USA). For all statistical analyses, significance was accepted at $P < 0.05$.

Results

Haemodynamics

Changes in haemodynamic parameters are shown in Fig. 2. Cardiac pacing was set at a rate predicted to be higher than the tachycardia induced by dobutamine infusion, in order to maintain HR constant throughout the protocol (Fig. 2A). In fact, only at the highest dobutamine dose, and before VS, was HR slightly but significantly higher than the pacing rate. VS reduced the spontaneous HR by approximately 30 beats min^{-1} . Atropine caused an increase in HR that reached values significantly higher than the pacing rate. dP/dt_{max} , an index of contractility, increased significantly during dobutamine infusion, although VS significantly attenuated the contractile response to β -adrenergic stimulation (Fig. 2B). Atropine reversed the vagal effect. Similar responses before and after VS were observed for LV systolic pressure and mean coronary blood flow (Fig. 2C and D). Mean arterial pressure did not change significantly after VS and during dobutamine infusion (Table 1). Ea, an index of

LV afterload, decreased significantly at the highest dose of dobutamine, but not during VS. In four additional dogs utilized to rule out the effect of dobutamine tachyphylaxis, we found the following changes in dP/dt_{max} , i.e. the parameter more characteristically affected by β -adrenoceptor stimulation. Considering for simplicity only baseline values and peak changes at $15 \mu\text{g kg}^{-1} \text{min}^{-1}$ dobutamine, dP/dt_{max} was, respectively: 2324.37 ± 129.48 and $4488.75 \pm 575.62 \text{ mmHg s}^{-1}$ ($P < 0.05$) during the first series of infusions and 2343.12 ± 324.53 and $4137.50 \pm 486.01 \text{ mmHg s}^{-1}$ during the second series, with no significant differences between the two phases of the protocol. Also other haemodynamic parameters were not significantly different during the second series of infusion compared with the first (data not shown).

Cardiac contractile performance, $M\dot{V}\text{O}_2$ and mechanical efficiency

End-diastolic diameter did not change significantly under any of the experimental conditions (Table 1).

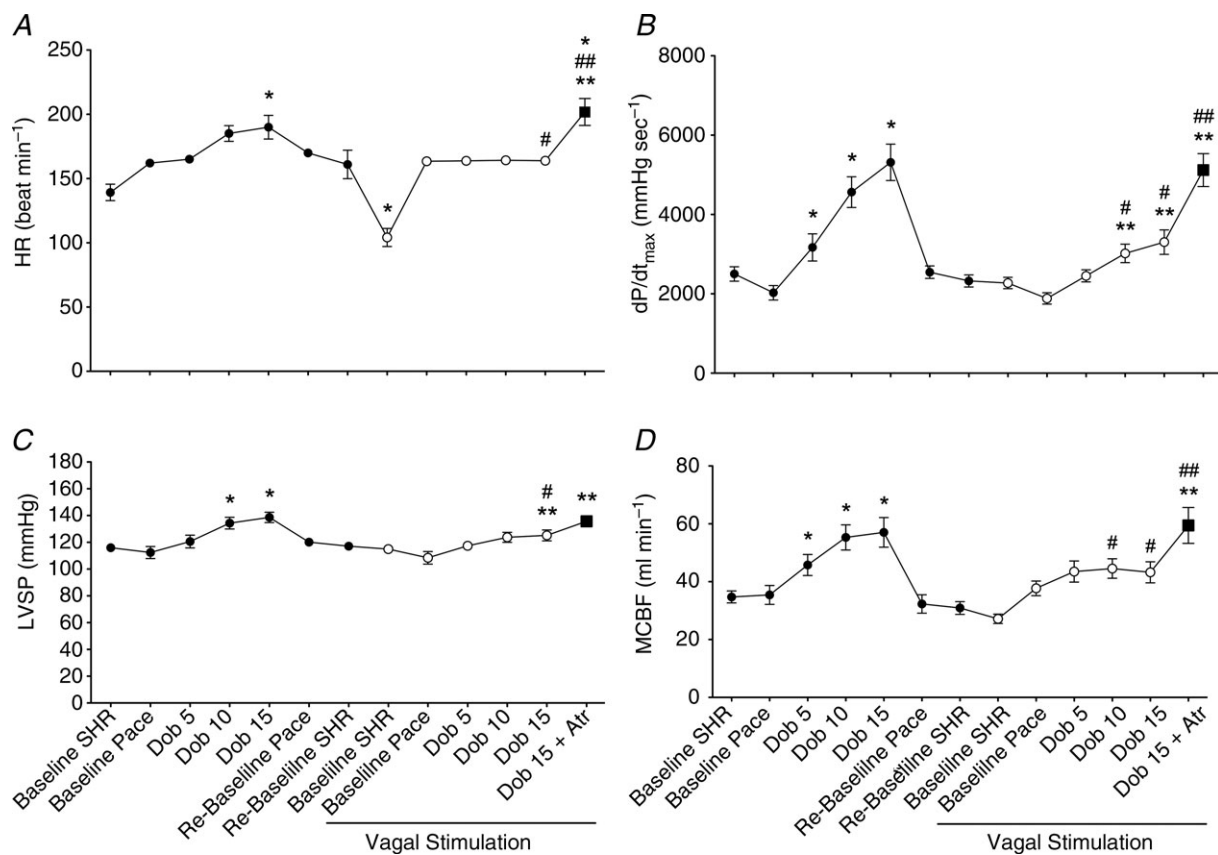


Figure 2. Changes in heart rate (HR), dP/dt_{max} , LV systolic pressure (LVSP) and mean coronary blood flow (MCBF) in the left circumflex coronary artery during dobutamine infusion, before (filled circles) and during (open circles) vagal stimulation

SHR, spontaneous heart rate; Pace, pacing at $165 \text{ beats min}^{-1}$; Dob, dobutamine; Atr, atropine. Data are presented as mean \pm SEM, $n = 11$. * $P < 0.05$ vs. pre-VS Baseline Pace; # $P < 0.05$ vs. corresponding time point pre-VS; ** $P < 0.05$ vs. post-VS Baseline Pace; ### $P < 0.05$ Dob 15+atropine vs. Dob 15 pre-VS.

Table 1. Changes in indexes of LV afterload and preload and in a calculated index of cardiac output with vagal stimulation (VS) on and off

	VS	Baseline	Dobutamine 5 $\mu\text{g kg min}^{-1}$	Dobutamine 10 $\mu\text{g kg min}^{-1}$	Dobutamine 15 $\mu\text{g kg min}^{-1}$	Re-baseline	Dobutamine 15+Atropine
Mean arterial pressure (mmHg)	OFF	95.5 \pm 4.0	96.9 \pm 4.1	104.6 \pm 3.3	101.9 \pm 2.6	101.3 \pm 2.6	
	ON	92.0 \pm 4.8	94.4 \pm 3.8	96.1 \pm 3.6	92.2 \pm 3.4		96.6 \pm 2.9
Ea (mmHg ml ⁻¹)	OFF	34.20 \pm 4.55	30.68 \pm 2.00	28.55 \pm 3.47	23.36 \pm 2.08	38.13 \pm 4.01	
	ON	40.96 \pm 4.69	34.38 \pm 3.11	32.64 \pm 3.05	32.45 \pm 4.31**		24.47 \pm 3.08**
End-diastolic diameter (mm)	OFF	42.62 \pm 0.73	42.33 \pm 0.73	42.89 \pm 0.91	43.75 \pm 1.01	42.57 \pm 0.87	
	ON	42.30 \pm 0.88	41.84 \pm 0.83	41.78 \pm 0.86	41.63 \pm 0.88		41.58 \pm 1.01
(EDD – ESD) \times HR (mm min ⁻¹)	OFF	451.45 \pm 47.91	576.43 \pm 61.22	766.20 \pm 60.43	794.40 \pm 52.35	491.08 \pm 36.35	
	ON	390.14 \pm 43.71	426.32 \pm 28.34	467.78 \pm 36.65**#	491.94 \pm 43.91**#		692.73 \pm 93.65**

Heart rate was fixed at 165 beats min⁻¹. Ea, arterial elastance; EDD, end-diastolic diameter; ESD, LV end-systolic diameter. Values are mean \pm SEM, $n = 11$. ** $P < 0.05$ vs. VS baseline; # $P < 0.05$ vs. corresponding time point pre-VS.

Table 2. Changes in oxygen content and energy substrate concentration in arterial blood with vagal stimulation (VS) on and off

	VS	Baseline	Dobutamine 5 $\mu\text{g kg min}^{-1}$	Dobutamine 10 $\mu\text{g kg min}^{-1}$	Dobutamine 15 $\mu\text{g kg min}^{-1}$	Re-baseline	Dobutamine 15+Atropine
Arterial O ₂ content (ml dl ⁻¹)	OFF	17.02 \pm 0.51	18.93 \pm 0.50	21.56 \pm 0.61	21.41 \pm 0.69	19.37 \pm 0.62	
	ON	16.25 \pm 1.06	18.39 \pm 1.49	19.46 \pm 1.16	19.97 \pm 1.05		19.25 \pm 1.15
Arterial free fatty acids (mmol l ⁻¹)	OFF	0.91 \pm 0.09	1.03 \pm 0.12	1.23 \pm 0.14	1.47 \pm 0.16*	1.18 \pm 0.14	
	ON	1.10 \pm 0.11	1.04 \pm 0.12	1.22 \pm 0.14	1.29 \pm 0.15		1.44 \pm 0.20
Arterial glucose (mmol l ⁻¹)	OFF	4.80 \pm 0.14	4.81 \pm 0.16	4.71 \pm 0.32	4.89 \pm 0.25	4.62 \pm 0.18	
	ON	5.13 \pm 0.25	5.41 \pm 0.24	5.34 \pm 0.26#	5.22 \pm 0.26		5.34 \pm 0.24
Arterial lactate (mmol l ⁻¹)	OFF	0.61 \pm 0.10	0.67 \pm 0.09	0.75 \pm 0.13	0.82 \pm 0.14	0.86 \pm 0.13	
	ON	0.83 \pm 0.14	0.79 \pm 0.13	0.88 \pm 0.14	0.93 \pm 0.14		0.98 \pm 0.14

Values are mean \pm SEM, $n = 8$. * $P < 0.05$ vs. pre-VS baseline; # $P < 0.05$ vs. corresponding time point pre-VS.

Nonetheless, LV stroke work, as measured by PDA, increased significantly during dobutamine infusion; however, VS significantly attenuated the contractile response to β -adrenergic stimulation, while atropine reversed the vagal effect (Fig. 3A), although this response displayed a high degree of variability. Similarly, the calculated index of cardiac output increased significantly during dobutamine infusion, but only before VS, while atropine reversed the depressant effects of vagus (Table 1). Ees, an afterload-independent index of contractility measured at spontaneous HR, was 31.8 ± 4.7 mmHg mm⁻¹ at baseline and 41.5 ± 4.9 mmHg mm⁻¹ (n.s.) during the post-dobutamine re-baseline, indicating that, even if the other functional parameters had returned to resting values, contractility was still recovering. After VS, Ees was 32.4 ± 7.7 mmHg mm⁻¹, not significantly different from the initial baseline.

Changes in $M\dot{V}_{O_2}$ are shown in Fig. 3B. As expected, $M\dot{V}_{O_2}$ beat⁻¹ increased significantly in response to adrenergic stress. VS did not affect $M\dot{V}_{O_2}$ beat⁻¹ at baseline, but significantly limited the enhanced oxygen consumption during dobutamine infusion, which was not significantly different from baseline, and atropine

could not reverse this effect. When compared side by side in terms of percentage changes, PDA and $M\dot{V}_{O_2}$ beat⁻¹ were similarly attenuated by VS during dobutamine stress, indicating that the mechanical efficiency, i.e. the ratio between external work and oxygen use, remained unchanged (Fig. 4). Interestingly, however, atropine caused a marked rebound of PDA but not of $M\dot{V}_{O_2}$ beat⁻¹, i.e. the heart was able to perform an external work comparable with the pre-VS dobutamine response, while maintaining low oxygen consumption, indicating an acute increase in mechanical efficiency.

Cardiac energy substrate utilization

Due to technical problems, a full analysis of cardiac substrate utilization could be obtained only in 8 of the 11 dogs. Figure 5 shows the changes in FFA, glucose and lactate utilization. During dobutamine stress, glucose oxidation and lactate uptake increased significantly to meet the higher metabolic demand, while FFA oxidation did not display significant changes. The post-dobutamine re-baseline was characterized by a fall in FFA oxidation below baseline levels. VS did not affect significantly glucose oxidation and lactate uptake in the resting heart, while FFA

oxidation returned to levels not significantly different from pre-VS baseline. During VS, β -adrenergic stress failed to increase significantly glucose oxidation and lactate uptake, which were indeed lower than control values. Interestingly, FFA oxidation fell significantly at the lowest dose of dobutamine ($5 \mu\text{g kg}^{-1} \text{min}^{-1}$), and then returned to baseline values. Finally, atropine could not re-establish pre-VS values of FFA and glucose oxidation and lactate uptake.

Cardiac energy substrate utilization can be markedly affected by circulating levels of these metabolites. The only small but statistically significant changes were found before VS: an increase in FFA concentration at the highest dobutamine dose and a decrease in glucose concentration at the intermediate dobutamine dose (Table 2). Atropine did not affect the concentration of these substrates.

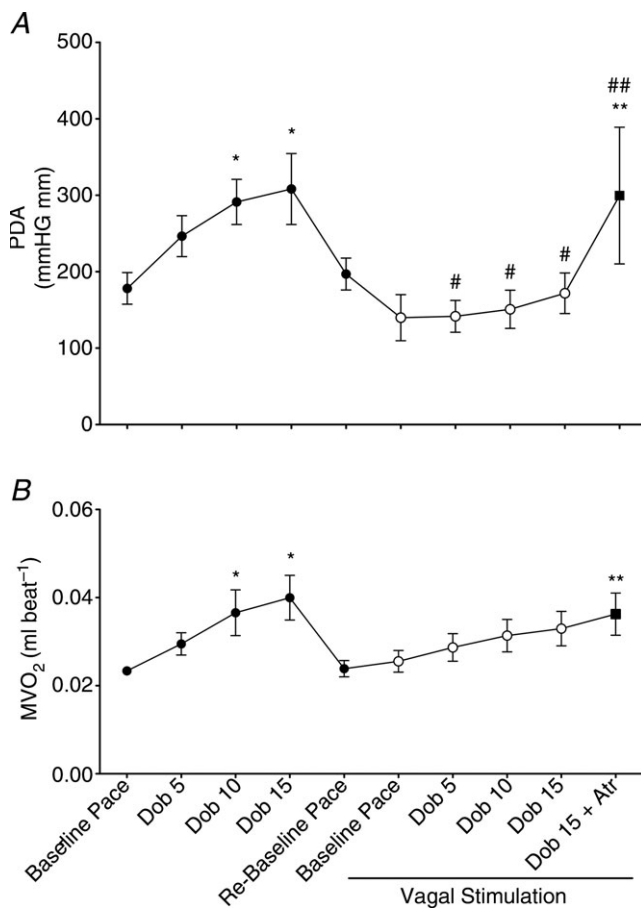


Figure 3. Changes in LV pressure-diameter area (PDA), an index of stroke work, and in $M\dot{V}O_2$, before (filled circles) and during (open circles) vagal stimulation

All measurements were taken with HR fixed at 165 beats min^{-1} (Pace). Data are presented as mean \pm SEM, $n = 7$ for PDA, due to the low quality of the loops in some dogs. Note $n = 8$ for $M\dot{V}O_2$. * $P < 0.05$ vs. pre-VS Baseline Pace; # $P < 0.05$ vs. corresponding time point pre-VS; ** $P < 0.05$ vs. post-VS Baseline Pace; ## $P < 0.05$ Dob 15+atropine vs. Dob 15 pre-VS.

Discussion

Our study shows that acute electrical stimulation of the efferent fibres in the intact right vagus nerve does not affect baseline cardiac function and metabolism, but markedly attenuates mechanical and metabolic response to β -adrenergic stress. External work and oxygen consumption during dobutamine infusion were similarly mitigated, indicating that VS preserved but did not enhance mechanical efficiency. Moreover, vagal efferent firing did not affect the balance between carbohydrate and FFA utilization in the resting heart, whereas during β -adrenergic stress it lowered glucose and lactate consumption compared with control, consistent with the reduced metabolic demand. Interestingly, VS selectively reduced oxidation of the less efficient substrate FFA only at the lowest dose of dobutamine, which more closely approximates physiological levels of sympathetic activation. Atropine reversed many of the functional effects of vagal stimulation, especially stroke work, which showed a marked rebound not paralleled by a similar increase in oxygen consumption and in carbohydrate utilization. As blood was sampled approximately 1 min after atropine administration to avoid the ensuing haemodynamic destabilization, it is possible that we missed the complete reversal of at least some metabolic changes which might have been slower than the functional reversal.

In our experiments, the confounding effects of bradycardia and changes in preload, the latter quantified as LV end-diastolic diameter, were prevented by pacing the heart at a fixed rate. Thus we can safely affirm that

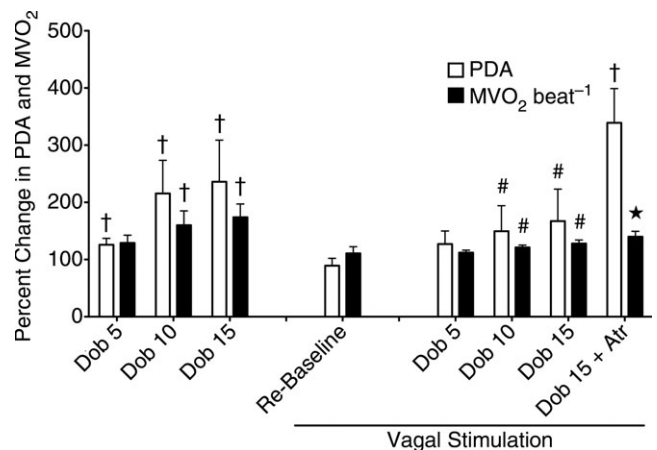


Figure 4. Percentage changes in LV pressure-diameter area (PDA), an index of stroke work, and in $M\dot{V}O_2$ for a normalized comparison of these two parameters

All measurements were taken with HR fixed at 165 beats min^{-1} (Pace). Data are presented as mean \pm SEM, $n = 7$ for PDA and $n = 8$ for $M\dot{V}O_2$. * $P < 0.05$ vs. pre-VS Baseline Pace; # $P < 0.05$ vs. corresponding time point pre-VS; ** $P < 0.05$ vs. post-VS Baseline Pace; † $P < 0.05$ PDA vs. $M\dot{V}O_2$.

the described metabolic changes were independent of HR and preload. The neurostimulator employed in the present study was set to deliver currents in the range 2.5–5 mA, which recruit mainly the efferent fibres (Anholt *et al.* 2011), also known as B-fibres. We cannot exclude that the afferent fibres (A-fibres) were in part recruited

too, although, if relevant, the expected consequence would have been a reflex sympathetic withdrawal. The absence of significant changes in ventricular afterload, as quantified by Ea, indicated that the afferent fibre stimulation, if any, was probably very small. Moreover, the rapid reversal following atropine administration provided further evidence of the involvement of the vagal efferent fibres acting on cardiac muscarinic receptors.

Classic studies in humans and animal models previously found that VS depresses cardiac contractility (DeGeest *et al.* 1964; Daggett *et al.* 1967; Henning *et al.* 1990; Lewis *et al.* 2001; Xenopoulos & Applegate, 1994). Just a few have focused also on cardiac oxygen consumption (Daggett *et al.* 1967; Downing *et al.* 1977), albeit in the absence of β -adrenergic stress, and none on the modulation of energy substrate utilization. The normo-perfused, healthy heart utilizes FFA as the preferential energy substrate. Under pathological conditions, such as ischaemia and failure, the balance between FFA and carbohydrate oxidation changes dramatically and this alteration may play an important role in the pathophysiological processes (Stanley *et al.* 2005). For instance, we have previously shown that the oxidation of glucose in the pentose phosphate pathway might fuel superoxide generation in myocardial tissue harvested from canine and human failing hearts (Gupte *et al.* 2006, 2007). It is difficult, based on the present data, to establish whether the inhibition of glucose and lactate consumption found during VS in stressed hearts was the cause or the consequence of reduced oxygen utilization. In both cases, our results are consistent with the direct inhibitory effects of nitric oxide, i.e. the mediator of the vagal negative inotropic action (Hare *et al.* 1995), on myocardial respiration and glucose utilization (Loke *et al.* 1999; Lei *et al.* 2005). As the stimulus intensity applied to the right vagus was very strong (reducing HR by 30 beats min^{-1}), another question is whether this metabolic control is physiologically relevant. We purposely tested extreme experimental conditions, such as superphysiological vagal firing and pharmacological β -adrenoreceptor activation, to amplify the measurable metabolic effects, but it is conceivable that the same mechanisms operate on a smaller scale as part of the physiological parasympathetic–sympathetic antagonism.

While our study provides further evidence that an exogenous activation of the vagal efferent branch minimally affects cardiac function under resting conditions, the interesting mechanisms occurring during β -adrenergic stress can be exploited for clinical use. The potential therapeutic implications of superphysiological vagal efferent output have been recently highlighted by studies in animal models of heart failure and pilot clinical trials (De Ferrari & Schwartz, 2011). Vagus nerve activity is notoriously impaired in heart failure (Chen *et al.* 1991; Bibevski & Dunlap, 2011). In rats subjected to coronary ligation, VS markedly improved long-term

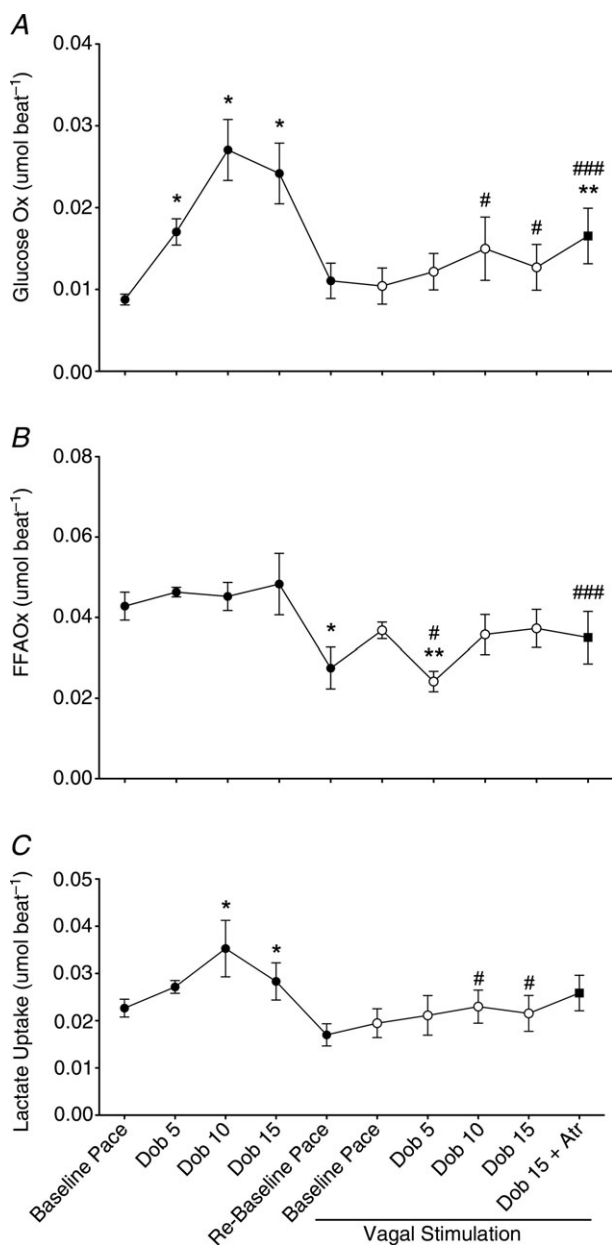


Figure 5. Changes in cardiac glucose and free fatty acid (FFA) oxidation and in lactate uptake during dobutamine infusion, before (filled circles) and during (open circles) vagal stimulation

All measurements were taken with HR fixed at 165 beats min^{-1} . Data are presented as mean \pm SEM, $n = 11$. * $P < 0.05$ vs. pre-VS Baseline Pace; # $P < 0.05$ vs. corresponding time point pre-VS; ** $P < 0.05$ vs. post-VS Baseline Pace; ### $P < 0.05$ vs. Dob 15+atropine vs. Dob 15 pre-VS.

survival through the prevention of pumping failure and cardiac remodelling (Li *et al.* 2004). In a pilot study, we chronically implanted the CardioFit stimulator in dogs with microembolism-induced heart failure and found improved LV function and biomarkers (Sabbah *et al.* 2010). Consistently, other investigators have documented a marked attenuation of cardiac dysfunction in dogs with tachypacing-induced heart failure when chronic stimulation of the cervical vagus was concurrently applied (Zhang *et al.* 2009). Finally, a multi-centre, open-labelled phase II study in New York Heart Association class II–IV heart failure patients showed that right cervical VS was well tolerated and produced significant improvements in quality of life, walk test and LV ejection fraction, maintained at 1 year (De Ferrari *et al.* 2011). This first trial might open a new avenue for the treatment of heart failure and calls for more extensive evaluations of VS.

The mechanisms underlying the beneficial effects of VS are multiple and only partially understood. Based on previous investigations and on the present one, this type of therapeutic intervention can be envisaged as a cardio-selective β -blockade, devoid of systemic side effects. In particular, our results suggest that VS can buffer metabolic peaks following episodes of pronounced sympathetic discharge, a mechanism that should be protective in cardiac ischaemia and/or failure.

Several limitations of the present study should be noted. First, the use of light propofol anaesthesia: VS causes discomfort when initially activated and we opted for an acute implantation of the stimulator on the day of the experiment, and hence we could exploit only in part the advantages of chronic animal preparations. A second limitation was the specific focus on right vagus, which may not account for other actions exerted by the left nerve. However, other authors have compared the stimulation of the right and left vagus and found no differences in their negative inotropic effects (DeGeest *et al.* 1964; Daggett *et al.* 1967). Third, we used atropine to block the cardiac muscarinic acetylcholine M2 receptors, although its partial efficacy in reversing metabolic changes might suggest the involvement of different acetylcholine receptors, such as the $\alpha 7$ nicotinic receptor, whose protective role in the heart has been recently shown in a rat model of ischaemia/reperfusion (Calvillo *et al.* 2011). Unfortunately, this potential mechanism is hardly testable in intact dog preparations, as the infusion of pharmacological inhibitors of nicotinic receptors would cause confounding effects due to parasympathetic ganglionic transmission (Bibevski *et al.* 2000).

In conclusion, we performed a systematic study on a specific vagal function that was previously unexplored, namely the control of cardiac metabolism in relation to various functional states. While our results do not support appreciable effects in the resting heart, they suggest a significant role under conditions of β -adrenergic

stress. Besides the physiological implications, our findings may contribute to better understanding the therapeutic effects of VS, recently proposed for the treatment of heart failure.

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Author contributions

Conception and design of the experiments: C.V., H.N.S. and F.A.R. Collection, analysis and interpretation of data K.Q., I.I., G.M. and R.S. Drafting/revising the article: C.V., W.C.S., H.N.S. and F.A.R. All authors approved the final version. I.I. is a full time employee of BioControl Medical, Ltd. The entire study was performed at the New York Medical College, Valhalla, NY.

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