Cardiac vagus and exercise

Alexander V. Gourine[1] and Gareth L. Ackland[2]

[1]Centre for Cardiovascular and Metabolic Neuroscience, Neuroscience, Physiology and Pharmacology, University College London, London WC1E 6BT, UK;

[2]William Harvey Research Institute, Barts and The London School of Medicine and Dentistry, Queen Mary University of London, London EC1M 6BQ, UK.

Abstract

Lower resting heart rate and high autonomic vagal activity are strongly associated with superior exercise capacity, maintenance of which is essential for general well-being and healthy ageing. Recent evidence obtained in experimental studies using the latest advances in molecular neuroscience, combined with human exercise physiology, physiological modelling and genomic data suggest that the strength of cardiac vagal activity causally determines our ability to exercise.

Correspondence to:

A.V.G., a.gourine@ucl.ac.uk or G.L.A., g.ackland@qmul.ac.uk

Introduction

Maintaining physical activity is essential for general well-being and healthy ageing. Higher exercise capacity is strongly associated with reduced risk of cardiovascular disease, type 2 diabetes, malignancy, osteoporosis, depression and premature death (14). Regular moderate exercise provides further health benefits (13, 50). It is well known that higher exercise capacity is strongly associated with lower resting heart rate and indirect measures of high cardiac vagal activity (16, 39), suggesting that the parasympathetic branch of the autonomic nervous system may play a key role in optimizing exercise performance. Despite the strong association between parasympathetic activity and exercise capacity, these data have long been interpreted as vagal activity merely being a marker for physical fitness. However, several lines of evidence obtained in studies of human exercise, physiological modelling, genome-wide association analysis and experimental animal studies now challenge this assertion. In this article we review these data, which collectively support the hypothesis that the strength of cardiac vagal activity directly determines the individual ability to exercise.

Exercise and resting heart rate

Resting heart rate in healthy individuals is largely determined by the restraining influence of the vagus nerve on cardiac pacemaker cells located within the atrial nodal tissue, which typically generate action potentials at an intrinsic rate of ~ 105 min^{-1}(44). Experimental pharmacological blockade or surgical interruption of vagal cardiac innervation increases heart rate above this intrinsic rate (58). The resting heart rate in elite endurance athletes is low (values as low as 30 beats min^{-1} have been reported) and has been traditionally attributed to exceptionally high parasympathetic vagal tone in these select individuals (19, 76). D'Souza and colleagues, however, reported evidence (obtained in animal models) suggesting that the resting bradycardia associated with higher exercise capacity is primarily due to decreased intrinsic rate of cardiac pacemaker cells, established by training-induced downregulation of the pacemaker channel HCN4 expression (27). This view is also supported by the results of an earlier report which demonstrated reduced intrinsic rate of atria isolated from rats following exercise training (15). It remains to be determined whether the expression of HCN4 is modulated by autonomic activity with high vagal tone potentially restraining HCN4 channel expression in the nodal tissue. Indeed, there is evidence that removal of

cardiac vagal innervation prevents the (lowering) effect of endurance exercise training on resting heart rate in dogs (67). Parasympathetic vagal dysfunction induced by sinoaortic denervation was also reported to prevent endurance training-induced increases in baroreflex sensitivity and decreases in resting heart rate in rats (21). Although the mechanisms underlying resting bradycardia in athletes are under debate, the general consensus is that in humans superior exercise performance is strongly associated with lower resting heart rate as well as indirect measures of high cardiac vagal activity which will be discussed next.

Assessment of vagal parasympathetic activity in humans by measuring heart rate recovery after exercise

While resting heart rate is largely determined by the vagus nerve, additional robust measures of cardiac vagal activity in humans were required. The interpretation of the most common measure, heart rate variability, is complicated by the fact that it appears to be strongly dependent on resting heart rate (63) as well as the frequency and depth of respiration (1). Although this measure is sensitive to systemic cholinergic blockade (5, 72), more recent analysis provided further evidence that even nonlinear indices of heart rate variability are not reliable measures of cardiac vagal activity in healthy humans (20).

An alternative, highly reproducible measure of an individual's ability to recruit cardiac vagal tone is the speed of heart rate recovery (HRR) after exercise. After heart rate peaks during exercise, a variably steep decline occurs upon cessation of exercise, termed heart rate recovery (Figure 1A). HRR after exercise is mediated by rapid vagal reactivation (25) as demonstrated by markedly reduced rate of HRR in conditions of systemic muscarinic receptor blockade with atropine (43). Indicative of a causal relationship between the rate of HRR and exercise capacity, there is a dramatic absolute difference in HRR between the athletes and heart failure patients (43). Assessment of individual ability to recruit parasympathetic vagal tone based on HRR after exercise is independent of other physiological measures, including resting heart rate (a significant confounder in heart rate variability analysis (63)), tidal volume and breathing frequency (1). Yet, reduced rate of HRR after exercise correlates with other indirect measures of cardiac vagal tone, including heart rate variability and baroreflex sensitivity, if these also suggest potential impairment of autonomic function (2, 3, 83).

Four large clinical studies demonstrated a strong association between vagal dysfunction (as evident from a reduced HRR), cardiovascular morbidity and all-cause mortality (22, 23, 45, 66). A six year follow-up study in 2,428 middle-aged men, without a history of heart failure or coronary revascularization, reported that impaired HRR (defined as a reduction of 12 beats min^{-1} or less during the first minute after cessation of graded exercise) (Figure 1A) was strongly and independently associated with mortality (22). HRR is a strong predictor independently of workload, the presence of myocardial perfusion abnormalities, and/or absolute changes in heart rate during exercise. Subsequent studies including more than 20,000 individuals found a similar association, implicating parasympathetic autonomic dysfunction as a key factor contributing to cardiovascular morbidity and mortality (22, 23, 45, 66) These data are also supported by the evidence showing strong association between mortality and baroreflex function (62) as well as resting heart rate (32, 89), the major autonomic determinant of which is cardiac vagal activity.

The strong association between low resting heart rate, measures of high cardiac vagal activity and superior exercise capacity implies that cardiac vagal tone can be enhanced by exercise training. One longitudinal study performed in a group of untrained young individuals demonstrated that 6 weeks of aerobic exercise training reduced resting heart rate and increased cardiac vagal tone, as assessed by heart rate variability analysis (6). Studies conducted in athletes (28, 59), patients with heart failure (64, 70, 82) and type 2 diabetes (11) have demonstrated that exercise training accelerates HRR, indicative of enhanced cardiac vagal function. These reports suggest that the vagal tone can indeed be "trained" by exercise, yet potential neurophysiological mechanisms (e.g. recruitment of more vagal neurons, increased excitability and activity of vagal neurons, facilitated transmission at the level of the ganglia) underlying this apparent plasticity of the autonomic nervous system remain unknown.

Genetic determinants of cardiac vagal tone

Although the emerging evidence suggests that cardiac vagal tone in healthy humans can be enhanced by exercise training, it is estimated from the results of twin studies that approximately 60% of HRR (and, hence, cardiac vagal activity) is determined genetically (65). Two recent genome-wide association studies conducted using data

obtained in over 50,000 participants undergoing an exercise test (United Kingdom Biobank) aimed to identify genes that may be associated with heart rate response to exercise and HRR (73, 86). There was a broad overlap between the results of two studies, which identified at least 16 independent genome-wide significant signals across 23 genetic loci. There was, however, some discordance between the results of two studies, which may be related to the methodological differences in the assessment of HRR. Nevertheless, both studies found strong associations between the resting heart rate, HRR and several genes expressed by the key components of the autonomic parasympathetic reflex signalling pathways including acetylcholinesterase and muscarinic M2 receptors. Other "neuronal" genes at the various loci prominently associated with HRR are implicated in the processes such as the control of neurite outgrowth, synaptic plasticity, neurotransmission and neuronal exocytosis.

Autonomic control of the heart during exercise

The conventional view of autonomic control of the heart during exercise has long held that increases in heart rate from resting baseline initially occur through (complete) vagal withdrawal, followed by progressive activation of the sympathetic input (75) (Figure 2A). However, it appears that significant cardiac vagal activity is maintained *during* exercise (Figure 2B), as suggested by the results obtained in studies using autonomic blockade and baroreflex stimulation during exercise (89). Indeed, the model of on/off thresholds for vagal/sympathetic withdrawal/activation appears to be over-simplistic, especially if we consider that the pressor response (35) and the arterial baroreflex (34) contribute to the resetting of the heart rate control during exercise. The concept that cardiac vagal activity is maintained throughout exercise is based on the analysis and modelling of the results of experimental exercise studies involving pharmacological blockade of autonomic limbs in healthy human volunteers. At the onset of exercise, vagal activity indeed decreases as a result of central command and resetting of the arterial baroreflex (38). However, subsequent rapid increase in venous return recruits/maintains cardiac vagal activity through increased baroreceptor loading (88). Combined withdrawal of central command and attenuation of exercise pressor reflex sensitivity increase cardiac output without significant changes in the arterial blood pressure (81). As exercise workload increases these mechanisms continually reset the arterial baroreflex (81). Accordingly, during exercise the heart receives strong vagal and sympathetic modulation at rates below 140 beats min^{-1} (Figure 2B).

At ~140 beats min^{-1}, sympathetic and parasympathetic influences are estimated to be approximately equal in strength, with sympathetic activity dominating heart rate control at higher exercise intensities (Figure 2B). In summary, studies of human heart rate control suggest that the resting heart rate is by ~80% attributable to vagal modulation, while heart rate control during intense exercise is ~80% dependent on sympathetic nerve activity (89). Thus, even at high exercise loads, vagal activity continues to modulate cardiac function alongside with heightened activity of the sympathetic nervous system. It is important to emphasise that the data supporting this concept are circumstantial and the dynamic changes in cardiac vagal activity during exercise with increasing workload have never been directly assessed. Although, recordings of cardiac vagus in humans and animals are not feasible, changes in vagal activity during exercise can potentially be inferred from direct recordings of vagal preganglionic neuron firing during dynamic exercise in experimental animals. To the best of our knowledge this experiment has never been performed. Yet, there is evidence obtained in experimental animal studies indicating that simultaneous co-activation of both autonomic limbs do occur in various conditions (68).

Significant cardiac vagal activity which is maintained during exercise could be functionally important in optimizing exercise performance. If this hypothesis is correct, then parasympathetic vagal dysfunction would be expected to be associated with impaired exercise capacity. We specifically determined the relationship between HRR and exercise capacity in 1,293 human participants, over an age range (>65 years old) when vagal function declines variably (57). A graded relationship between the speed of HRR, resting heart rate and all prognostically relevant measures of exercise performance was found (Figure 1B,C). Decrements in HRR were also found to be strongly associated with reduced oxygen pulse (57), a robust surrogate for left ventricular stroke volume, suggesting that cardiac vagal activity may play an important role in modulation of ventricular contractility.

CNS origins of cardiac vagal activity

Cardiac vagal activity is generated by populations of vagal preganglionic neurons residing in two brainstem nuclei, – the dorsal vagal motor nucleus and the nucleus ambiguus. These two distinct populations of neurons have different patterns of discharge and provide differential control of the cardiac function (37; for a recent

review see [40]). Neurons of the nucleus ambiguus are rhythmic, entrained by the neighbouring respiratory network, and appear to exclusively control the nodal tissue and, therefore, the heart rate. Vagal preganglionic neurons residing in the dorsal vagal motor nucleus display tonic pattern of discharge which is maintained in an acute slice preparation (i.e. they are active in the absence of afferent input from the periphery and the rest of the CNS). A subpopulation of these neurons with cardiac projections is responsible for vagal modulation of the ventricular function (56).

It is important to keep in mind that all the existing measures of cardiac vagal tone in humans (including HRR), assess the activity of vagal projections which innervate the cardiac pacemaker cells and, thus, provide chronotropic control of the heart. For the purpose of this consideration, we assume (but cannot be sure without experimental evidence) that the strength of chronotropic vagal tone (generated by the nucleus ambiguus) parallels the activity of vagal projections innervating the atria and the ventricles (generated by the dorsal vagal motor nucleus). The functional significance of parasympathetic ventricular innervation is considered next.

Vagal innervation of the ventricles: anatomical evidence

Appropriate increases in cardiac contractility are essential to support the metabolic demands of the exercise. If cardiac vagal activity contributes to optimizing exercise performance, vagal innervation of the ventricles is likely to play an important role. Intrinsic cardiac ganglia harbouring clusters of vagal neurons are located in the epicardium of the atria and the ventricular septum (31, 36, 80). There is significant anatomical evidence that these vagal neurons send extensive projections to all four chambers of the heart (8, 12, 74, 77, 90). Staining for acetylcholinesterase activity, immunohistochemical detection of choline acetyltransferase and other anatomical methods demonstrated significant cholinergic innervation of the ventricular myocardium in all species studied so far (24), including mice (54), rats (34, 61, 67), dogs (47), pigs (26, 84) and humans (47, 69). A proportion of these cardiac vagal fibres synapse on postganglionic sympathetic terminals (inhibiting the release of norepinephrine presynaptically) while the others may directly innervate the ventricular muscle (51) (Figure 3).

Vagal innervation of the ventricles: functional evidence

Studies conducted in several species of experimental animals have demonstrated that electrical stimulation of the cervical vagus nerve causes stimulation frequency-dependent release of acetylcholine and nitric oxide (NO) in the ventricular myocardium (4, 18, 33, 41, 79). Ablation of vagal neurons of the intrinsic cardiac ganglia in dogs abolished ventricular acetylcholinesterase activity and prevented the effect of vagal nerve stimulation on the ventricular electrical properties (47). Another study conducted in rats specifically targeted the dorsal group of the brainstem vagal preganglionic neurons to express an inhibitory GPCR from insects (61) and demonstrated that this population of neurons has a tonic effect on the electrical properties of the ventricular myocardium (54). Acute inhibition of the dorsal vagal motor nucleus by application of a specific ligand of this invertebrate receptor shortened the ventricular effective refractory period, lowered the ventricular tachycardia threshold, and prolonged the QT interval (54). The vagal influences on the ventricular electrical properties appear to be mediated by complex interactions between cholinergic mechanisms and these mediated by NO produced by the neuronal nitric oxide synthase (7, 17, 46, 54, 55).

Collectively, these studies demonstrated that the vagus nerve modulates electrical properties of the ventricular myocardium, suggestive of direct, functionally significant innervation. Further studies explored whether projections of dorsal vagal preganglionic neurons may also modulate left ventricular contractility. That the electrical stimulation of the vagus nerve results in load-independent reduction of left ventricular contractility is well known (29, 30, 52). Whether the modulation of contractility by vagal ventricular innervation is tonic and functionally significant (at rest and during exercise) is less clear. The only human evidence is provided by the report showing that intracoronary atropine potentiates dobutamine-induced inotropic responses, and this effect is absent in the transplanted (i.e. denervated) hearts (48). In rats under conditions of complete sympathetic blockade (systemic β-adrenoceptor blockade combined with spinal cord transection), a tonic inhibitory muscarinic influence on ventricular contractility was evident following systemic administration of atropine or direct pharmacological or genetic inhibition of the dorsal group of vagal preganglionic neurons (56). The strength of vagal modulation of ventricular contractility was dependent on the type of the anesthetic used in these studies, an important confounding factor in the experiments of this type, considering the high sensitivity of vagal neurons to these agents (87).

There is also evidence that the activity of dorsal vagal preganglionic neurons can effectively protect (via a muscarinic mechanism) ventricular cardiomyocytes against acute ischaemia/reperfusion injury (61). These neurons appear to be critically important for the operation of the innate reflex mechanisms which mediate inter-organ protection against ischaemia/reperfusion myocardial injury (through the phenomenon of remote ischaemic preconditioning cardioprotection) (61). These protective reflexes may recruit direct ventricular projections of vagal neurons as well as vagal innervation of the visceral organs and activation of mechanisms involving systemic actions of cardiotropic gut hormones, including glucagon-like peptide-1 (7, 10, 60).

Exercise capacity is determined by vagal activity: molecular neuroscience data demonstrating causality

Recent advances in molecular neuroscience allow precise control of neuronal activity with unprecedented specificity, spatial and temporal resolution (9). This is particularly important for studies of parasympathetic mechanisms, considering that the vagus is a mixed nerve containing motor (efferent) and sensory (afferent) fibres, making data obtained with the use of electrical stimulation sometimes difficult to interpret. Indeed, electrical stimulation of the vagus nerve at the cervical level has many shortcomings, not least because of the uncertainty as to which vagal fibres (afferent or efferent) are activated by electrical pulses, but also because of the potential recruitment of sympathetic axons which join the vagal trunk (78, 85). A refined approach (to avoid the drawbacks inherent to indiscriminate nature of nerve sectioning, systemic pharmacological blockade and/or electrical stimulation) to inhibit or stimulate vagal efferent activity would require cell-specific genetic targeting of vagal preganglionic neurons to express membrane proteins allowing control of their activity using light or specific ligands. This was achieved in rats by using viral vectors to target dorsal vagal preganglionic neurons to express either inhibitory GPCRs from insects (which could be activated by invertebrate peptides which do not have endogenous receptors in mammals (49)) (Figure 4A) or light-sensitive cation channels from algae (53, 61).

Exercise capacity was assessed in animals transduced to express the insect inhibitory GPCR, light sensitive channels and respective control transgenes in neurons of the dorsal vagal motor nucleus (57). Experimental and control groups shared similar exercise capacity at baseline, however, acute silencing of the dorsal vagal

preganglionic neurons reversibly reduced exercise capacity by ~80% (Figure 4B). As chronotropic control of the heart is provided by neurons of the nucleus ambiguus located ventrally in the brainstem (37, 40), the effect of silencing the dorsal population of vagal neurons is likely to be due to impaired exercise-evoked ventricular and/or functional systemic hemodynamic changes, as suggested by the results of human studies.

The same study demonstrated that optogenetic stimulation of this population of vagal neurons enhances cardiac contractility and prolongs exercise endurance (57). Mechanistic insight was provided by the evidence showing that recruitment of vagal activity by optogenetic stimulation of these neurons was associated with reduced myocardial expression of G-protein-coupled receptor kinase 2 (GRK2) and β-arrestin 2 (57). This observation is important since these proteins are critical components of the intracellular machinery which controls the efficacy of β-adrenoceptor (sympathetic) signalling in cardiomyocytes. GRKs promote phosphorylation of the intracellular domains of β-adrenoceptors, recruiting arrestins to block receptor coupling to G-proteins resulting in receptor desensitization and internalization (42) (Figure 3). Reduced, or inducible ablation of, cardiac GRK2 expression improves cardiac function in low exercise capacity states such as heart failure (71). These data obtained in experimental animals suggest that vagal activity may control cardiac responsiveness to sympathetic stimulation via modulation of expression of key negative regulators of β-adrenoceptor-mediated signalling.

Conclusions

The basic science and clinical data discussed in this essay challenge the long-held prevailing view that the strength of cardiac vagal tone is merely a marker of physical fitness. The key points discussed above can be condensed as follows:

- The speed of heart rate recovery after exercise provides a robust measure of cardiac vagal activity, which is strongly associated with exercise capacity, cardiovascular morbidity and all-cause mortality in humans.

- In health, the cardiac vagal activity is largely determined by the genetic factors, with strong associations identified between HRR and several genes expressed by the key components of autonomic reflex signalling pathways.

- Cardiac vagal activity may be enhanced further even in healthy individuals through exercise training.

- A significant level of cardiac vagal activity is maintained during exercise.

- The vagus nerve provides functional innervation of the ventricles. Cardiac projections of a dorsal group of vagal preganglionic neurons modulate ventricular excitability, contractility and responsiveness of the ventricular myocardium to β-adrenoceptor stimulation.

- Inhibition of vagal neurons impairs exercise capacity, while vagal stimulation enhances cardiac contractility and prolongs exercise endurance (in experimental animals).

The most logical conclusion from these lines of evidence is that the strength of cardiac vagal activity causally determines our ability to exercise. High cardiac vagal tone in elite athletes may be critically important to confer higher tolerance for intense training regimes, essential to achieve superior athletic performance. On the other hand, and in accord with this hypothesis, development of the pathology associated with progressive or acute parasympathetic dysfunction invariably parallels the decline in aerobic exercise capacity. Pharmacological or device-based neuromodulation approaches allowing selective recruitment of cardiac vagal activity may prove to be effective in enhancing the exercise capacity and promoting cardiovascular health across the lifespan.

References

1. Heart rate variability: standards of measurement, physiological interpretation and clinical use. Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology. *Circulation* 93: 1043-1065, 1996.

2. **Ackland GL, Minto G, Clark M, Whittle J, Stephens RCM, Owen T, Prabhu P, and Del Arroyo AG.** Autonomic regulation of systemic inflammation in humans: A multi-center, blinded observational cohort study. *Brain Behav Immun* 67: 47-53, 2018.

3. **Ackland GL, Whittle J, Toner A, Machhada A, Del Arroyo AG, Sciuso A, Jenkins N, Dyson A, Struthers R, Sneyd JR, Minto G, Singer M, Shah AM, and Gourine AV.** Molecular Mechanisms Linking Autonomic Dysfunction and Impaired Cardiac Contractility in Critical Illness. *Crit Care Med* 44: e614-624, 2016.

4. **Akiyama T and Yamazaki T.** Effects of right and left vagal stimulation on left ventricular acetylcholine levels in the cat. *Acta Physiol Scand* 172: 11-16, 2001.

5. **Akselrod S, Gordon D, Ubel FA, Shannon DC, Berger AC, and Cohen RJ.** Power spectrum analysis of heart rate fluctuation: a quantitative probe of beat-to-beat cardiovascular control. *Science* 213: 220-222, 1981.

6. **al-Ani M, Munir SM, White M, Townend J, and Coote JH.** Changes in R-R variability before and after endurance training measured by power spectral analysis and by the effect of isometric muscle contraction. *Eur J Appl Physiol Occup Physiol* 74: 397-403, 1996.

7. **Ang R, Mastitskaya S, Hosford PS, Basalay M, Specterman M, Aziz Q, Li Y, Orini M, Taggart P, Lambiase PD, Gourine A, Tinker A, and Gourine AV.** Modulation of cardiac ventricular excitability by GLP-1 (Glucagon-Like Peptide-1). *Circ Arrhythm Electrophysiol* 11: e006740, 2018.

8. **Armour JA.** Potential clinical relevance of the 'little brain' on the mammalian heart. *Exp Physiol* 93: 165-176, 2008.

9. **Aston-Jones G and Deisseroth K.** Recent advances in optogenetics and pharmacogenetics. *Brain Res* 1511: 1-5, 2013.

10. **Basalay MV, Mastitskaya S, Mrochek A, Ackland GL, Del Arroyo AG, Sanchez J, Sjoquist PO, Pernow J, Gourine AV, and Gourine A.** Glucagon-like peptide-1 (GLP-1) mediates cardioprotection by remote ischaemic conditioning. *Cardiovasc Res* 112: 669-676, 2016.

11. **Bhati P, Shenoy S, and Hussain ME.** Exercise training and cardiac autonomic function in type 2 diabetes mellitus: A systematic review. *Diabetes Metab Syndr* 12: 69-78, 2018.

12. **Billman GE, Hoskins RS, Randall DC, Randall WC, Hamlin RL, and Lin YC.** Selective vagal postganglionic innervation of the sinoatrial and atrioventricular nodes in the non-human primate. *J Auton Nerv Syst* 26: 27-36, 1989.

13. **Blair SN, Kohl HW, 3rd, Barlow CE, Paffenbarger RS, Jr., Gibbons LW, and Macera CA.** Changes in physical fitness and all-cause mortality. A prospective study of healthy and unhealthy men. *JAMA* 273: 1093-1098, 1995.

14. **Blair SN, Kohl HW, 3rd, Paffenbarger RS, Jr., Clark DG, Cooper KH, and Gibbons LW.** Physical fitness and all-cause mortality. A prospective study of healthy men and women. *JAMA* 262: 2395-2401, 1989.

15. **Bolter CP, Hughson RL, and Critz JB.** Intrinsic rate and cholinergic sensitivity of isolated atria from trained and sedentary rats. *Proc Soc Exp Biol Med* 144: 364-367, 1973.

16. **Boutcher SH, and Stein P.** Association between heart rate variability and training response in sedentary middle-aged men. *Eur J Appl Physiol Occup Physiol* 70: 75-80, 1995.

17. **Brack KE, Coote JH, and Ng GA.** Vagus nerve stimulation protects against ventricular fibrillation independent of muscarinic receptor activation. *Cardiovasc Res* 91: 437-446, 2011.

18.   **Brack KE, Patel VH, Mantravardi R, Coote JH, and Ng GA.** Direct evidence of nitric oxide release from neuronal nitric oxide synthase activation in the left ventricle as a result of cervical vagus nerve stimulation. *J Physiol* 587: 3045-3054, 2009.

19.   **Carter JB, Banister EW, and Blaber AP.** Effect of endurance exercise on autonomic control of heart rate. *Sports Med* 33: 33-46, 2003.

20.   **Cepeda FX, Lapointe M, Tan CO, and Taylor AJ.** Inconsistent relation of nonlinear heart rate variability indices to increasing vagal tone in healthy humans. *Auton Neurosci* 213: 1-7, 2018.

21.   **Ceroni A, Chaar LJ, Bombein RL, and Michelini LC.** Chronic absence of baroreceptor inputs prevents training-induced cardiovascular adjustments in normotensive and spontaneously hypertensive rats. *Exp Physiol* 94: 630-640, 2009.

22.   **Cole CR, Blackstone EH, Pashkow FJ, Snader CE, and Lauer MS.** Heart-rate recovery immediately after exercise as a predictor of mortality. *N Engl J Med* 341: 1351-1357, 1999.

23.   **Cole CR, Foody JM, Blackstone EH, and Lauer MS.** Heart rate recovery after submaximal exercise testing as a predictor of mortality in a cardiovascularly healthy cohort. *Ann Intern Med* 132: 552-555, 2000.

24.   **Coote JH.** Myths and realities of the cardiac vagus. *J Physiol* 591: 4073-4085, 2013.

25.   **Coote JH.** Recovery of heart rate following intense dynamic exercise. *Exp Physiol* 95: 431-440, 2010.

26.   **Crick SJ, Anderson RH, Ho SY, and Sheppard MN.** Localisation and quantitation of autonomic innervation in the porcine heart II: endocardium, myocardium and epicardium. *J Anat* 195: 359-373, 1999.

27.   **D'Souza A, Bucchi A, Johnsen AB, Logantha SJ, Monfredi O, Yanni J, Prehar S, Hart G, Cartwright E, Wisloff U, Dobryznski H, DiFrancesco D, Morris GM, and Boyett MR.** Exercise training reduces resting heart rate via downregulation of the funny channel HCN4. *Nat Commun* 5: 3775, 2014.

28.   **Daanen HA, Lamberts RP, Kallen VL, Jin A, and Van Meeteren NL.** A systematic review on heart-rate recovery to monitor changes in training status in athletes. *Int J Sports Physiol Perform* 7: 251-260, 2012.

29.   **Degeest H, Levy MN, and Zieske H.** Negative Inotropic Effect of the Vagus Nerves Upon the Canine Ventricle. *Science* 144: 1223-1225, 1964.

30.   **Degeest H, Levy MN, Zieske H, and Lipman RI.** Depression of Ventricular Contractility by Stimulation of the Vagus Nerves. *Circ Res* 17: 222-235, 1965.

31.   **Dickerson LW, Rodak DJ, Fleming TJ, Gatti PJ, Massari VJ, McKenzie JC, and Gillis RA.** Parasympathetic neurons in the cranial medial ventricular fat pad on the dog heart selectively decrease ventricular contractility. *J Auton Nerv Syst* 70: 129-141, 1998.

32.   **Eckberg DL, Drabinsky M, and Braunwald E.** Defective cardiac parasympathetic control in patients with heart disease. *N Engl J Med* 285: 877-883, 1971.

33.   **Eliakim M, Bellet S, Tawil E, and Muller O.** Effect of vagal stimulation and acetylcholine on the ventricle. Studies in dogs with complete atrioventricular block. *Circ Res* 9: 1372-1379, 1961.

34.   **Fadel PJ, Ogoh S, Watenpaugh DE, Wasmund W, Olivencia-Yurvati A, Smith ML, and Raven PB.** Carotid baroreflex regulation of sympathetic nerve activity during dynamic exercise in humans. *Am J Physiol Heart Circ Physiol* 280: H1383-1390, 2001.

35.   **Fisher JP.** Autonomic control of the heart during exercise in humans: role of skeletal muscle afferents. *Exp Physiol* 99: 300-305, 2014.

36.   **Gatti PJ, Johnson TA, McKenzie J, Lauenstein JM, Gray A, and Massari VJ.** Vagal control of left ventricular contractility is selectively mediated by a cranioventricular intracardiac ganglion in the cat. *J Auton Nerv Syst* 66: 138-144, 1997.

37.   **Geis GS, and Wurster RD.** Cardiac responses during stimulation of the dorsal motor nucleus and nucleus ambiguus in the cat. *Circ Res* 46: 606-611, 1980.

38.   **Gladwell VF, and Coote JH.** Heart rate at the onset of muscle contraction and during passive muscle stretch in humans: a role for mechanoreceptors. *J Physiol* 540: 1095-1102, 2002.

39.   **Goldsmith RL, Bigger JT, Jr., Bloomfield DM, and Steinman RC.** Physical fitness as a determinant of vagal modulation. *Med Sci Sports Exerc* 29: 812-817, 1997.

40.   **Gourine AV, Machhada A, Trapp S, and Spyer KM.** Cardiac vagal preganglionic neurones: An update. *Auton Neurosci* 199: 24-28, 2016.

41.   **Hare JM, Keaney JF, Jr., Balligand JL, Loscalzo J, Smith TW, and Colucci WS.** Role of nitric oxide in parasympathetic modulation of beta-adrenergic myocardial contractility in normal dogs. *J Clin Invest* 95: 360-366, 1995.

42.   **Hullmann J, Traynham CJ, Coleman RC, and Koch WJ.** The expanding GRK interactome: Implications in cardiovascular disease and potential for therapeutic development. *Pharmacol Res* 110: 52-64, 2016.

43.   **Imai K, Sato H, Hori M, Kusuoka H, Ozaki H, Yokoyama H, Takeda H, Inoue M, and Kamada T.** Vagally mediated heart rate recovery after exercise is accelerated in athletes but blunted in patients with chronic heart failure. *Journal of the American College of Cardiology* 24: 1529-1535, 1994.

44.   **Jose AD, and Collison D.** The normal range and determinants of the intrinsic heart rate in man. *Cardiovasc Res* 4: 160-167, 1970.

45.   **Jouven X, Empana JP, Schwartz PJ, Desnos M, Courbon D, and Ducimetiere P.** Heart-rate profile during exercise as a predictor of sudden death. *N Engl J Med* 352: 1951-1958, 2005.

46.   **Kalla M, Chotalia M, Coughlan C, Hao G, Crabtree MJ, Tomek J, Bub G, Paterson DJ, and Herring N.** Protection against ventricular fibrillation via cholinergic receptor stimulation and the generation of nitric oxide. *J Physiol* 594: 3981-3992, 2016.

47.   **Kent KM, Epstein SE, Cooper T, and Jacobowitz DM.** Cholinergic innervation of the canine and human ventricular conducting system. Anatomic and electrophysiologic correlations. *Circulation* 50: 948-955, 1974.

48.   **Landzberg JS, Parker JD, Gauthier DF, and Colucci WS.** Effects of intracoronary acetylcholine and atropine on basal and dobutamine-stimulated left ventricular contractility. *Circulation* 89: 164-168, 1994.

49.   **Lechner HA, Lein ES, and Callaway EM.** A genetic method for selective and quickly reversible silencing of Mammalian neurons. *J Neurosci* 22: 5287-5290, 2002.

50.   **Lee IM, Hsieh CC, and Paffenbarger RS, Jr.** Exercise intensity and longevity in men. The Harvard Alumni Health Study. *JAMA* 273: 1179-1184, 1995.

51.   **Levy MN.** Sympathetic-parasympathetic interactions in the heart. *Circ Res* 29: 437-445, 1971.

52.   **Lewis ME, Al-Khalidi AH, Bonser RS, Clutton-Brock T, Morton D, Paterson D, Townend JN, and Coote JH.** Vagus nerve stimulation decreases left ventricular contractility in vivo in the human and pig heart. *J Physiol* 534: 547-552, 2001.

53.   **Lin JY, Lin MZ, Steinbach P, and Tsien RY.** Characterization of engineered channelrhodopsin variants with improved properties and kinetics. *Biophys J* 96: 1803-1814, 2009.

54.   **Machhada A, Ang R, Ackland GL, Ninkina N, Buchman VL, Lythgoe MF, Trapp S, Tinker A, Marina N, and Gourine AV.** Control of ventricular excitability by neurons of the dorsal motor nucleus of the vagus nerve. *Heart Rhythm* 12: 2285-2293, 2015.

55.   **Machhada A, Ang R, Ackland GL, Ninkina NN, Buchman VL, Lythgoe MF, Trapp S, Tinker A, Marina N, and Gourine AV.** Reduced activity of dorsal vagal preganglionic neurones associated with synuclein pathology predisposes the heart to ventricular arrhythmia. *Eur Heart J* 36: 215-215, 2015

56.   **Machhada A, Marina N, Korsak A, Stuckey DJ, Lythgoe MF, and Gourine AV.** Origins of the vagal drive controlling left ventricular contractility. *J Physiol* 594: 4017-4030, 2016.

57.   **Machhada A, Trapp S, Marina N, Stephens RCM, Whittle J, Lythgoe MF, Kasparov S, Ackland GL, and Gourine AV.** Vagal determinants of exercise capacity. *Nat Commun* 8: 15097, 2017.

58.   **Maciel BC, Gallo Junior L, Marin Neto JA, Lima Filho EC, Terra Filho J, and Manco JC.** Parasympathetic contribution to bradycardia induced by endurance training in man. *Cardiovasc Res* 19: 642-648, 1985.

59.   **Mann TN, Webster C, Lamberts RP, and Lambert MI.** Effect of exercise intensity on post-exercise oxygen consumption and heart rate recovery. *Eur J Appl Physiol* 114: 1809-1820, 2014.

60.   **Mastitskaya S, Basalay M, Hosford PS, Ramage AG, Gourine A, and Gourine AV.** Identifying the Source of a Humoral Factor of Remote (Pre)Conditioning Cardioprotection. *PLoS One* 11: e0150108, 2016.

61.   **Mastitskaya S, Marina N, Gourine A, Gilbey MP, Spyer KM, Teschemacher AG, Kasparov S, Trapp S, Ackland GL, and Gourine AV.** Cardioprotection evoked by remote ischaemic preconditioning is critically dependent on the activity of vagal pre-ganglionic neurones. *Cardiovasc Res* 95: 487-494, 2012.

62.   **McCrory C, Berkman LF, Nolan H, O'Leary N, Foley M, and Kenny RA.** Speed of Heart Rate Recovery in Response to Orthostatic Challenge. *Circ Res* 119: 666-675, 2016.

63.   **Monfredi O, Lyashkov AE, Johnsen AB, Inada S, Schneider H, Wang R, Nirmalan M, Wisloff U, Maltsev VA, Lakatta EG, Zhang H, and Boyett MR.** Biophysical characterization of the underappreciated and important relationship between heart rate variability and heart rate. *Hypertension* 64: 1334-1343, 2014.

64.   **Myers J, Hadley D, Oswald U, Bruner K, Kottman W, Hsu L, and Dubach P.** Effects of exercise training on heart rate recovery in patients with chronic heart failure. *Am Heart J* 153: 1056-1063, 2007.

65.   **Nederend I, Schutte NM, Bartels M, Ten Harkel AD, and de Geus EJ.** Heritability of heart rate recovery and vagal rebound after exercise. *Eur J Appl Physiol* 116: 2167-2176, 2016.

66.   **Nishime EO, Cole CR, Blackstone EH, Pashkow FJ, and Lauer MS.** Heart rate recovery and treadmill exercise score as predictors of mortality in patients referred for exercise ECG. *JAMA* 284: 1392-1398, 2000.

67.   **Ordway GA, Charles JB, Randall DC, Billman GE, and Wekstein DR.** Heart rate adaptation to exercise training in cardiac-denervated dogs. *J Appl Physiol Respir Environ Exerc Physiol* 52: 1586-1590, 1982.

68.   **Paton JF, Boscan P, Pickering AE, and Nalivaiko E.** The yin and yang of cardiac autonomic control: vago-sympathetic interactions revisited. *Brain Res Brain Res Rev* 49: 555-565, 2005.

69.   **Pauza DH, Skripka V, Pauziene N, and Stropus R.** Morphology, distribution, and variability of the epicardiac neural ganglionated subplexuses in the human heart. *Anat Rec* 259: 353-382, 2000.

70.   **Pearson MJ and Smart NA.** Exercise therapy and autonomic function in heart failure patients: a systematic review and meta-analysis. *Heart Fail Rev* 23: 91-108, 2018.

71.   **Penela P, Murga C, Ribas C, Tutor AS, Peregrin S, and Mayor F, Jr.** Mechanisms of regulation of G protein-coupled receptor kinases (GRKs) and cardiovascular disease. *Cardiovasc Res* 69: 46-56, 2006.

72.   **Pomeranz B, Macaulay RJ, Caudill MA, Kutz I, Adam D, Gordon D, Kilborn KM, Barger AC, Shannon DC, Cohen RJ, and et al.** Assessment of autonomic function in humans by heart rate spectral analysis. *Am J Physiol* 248: H151-153, 1985.

73.   **Ramirez J, Duijvenboden SV, Ntalla I, Mifsud B, Warren HR, Tzanis E, Orini M, Tinker A, Lambiase PD, and Munroe PB.** Thirty loci identified for heart rate response to exercise and recovery implicate autonomic nervous system. *Nat Commun* 9: 1947, 2018.

74.   **Randall WC, Ardell JL, Wurster RD, and Milosavljevic M.** Vagal postganglionic innervation of the canine sinoatrial node. *J Auton Nerv Syst* 20: 13-23, 1987.

75.   **Rowell LB.** *Human Cardiovascular Control.* New York, NY: Oxford University Press, 1993.

76.   **Sacknoff DM, Gleim GW, Stachenfeld N, and Coplan NL.** Effect of athletic training on heart rate variability. *Am Heart J* 127: 1275-1278, 1994.

77.   **Sampaio KN, Mauad H, Spyer KM, and Ford TW.** Differential chronotropic and dromotropic responses to focal stimulation of cardiac vagal ganglia in the rat. *Exp Physiol* 88: 315-327, 2003.

78.   **Seki A, Green HR, Lee TD, Hong L, Tan J, Vinters HV, Chen PS, and Fishbein MC.** Sympathetic nerve fibers in human cervical and thoracic vagus nerves. *Heart Rhythm* 11: 1411-1417, 2014.

79.   **Shimizu S, Akiyama T, Kawada T, Shishido T, Yamazaki T, Kamiya A, Mizuno M, Sano S, and Sugimachi M.** In vivo direct monitoring of vagal acetylcholine release to the sinoatrial node. *Auton Neurosci* 148: 44-49, 2009.

80.   **Singh S, Johnson PI, Javed A, Gray TS, Lonchyna VA, and Wurster RD.** Monoamine- and histamine-synthesizing enzymes and neurotransmitters within neurons of adult human cardiac ganglia. *Circulation* 99: 411-419, 1999.

81.   **Strange S, Secher NH, Pawelczyk JA, Karpakka J, Christensen NJ, Mitchell JH, and Saltin B.** Neural control of cardiovascular responses and of ventilation during dynamic exercise in man. *J Physiol* 470: 693-704, 1993.

82.   **Streuber SD, Amsterdam EA, and Stebbins CL.** Heart rate recovery in heart failure patients after a 12-week cardiac rehabilitation program. *Am J Cardiol* 97: 694-698, 2006.

83.   **Toner A, Jenkins N, Ackland GL, and Investigators P-OS.** Baroreflex impairment and morbidity after major surgery. *Br J Anaesth* 117: 324-331, 2016.

84.   **Ulphani JS, Cain JH, Inderyas F, Gordon D, Gikas PV, Shade G, Mayor D, Arora R, Kadish AH, and Goldberger JJ.** Quantitative analysis of parasympathetic innervation of the porcine heart. *Heart Rhythm* 7: 1113-1119, 2010.

85.   **Verlinden TJ, Rijkers K, Hoogland G, and Herrler A.** Morphology of the human cervical vagus nerve: implications for vagus nerve stimulation treatment. *Acta Neurol Scand* 133: 173-182, 2016.

86.   **Verweij N, van de Vegte YJ, and van der Harst P.** Genetic study links components of the autonomous nervous system to heart-rate profile during exercise. *Nat Commun* 9: 898, 2018.

87.   **Wang X, Huang ZG, Gold A, Bouairi E, Evans C, Andresen MC, and Mendelowitz D.** Propofol modulates gamma-aminobutyric acid-mediated inhibitory neurotransmission to cardiac vagal neurons in the nucleus ambiguus. *Anesthesiology* 100: 1198-1205, 2004.

88.   **Wasserman K.** Coupling of external to cellular respiration during exercise: the wisdom of the body revisited. *Am J Physiol* 266: E519-539, 1994.

89.   **White DW and Raven PB.** Autonomic neural control of heart rate during dynamic exercise: revisited. *J Physiol* 592: 2491-2500, 2014.

90.   **Yuan BX, Ardell JL, Hopkins DA, Losier AM, and Armour JA.** Gross and microscopic anatomy of the canine intrinsic cardiac nervous system. *Anat Rec* 239: 75-87, 1994.

Figure legends

Figure 1| Exercise capacity is reduced in subjects with cardiac vagal dysfunction. (A) Cardiopulmonary exercise test protocol for measuring the speed of heart rate recovery (HRR) after reaching peak tolerance. (B) Resting heart rate in subjects (n=1,293), stratified by heart rate recovery 1 minute after cessation of cardiopulmonary exercise testing (HRR$_1$). Numbers of subjects are indicated within bars. Resting heart rate is higher in the participants with reduced HRR. (C) In the same subjects, percentage population-predicted peak oxygen consumption decreases with reduced HRR. Data are presented as means±s.e.m. (panels B and C are adapted from Ref. 57).

Figure 2| Autonomic control of the heart during exercise. (A) Schematic representation of the prevailing concept of parasympathetic and sympathetic control of heart rate during exercise. According to this concept, vagal withdrawal is responsible for heart rate increases up to 100 beats min^{-1}, followed by activation of the sympathetic input mediating further heart rate increases above 100 beats min^{-1}. (B) Schematic representation of the parasympathetic and sympathetic contributions to the control of heart rate during exercise proposed by White and Raven (ref. 89). According to this model, significant cardiac vagal activity is maintained during exercise. In healthy humans, at ~140 beats min^{-1} sympathetic and parasympathetic influences are estimated to be approximately equal in strength, with sympathetic activity dominating heart rate control at higher exercise intensities. Yet, even at high exercise loads, vagal activity continues to modulate cardiac function alongside with heightened sympathetic activity (schematic adapted from Ref. 89).

Figure 3| Schematic illustration of dual innervation and key signalling mechanisms underlying control of cardiac function by the autonomic nervous system. Reciprocal inhibition prevents competitive effects taking place within the heart and is responsible for the phenomenon of a so-called "accentuated antagonism" whereby the effect of increased activity of one branch of the autonomic nervous system is accentuated by cross-inhibition of transmitter release from the nerve endings of the other branch (51). Mechanisms underlying modulation of G Protein-Coupled Receptor Kinase 2 (GRK2) and β-arrestin-2 (β-arr) expression by the vagus nerve remain to be determined. Cellular mechanisms downstream of cAMP and cGMP generation are not illustrated. AC - adenylyl cyclase; cAMP - cyclic adenosine monophosphate; M2R - muscarinic

acetylcholine receptor M2; sGC - soluble guanylyl cyclase; sGMP - cyclic guanosine monophosphate; β-AR - β-adrenoceptor.

Figure 4| Exercise capacity is determined by the activity of vagal preganglionic neurons that reside in the dorsal vagal motor nucleus (DVMN) of the brainstem. (A) Genetic targeting of the DVMN vagal preganglionic neurons in rats to express an inhibitory G_i-protein-coupled *Drosophila* allatostatin receptor. *Left*: Schematic drawings of the rat brain in saggital and coronal projections illustrating the anatomical location of the DVMN, *Right*: photomicrograph of a representative coronal section of the rat dorsal brainstem illustrating the distribution of the DVMN neurons transduced to express the receptor in the caudal region of the nucleus. Arrows point at ventrally projecting axons of the transduced vagal neurons (forming the efferent vagus nerve). XII, hypoglossal motor nucleus (cells are not visible); eGFP-enhanced green fluorescence protein; CC, central canal. Scale bar: 200 μm. (B) Summary data illustrating the effect of allatostatin receptor ligand (insect peptide allatostatin) administration on exercise capacity in rats transduced to express control transgene (left) or allatostatin receptor (right) by the DVMN neurons. Allatostatin binding to allatostatin receptor results in rapid and reversible silencing of mammalian neurons transduced to express this receptor (49, 61). Data are presented as individual values and means±s.e.m. Comparisons are made using ANOVA. (Figure adapted from Ref. 57).

A

Heart rate (bpm) vs Time (min); graded exercise and recovery phases.

cardiac vagal dysfunction (minimal HRR)

preserved cardiac vagal activity (sharp decline from peak heart rate)

graded exercise

recovery

B

Resting heart rate (bpm) vs HRR_1

| <0 | 0-12 | 12-24 | 24-36 | >36 |
| 69 | 432 | 509 | 208 | 75 |

*

C

Peak V_{O_2} (% predicted) vs HRR_1

| <0 | 0-12 | 12-24 | 24-36 | >36 |
| 69 | 432 | 509 | 208 | 75 |

*

A

strength of heart rate modulation

workload →

vagus

sympathetic

heart rate (bpm)

B

relative contribution to heart rate control (%)

workload →

vagus

sympathetic

heart rate (bpm)

A

eGFP

DVMN

XII   CC

5 mm   1 mm

○— control transgene (n=8)
●— allatostatin receptor (n=8)

B

Work done (kJ)

4.0

3.0

2.0

1.0

baseline   ligand
control   +24 h

Work done (kJ)

4.0

3.0

2.0

1.0

p<0.001   p<0.001

baseline   DVMN
inhibited   +24 h