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Integrated Cardio-Respiratory Control: Insight in Diabetes

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Abstract Autonomic dysfunction is a frequent and relevant complication of diabetes mellitus, as it is associated with increased morbidity and mortality. In addition, it is today considered as predictive of the most severe diabetic complications, like nephropathy and retinopathy. The classical methods of screening are the cardiovascular reflex tests and were originally interpreted as evidence of nerve damage. A more modern approach, based on the integrated control of cardiovascular and respiratory function, reveals that these abnormalities are to a great extent functional, at least in the early stage of the disease, thus suggesting new potential interventions. Therefore, this review aims to go further investigating how the imbalance of the autonomic nervous system is altered and can be influenced in many chronic pathologies through a global view of cardio-respiratory and metabolic interactions and how the same mechanisms are applicable to diabetes.

Keywords Chemoreflex · Baroreflex · Heart rate variability · Diabetic neuropathy · Hypoxia · Autonomic nervous system · Cardio-respiratory interactions

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Introduction

Autonomic abnormalities have long been described in patients with diabetes. These include both clinical symptoms related to many organs (e.g., gut, bladder, reproductive system) and functions (e.g., sweating, posture) but also subclinical abnormalities that could be revealed by four or five laboratory tests, originally described by Ewing [1] and recently reviewed [2••], and based on cardiovascular reflexes: heart rate variation during deep breathing, heart rate and blood pressure variation at the beginning of reaching the upright posture, heart rate changes during the Valsalva maneuver, and the blood pressure response to handgrip. Since the clinical symptoms are relatively infrequent and the laboratory abnormalities are not clinically appreciable by the patient, the relevance of the autonomic dysfunction is often dismissed.

Nevertheless, subclinical autonomic dysfunction in diabetes mellitus is probably one of the most relevant complications, as it is associated with increased morbidity and mortality [3], and in addition it is today considered as predictive of the most severe diabetic complications, like nephropathy and retinopathy [4–6].

In diabetic research, the abnormalities in the cardiovascular reflex test are considered an evidence of "neuropathy" (e.g., nerve damage). This concept in fact derives from the first observations of highly compromised, almost denervated patients [7, 8]. However, nowadays those extreme observations are much less frequent, due to the tremendous improvement in diabetic care, while still the frequency of subclinical autonomic abnormalities remains very high, even before full-blown diabetes develops [9]. These evidences make inconsistent the attribution to "neuropathy," at least in the initial or uncomplicated stage of diabetes, and point to a different interpretation of autonomic abnormalities.

Indeed, the same alterations in the cardiovascular autonomic tests can easily result from an imbalance of the autonomic nervous system, with parasympathetic withdrawal and sympathetic overactivation, rather than a nerve loss. This concept is largely supported by observations in other clinical conditions, like heart failure, hypertension, and chronic respiratory diseases, where the same observations are considered as evidence of autonomic "dysfunction" or "imbalance." There are two major theoretical and practical consequences of this different interpretation: (1) if indeed the neuropathy is preceded by a period more or less prolonged of dysfunction, this implies the potential of reversibility of this condition in diabetes and, in view of the established prognostic and predictive relevance of autonomic dysfunction, this also paves the way to new potential preventive strategies for the diabetic complications, and (2) a better understanding of the mechanisms underlying the cardiovascular autonomic tests and their abnormalities becomes essential.

A comprehensive evaluation of the autonomic function should therefore consider not just changes in heart rate in response to some stimulus, but both the mechanisms that determine such changes. These are multifarious, but mainly involve the interplay of the control of blood pressure (mainly via the baroreflexes) together with the control of respiration (via the chemoreflexes) and their interaction.

Chemoreflexes and baroreflexes are tightly related (Fig. 1): the chemoreflex induces a ventilatory response that, via the sympathetic activation, is one of the most powerful modulators of the arterial baroreflex, through direct interactions at the integration centers and through respiratory-induced changes in venous return, stroke volume, and blood pressure, which modulate heart rate through the baroreflex [10, 11]. Baroreflex alterations in turn modulate ventilation [12••]. Only with a global view on the autonomic abnormalities can we understand their real nature, hence we treat them appropriately.

Blood Pressure Regulation: the Baroreflex

The arterial baroreceptors, located mainly in the carotid bodies and in the aortic arch, respond to acute increases (or decreases) in blood pressure with both an increase (or decrease) in parasympathetic (vagal) firing rate to the heart, leading to a reduction (or increase) in heart rate and thus a transient reduction (or increase) in cardiac output and blood pressure, and also to a reduction (or increase) in the sympathetic firing rate to the vessels, leading to a decrease (or increase) in peripheral resistances. This reflex has the effect of stabilizing the blood pressure. The arterial baroreflex, though only one of the many factors determining the blood pressure and its variations, is considered the most important factor regulating the blood pressure in the short term.

Baroreflex Sensitivity—Methodology

The parasympathetic cardiac arm of the baroreflex can be easily measured, either by evaluating the heart rate change to pharmacological interventions transiently increasing or decreasing the blood pressure as originally proposed [13], or more recently, by examining the heart rate responses to spontaneous fluctuations in blood pressure (for example, periods of spontaneous increases or decreases in blood pressure, or else fluctuations at 0.1 Hz or at the frequency of respiration). The spontaneous methods showed to be a robust measure of baroreflex sensitivity (BRS) with the advantage of not requiring interventions [14–16].

A detailed description of the methods for obtaining the baroreflex sensitivity is reported in previous publications [16, 17•, 18••]. In practice, many methods are available, even based on complex mathematical algorithms, but often these methods do not agree with each other. To overcome these problems, our group recently proposed a new technique, based on a simple mathematical algorithm (the ratio of standard deviation of RR intervals/standard deviation of systolic blood pressure) which showed the best agreement with all other established methods [17•, 19]. Alternatively, the average or the median of all main available methods is a cumbersome but more robust option than using a single standard method [17•, 19, 20].

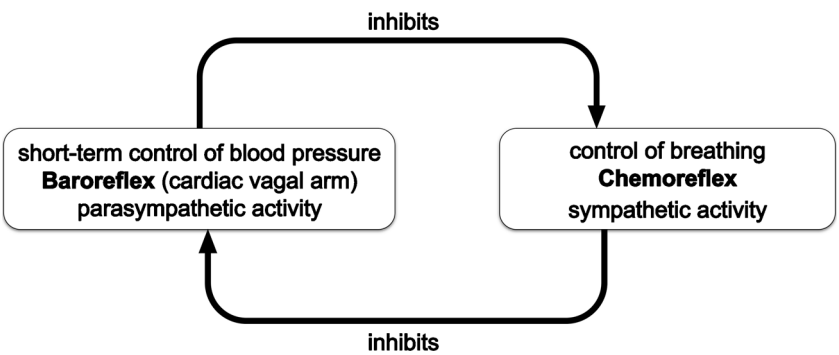

Fig. 1 Simplified *diagram* of the interactions between the control of blood pressure and the control of breathing

Clinical and Prognostic Significance of Baroreflex Abnormalities

The analysis of the spontaneous baroreflex can provide a more sensitive diagnosis of autonomic dysfunction than cardiovascular reflex tests as repeatedly shown in several studies [21, 22••], thereby allowing abnormalities of autonomic cardiac modulation to be identified at an early stage and quantified in a graded fashion [21, 22••]. It also showed a predictive value for hypertension in a 5-year follow-up [23].

In cardiology, the baroreflex sensitivity is a well-established predictor of cardiovascular mortality. The multicenter Autonomic Tone and Reflexes After Myocardial Infarction (ATRAMI) study provides clinical evidence that after myocardial infarction the analysis of BRS has significant prognostic value independently of left ventricular ejection fraction and of ventricular arrhythmias [24••]. The Cardiovascular Autonomic Neuropathy (CAN) Subcommittee of the Toronto Diabetic Neuropathy Expert Group consider heart rate variability and BRS (especially the cardiac-vagal arm) as important tests to evaluate the autonomic function [18••]. The BRS assessment is an important component of autonomic testing as it combines information derived from both heart rate and blood pressure; moreover, it has an independent prognostic value in cardiac and diabetic patients as it is a predictor of sudden death and all-cause mortality [25•, 26•].

Regulation of Breathing: the Chemoreflex

There are at least two types of receptors directly connected to the regulation of breathing: the "central chemoreceptors," located in brain tissue of the medulla, more sensible to local hydrogen ions and to carbon dioxide, and the "peripheral" chemoreceptors, located in the carotid bodies, mainly sensible to hypoxia and hydrogen ions [27]. Other receptors located in the muscles play also an important role in heart failure [28••], but still need to be studied in diabetes.

The chemoreceptors increase the frequency and depth of breathing in response to a drop in the oxygen arterial partial pressure or to an increase in the arterial partial pressure of carbon dioxide, potassium, or hydrogen ions.

Chemoreflex Sensitivity—Methodology

The central and the peripheral chemoreflex responses may be individually evaluated by specific techniques. The most used today is the "rebreathing method." Rebreathing into a closed circuit causes a progressive reduction in the inspired oxygen and an increase in the carbon dioxide concentration, both of which stimulate ventilation. To assess the response to varying oxygen concentrations (hypoxic chemoreflex), the end-tidal carbon dioxide pressure ($CO_{2\text{-et}}$) is kept constant at baseline values by absorbing its excess with soda lime. To test the response to changes in carbon dioxide concentration (hypercapnic chemoreflex), oxygen is continuously supplied at a very low flow to maintain the percentage of arterial oxygen saturation (SaO_2) at baseline values (>97 %). The tests end when SaO_2 or $CO_{2\text{-et}}$ reach a target value (80 % and 10–15 mmHg above baseline, respectively). The chemoreflex sensitivity to hypoxia (hypoxic ventilatory response) or hypercapnia (hypercapnic ventilatory response) is obtained from the slope of the linear regression linking minute ventilation vs SaO_2 or CO_{2et}, respectively [11]. Alternatively, the gas concentration can be varied in several steps by inspiring different gas mixtures (hypoxic or hypercapnic) during either single breaths or fixed periods of time (e.g., 5-min, steady-state method). Despite the theoretical precision of the steady-state method, the fairly long time needed to reach a steady-state ventilation in response to a given concentration of inhaled gas caused severe problems in patients with chemoreflex abnormalities [29, 30]. Conversely, the rebreathing method resulted to be entirely safe [31]. Other simpler methods measure the minute ventilation in response to the carbon dioxide production (VE/VCO_2 ratio), which may prove useful particularly during exercise [32••], and the even simpler ratio of tidal volume/inspiratory time [33]. These simpler methods estimate a "global chemosensitivity" and can be applied to continuous monitoring, but do not provide specific information on the central or peripheral chemoreceptors.

Clinical and Prognostic Significance of Chemoreflex Abnormalities

Chemoreflex abnormalities play an important role in the pathogenesis and progression of heart failure. An augmented peripheral chemoreflex in chronic heart failure patients is associated with hyperpnoea and tachycardia which increase the severity of this pathology in terms of symptoms and exercise limitation [34]. In turn, the tonic activation of excitatory chemoreflex afferents contributes to increased efferent sympathetic activity that worsens cardiovascular prognosis [35]. Thus, this process initiates a vicious circle in which the sympathetic activity increases and the clinical condition worsens. An increased chemoreflex highlights patients with more severe heart failure and is an independent negative prognostic marker [36•]. Recent studies show that ablation of the carotid body chemoreceptors improves autonomic function and breathing control in heart failure and improves survival [37, 38]. Additionally, the autonomic dysfunction assessed by altered chemoreflex sensitivity predicts mortality in patients with multiple organ dysfunction syndrome [39].

Baroreflex-Chemoreflex Interaction

Evidence from animal models and human studies showed that there is an antagonistic interaction between the chemoreflex and the baroreflex, comprehensively reviewed in [12••] and outlined in Fig. 1. For instance, in man [40], baroreflex activation selectively abolished the sympathetic nerve activity response to hypoxia (move above citation here). This specific interaction between the baroreceptors and the peripheral chemoreceptors was explained by the convergence of baroreceptor and peripheral chemoreceptor afferents on neurons in the medulla. Thus, baroreceptors exert a restraining influence on the excitatory effect of chemoreceptors during hypoxia. In another study that included 13 healthy volunteers, stimulation of chemoreceptors by hypercapnia or hypoxia reduced heart rate variability and baroreflex sensitivity. Conversely, stimulation of chemoreceptors by hypercapnia or hypoxia reduces heart rate variability and baroreflex sensitivity [11]. Combined hypoxia and hypercapnia have a synergistic effect on sympathetic activity as well as on minute ventilation [41].

Baro-chemoreflex Interaction and Sympathovagal Imbalance in Chronic Pathologies

Several studies showed that many relevant cardiovascular/respiratory diseases (such as heart failure, hypertension, obstructive sleep apnea syndrome, and chronic obstructive pulmonary diseases) exhibit potential vicious circles of reflex derangements: the loss of cardiovascular control (baroreflex attenuation) interplays with increased breathing stimulation (chemoreflex augmentation) through a sustained sympathetic activation and a reduced vagal activity [28••, 42••].

Congestive Heart Failure

Chronic congestive heart failure (CHF) is characterized by a loss of pump function of the heart. The reduced cardiac output and the resulting tissue hypoxia stimulate the sympathetic activity leading to vasoconstriction, with increased afterload, and hyperventilation. The increased afterload intensifies the heart work, while the increased ventilation lowers the threshold for the onset of dyspnea and thus reduces the exercise performance [36•]. Peripheral vasoconstriction aggravates tissue hypoxia, which, together with the sympathetic activation, leads to chronic low-grade inflammation, insulin resistance (both often promoting diabetes development), endothelial dysfunction, and oxidative stress.

The impaired cardiac autonomic activity in CHF (expressed as reduced heart rate variability or depressed arterial baroreflex sensitivity) is associated with an increased peripheral chemosensitivity, suggesting a link between the chemoreflex and the baroreflex in a clear inverse relationship [43•], contributing further to sympathetic overactivity, blunted baroreflex function, and ultimately poor prognosis in CHF patients [44].

Thus, these autonomic, metabolic, and cardiovascular abnormalities are linked in a progressive vicious circle (Fig. 2). For instance, a study conducted in 14 men with CHF demonstrated a significant positive correlation between sympathetic nerve activity and breathing frequency and an inverse correlation between sympathetic activity and tidal volume [45••]. Hence, the patients with rapid shallow breathing seem to exhibit the highest degree of sympathetic activation and the worse prognosis [45••]. Slow-deep breathing training improves oxygenation, heart rate variability, and exercise performance in heart failure [46]. The abnormalities described above are thus implicated in the reduced exercise performance of the patients with heart failure, and interventions aimed at restoring the cardio-respiratory control (e.g., physical exercise) improved both cardiovascular regulation and exercise tolerance, hence resulting in important clinical improvement in these patients [47].

Obstructive Sleep Apnea Syndrome

Obstructive sleep apnea syndrome (OSAS) is frequent in type 2 diabetic patients. Diabetes and OSAS exert a bidirectional influence, with diabetes predisposing to or aggravating OSAS, and OSAS predisposing to or aggravating diabetes [48••]. Furthermore, there is a large amount of evidence linking OSAS, the intermittent hypoxia, and the carbon dioxide retention that accompany it, with the development of hypertension [49•, 50]. The chemoreflex responds acutely to intermittent hypoxic exposure with an increase in sympathetic activity; the subsequent rise in blood pressure initiates a compensatory baroreflex response that attempts to buffer these changes. This mechanism was originally described only for the central apneas, but it is now clear that the autonomic nervous system is highly involved also in the obstructive apneas [51]. With chronic exposure to intermittent hypoxia, a sensitization of peripheral chemoreflex occurs. Conversely, baroreflex may become desensitized: the exposure to intermittent hypoxia resets the baroreflex to operate at higher levels of sympathetic activity and blood pressure [52]. During the early phase of apnea, the muscle sympathetic nerve activity (MSNA) is suppressed; it then increases constantly and reaches a peak at the end of apnea and on arousal. The resumption of ventilation occurs in the context of peripheral vasoconstriction and increased peripheral resistance. This situation persists for several seconds after the MSNA has ceased, due to the kinetics of norepinephrine uptake, release, and washout at the neurovascular junction. The alterations in the autonomic nervous system are carried over into wakefulness and may contribute to the development of the cardiovascular disorders associated with OSAS, including sympathovagal

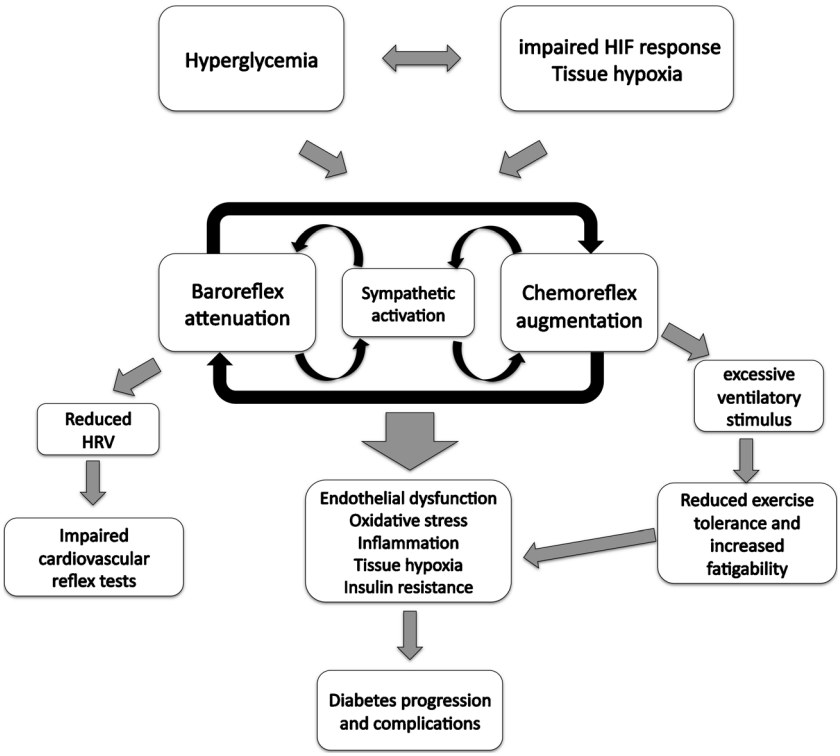

Fig. 2 *Diagram* of the abnormal interactions between the control of blood pressure and the control of breathing in diabetes

imbalance. Several neural and humoral mechanisms may contribute to maintenance of higher sympathetic activity and blood pressure even during daytime wakefulness when subjects are breathing normally, and no evidence of hypoxia or chemoreflex activation is apparent. These mechanisms include chemoreflex and baroreflex dysfunction, altered cardiovascular variability, vasoconstrictor effects of nocturnal endothelin release, and endothelial dysfunction [53]. Notably, it has been reported that hemodynamic and autonomic dysfunction associated with OSAS may improve with continuous positive airway pressure (CPAP) [54, 55]). One month of treatment of nasal CPAP readjusted the peripheral oxygen chemosensitivity (normocapnic hypoxic ventilatory response reduced in patients but not in controls). This change may be a side effect of both reduced sympathetic activity and increased baroreflex activity, or a possible CPAP-related mechanism leading to a reduced activation of autonomic nervous system per se [56].

Obstructive and Restrictive Lung Diseases

Conditions like chronic obstructive pulmonary disease (COPD) and restrictive lung diseases that induce respiratory failure and chronic hypoxemia may lead to autonomic imbalance with an increased sympathetic activity. MSNA was higher in patients with chronic respiratory failure compared with healthy matched subjects. Additionally, the sympathetic activity decreased during oxygen administration in the patients but not in the controls. The higher sympathetic tone could be explained by the arterial chemoreflex activation present in these patients and may play an important role in the pathogenesis of the disease [57]. COPD causes neurohumoral activation, and its negative consequences, namely, inflammation, cachexia, skeletal muscle dysfunction, and insulin-resistance, give rise to a self-perpetuating cycle that contributes to the pathogenesis of COPD [58], similar to CHF (Fig. 2). In COPD, MSNA showed evidence of sympathetic overactivation and decreased baroreflex sensitivity even in normoxic patients. These findings suggest the sympathovagal imbalance as a pathophysiological phenomenon in COPD [59•]. A study testing the effect of hypoxic training in patients with mild COPD demonstrated the presence of cardiovascular autonomic abnormalities (depressed baroreflex sensitivity) even if there were no chemoreflex abnormalities. After training, baroreflex sensitivity increased up to normal levels and hypercapnic ventilatory response increased as well without changes in hypoxic ventilatory response [60]. The administration of oxygen or the slowing of breathing rate resulted in significant improvement of BRS and a decrease in mean heart rate and arterial pulse pressure in patients with COPD [59•, 61].

Cardio-Respiratory Control in Diabetes

Although some studies have investigated the hypothesis of an altered cardiovascular or respiratory control in diabetes, the chemoreflexes and baroreflexes were tested only in a small number of patients and only separately. Thus, in diabetes, the cardio-respiratory-integrated control remains to a large extent to be investigated.

Table 1 Comparative schematic effects of slow breathing and physical training, with respect to the baroreflex-chemoreflex interaction, as demonstrated in different pathologies (CHF, COPD, hypertension, diabetes)

Effect	Slow breathing		Physical training	
	Short-term	Long-term	Short-term	Long-term
Sympathetic suppression	+	?	−	+
Parasympathetic enhancement	+	?	−	+
Increase in BRS	+	?	−	+
Increase in heart rate variability	+	?	−	+
Reduced chemoreflex hyperactivity	+	+	−	+
Arterial/tissue oxygenation	+	?	+	+
Improved exercise capacity		+		+
Anti-inflammatory effect	?	?	−	+
Antioxidant effect	+	?	−	+

+ positive effect, − negative effect, *?* unknown

It is known that the impairment of autonomic function could be observed at an early stage of diabetes by a depressed BRS [14, 21]. Nevertheless, diabetic patients are able to increase BRS in response to slow deep breathing, an intervention capable of reducing the sympathetic and enhancing the parasympathetic activity. In contrast, really denervated patients (as for instance after heart transplantation) could not increase BRS. These findings indicate that the autonomic nervous system of type 1 diabetic patients may still be able to react to a simple intervention and thus that the abnormalities in type 1 diabetes are to some extent functional at least at an early stage of the disease [22••]. Another study showed that both oxygen and slow breathing increased BRS in a group of 96 type 1 diabetic participants, in which BRS was depressed at baseline, equally or more than in healthy control participants. The larger-than-normal response to hyperoxia suggests a pre-existing condition of tissue hypoxia that functionally restrains parasympathetic activity in these patients. These studies indicate that autonomic abnormalities can be partially and temporarily reversed by simple maneuvers such as slow breathing or oxygen administration through enhancement of parasympathetic activity and/or correction of tissue hypoxia [62••]. Similar results (improvement in BRS) were found also in type 2 diabetic patients with chronic kidney disease after two different procedures that increase blood oxygenation (slow deep breathing and oxygen administration), suggesting a functional role of hypoxia even in complicated diabetic patients [63•]. All these findings suggest a potential link between baroreflex abnormalities and impaired control of respiration (chemoreflexes).

There are only a few studies evaluating the chemoreflexes in diabetes, indicating in general a reduced response to oxygen and a normal or exaggerated response to carbon dioxide. However, these studies were carried out in small groups of subjects, evaluating either the central [64••], the peripheral [65•] chemoreflex, or both [66•], but none included a measure of BRS. .

In a preliminary study carried out in 46 type-1 diabetic and 103 control subjects, we showed that in diabetic patients the reduced BRS is accompanied by an enhancement in the hypercapnic chemoreflex, whereas the responses to hypoxia showed a trend toward a reduction, despite that the resting oxygen saturation was significantly depressed [67].

These findings suggest a possible role of hypoxia (in association to hyperglycemia) in inducing BRS and chemoreflex dysfunction, as well as downstream diabetic complications [68•, 69]. Hyperglycemia and other cellular abnormalities (low-grade inflammation, oxidative stress) seem to blunt the function of hypoxia-inducible factor-1 (HIF-1), a transcription factor that is essential for adaptive responses of the cells to hypoxia [70•, 71••]. At the same time, the hypercapnia-induced sympathetic activation might in turn depress baroreflex, heart rate variability, and autonomic function tests [41]. A schematic representation of these mechanisms is shown in Fig. 2.

Conclusion

What We Learned and What Is the Practical Significance of the Abnormal Integration of Cardiovascular and Respiratory Reflexes in Diabetes?

This review underlines the basic concept that the regulations of ventilation and blood pressure are tightly interconnected, that this interplay is altered in diabetes, and that hypoxia, either/both as a cause or as a consequence, is present in diabetes [62••]. Starting from this new approach, we showed that early autonomic dysfunction is reversible [22••] and that rebalancing of the autonomic reflexes and correction of hypoxia are theoretically possible. Because respiration is not only automatic but also under volitional control, a specific respiratory pattern may interfere with both the cardiovascular and the respiratory systems, thus potentially helping the restoration of a more physiological balance. We and others showed that slow deep breathing transiently improves oxygen saturation, enhances baroreflex sensitivity, and reduces the chemoreflex response to both hypoxia and hypercapnia [11]

in healthy subjects; in patients with CHF [72], COPD [59•], or hypertension [73]; and in diabetes patients [22••], in addition to suppressing the sympathetic nerve activity in conditions of sympathetic activation such as CHF [45••, 74] and COPD [59•]: more recent results indicate that the vagal stimulation exerted by slow breathing reduces the free radical excess in diabetic patients [75], similar to what occurs with vagal nerve stimulators in heart failure [76–78]. This apparently simple intervention has in fact a multidimensional effect and reveals potentials in diabetic autonomic dysfunction, as it seems to correct several basic problems, at least in the short-term application. In the long term, promising results seem to occur in diabetes by effect of yoga, a technique in which various forms of slow breathing are largely employed [79, 80]. In addition, the same results (improvement in tissue oxygenation, reduction of chemoreflex activation, improvement in parasympathetic activity and BRS, increase in antioxidant reserve, and reduced free radical excess) that can be obtained with slow breathing also occur with physical exercise (Table 1). This review then suggests a rather new reason for encouraging physical exercise in diabetes, in addition to other potential treatments, like hypoxic training [20, 81] or drugs which stabilize and activate HIF [82]. All these interventions, based on the knowledge of the cardio-respiratory reflex interaction, have the potential to be helpful in improving the autonomic dysfunction and the sensitivity to hypoxia and finally prevent diabetic complications.

Compliance with Ethical Standards

Conflict of Interest Luciano Bernardi and Lucio Bianchi declare that they have no conflict of interest.

Human and Animal Rights and Informed Consent This article does not contain any studies with human or animal subjects performed by any of the authors.

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