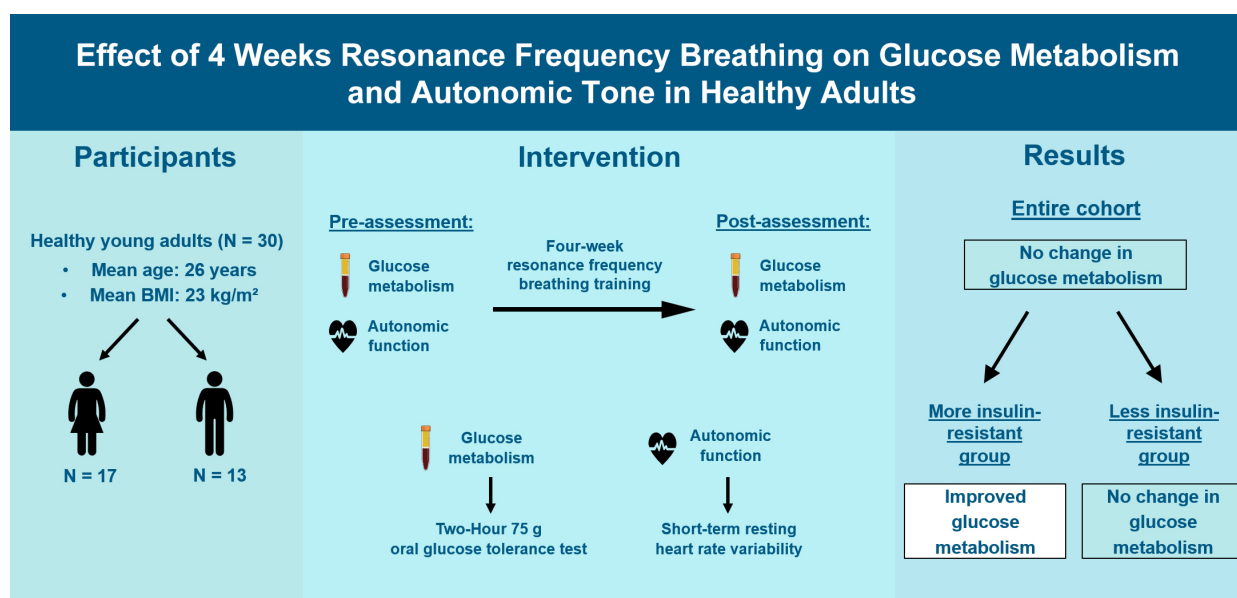


Effect of 4 Weeks Resonance Frequency Breathing on Glucose Metabolism and Autonomic Tone in Healthy Adults

Benedict Herhaus, Andreas Peter, Julia Hummel, Thomas Kubiak, Martin Heni, Katja Petrowski

Diabetes Metab J 2025;49:1219-1228 | <https://doi.org/10.4093/dmj.2024.0647>



Conclusion

Breathing intervention increases vagal activity in healthy individuals and may improve glucose metabolism in those with higher insulin resistance before the intervention.



Highlights

- Breathing intervention increased vagal activity in healthy adults
- Breathing intervention did not influence glucose metabolism in healthy adults
- More insulin-resistant adults improved glucose metabolism via breathing intervention

How to cite this article:

Herhaus B, Peter A, Hummel J, Kubiak T, Heni M, Petrowski K. Effect of 4 Weeks Resonance Frequency Breathing on Glucose Metabolism and Autonomic Tone in Healthy Adults. Diabetes Metab J 2025;49:1219-1228. <https://doi.org/10.4093/dmj.2024.0647>

Effect of 4 Weeks Resonance Frequency Breathing on Glucose Metabolism and Autonomic Tone in Healthy Adults

Benedict Herhaus¹, Andreas Peter², Julia Hummel³, Thomas Kubiak⁴, Martin Heni^{2,3,*}, Katja Petrowski^{1,*}

¹Medical Psychology and Medical Sociology, University Medical Center of the Johannes Gutenberg University Mainz, Mainz, Germany

²Department for Diagnostic Laboratory Medicine, Institute for Clinical Chemistry and Pathobiochemistry, University Hospital Tübingen, Tübingen, Germany

³Division of Endocrinology and Diabetology, Department of Internal Medicine I, Ulm University Hospital, Ulm, Germany

⁴Health Psychology, Institute for Psychology, Johannes Gutenberg University, Mainz, Germany

Background: The autonomic nervous system plays a crucial role in the brain's communication with metabolically important peripheral organs, modulating insulin sensitivity and secretion. Increased sympathetic tone is a common feature in prediabetes and diabetes. The parasympathetic nervous system activity might be improvable through resonance frequency breathing (RFB) with heart rate variability biofeedback (HRV-BF) training.

Methods: We here investigated the effect of a 4-week mobile RFB-HRV-BF intervention on glucose metabolism and HRV of 30 healthy adults (17 females; mean age 25.77 ± 3.64 years; mean body mass index 22.65 ± 2.95 kg/m²). Before and after the intervention, glucose metabolism was assessed by 75 g oral glucose tolerance tests (with blood sampling every 30 minutes over 2 hours) and HRV was measured through electrocardiography.

Results: RFB-HRV-BF training did not influence glucose metabolism in healthy adults but reduced fasting as well as 2-hour-post-load glucose in participants categorized as more insulin resistant before the intervention. In addition, RFB-HRV-BF training was associated with an increase in the time and frequency domain HRV parameters standard deviation of all NN-intervals, root mean square successive differences, HRV high-frequency and HRV low-frequency after 4 weeks of intervention.

Conclusion: Our findings introduce RFB-HRV-BF training as an effective tool to modulate the autonomic nervous system with a shift towards the parasympathetic tone. Along with the observed decrease in glycemia in those with lower insulin sensitivity, RFB-HRV-BF training emerges as a promising non-pharmacological approach to improve glucose metabolism which has to be further investigated in prediabetes and diabetes.

Keywords: Blood glucose; Breathing exercises; Parasympathetic nervous system; Vagus nerve; Young adult

INTRODUCTION

The global prevalence of diabetes has quadrupled from 1980 to 2014 and numbers are further increasing worldwide [1]. Dia-

betes is linked to cardiovascular disease, nephropathy, retinopathy, neuropathy, and further complications, with ultimately lead to higher mortality in those living with the disease [2].

The regulation of glucose metabolism is a complex process

Corresponding authors: Benedict Herhaus

 <https://orcid.org/0000-0002-3456-9735>

Medical Psychology and Medical Sociology, University Medical Center of the Johannes Gutenberg University Mainz, Duesbergweg 6, 55128 Mainz, Germany
E-mail: bherhaus@uni-mainz.de

Martin Heni  <https://orcid.org/0000-0002-8462-3832>

Division of Endocrinology and Diabetology, Department of Internal Medicine I, Ulm University Hospital, Ulm, Germany
E-mail: Martin.Heni@uniklinik-ulm.de

*Shared senior authorship.

This is an Open Access article distributed under the terms of the Creative Commons Attribution Non-Commercial License (<https://creativecommons.org/licenses/by-nc/4.0/>) which permits unrestricted non-commercial use, distribution, and reproduction in any medium, provided the original work is properly cited.

involving multiple organs that interact with each other [3]. While the autonomic nervous system (ANS) appears to play a significant role in this coordination, the extent of its contribution is still under investigation [4]. Nonetheless, increasing evidence indicates a critical connection between the ANS and glucose metabolism, suggesting that the ANS may have a crucial role in coordinating metabolic homeostasis [5]. Therefore, the brain communicates via the ANS with metabolically active organs to modulate insulin sensitivity and insulin secretion [3,6,7]. On the anatomical level, the ANS innervates key metabolic organs, including the liver and the pancreas to modulate glucose metabolism [8]. Of notice, brain derived modulation of both, insulin sensitivity and insulin secretion are thought to be impaired in people with type 2 diabetes mellitus [9]. In line, individuals with prediabetes and diabetes display an impaired balance between sympathetic and parasympathetic tone [10].

In humans, autonomic balance can be assessed through respiratory sinus arrhythmia, which measures natural variations in heart rate during the breathing cycle [11]. Respiratory sinus arrhythmia is regulated by vagal efferent pathways from the nucleus ambiguus of the brain [12]. Indeed, Heni et al. [13] found a positive correlation between changes in insulin sensitivity and the high-frequency (HF) band in the heart rate variability (HRV) spectrum, an index for respiratory sinus arrhythmia [14].

Additionally, the ANS affects glucose metabolism indirectly by regulating systemic inflammation through the cholinergic anti-inflammatory pathway. This pathway is primarily driven by stimulation of the dorsal motor nucleus of the vagus nerve, the main nerve of the parasympathetic nervous system [15]. There, efferent vagal neurons release acetylcholine, which binds to the $\alpha 7$ nicotinic receptors of cytokine-producing cells, including macrophages [16,17]. This anti-inflammatory pathway results in suppression of pro-inflammatory cytokine release and protection against systemic inflammation [18]. Impairments in the ANS thereby promote systemic subclinical inflammation, a pathophysiological contributor to whole-body insulin resistance [19].

There are still no specific therapeutic approaches to address the increased sympathetic tone in prediabetes and diabetes. Resonance frequency breathing (RFB) offers a potential method to stimulate the parasympathetic nervous system through slow-paced breathing frequencies. RFB maximizes HRV, a readout that mainly reflects the activity of the parasympathetic nervous system [11]. By rhythmizing breathing, heartbeat, and

blood pressure, RFB enhances parasympathetic activity while suppressing sympathetic activity [20].

To facilitate RFB, this approach is often paired with HRV-biofeedback (BF), i.e., the measurement and visualization of unconscious body processes causing variation in time between heartbeats, allowing individuals to actively influence these processes [11]. Several studies have demonstrated that RFB-HRV-BF interventions can improve HRV, reduce stress and anxiety, and alleviate symptoms in patients with various medical conditions [21].

Hence, we now tested the potential of RFB-HRV-BF training to affect whole-body glucose metabolism. To do this, we assessed glucose metabolism before and after a 4-week RFA-HRV-BF intervention in healthy adults.

METHODS

Study participants

A power analysis (G*power version 3.1.9.2., Heinrich Heine University, Düsseldorf, Germany) indicated that a sample size of 27 participants would be required to detect a medium effect size of Cohen's $d=0.5$ with a significance level of $P=0.05$ and a power of 80% ($1-\beta=0.80$), when using a dependent t -test to compare pre- and post-measurements. The study inclusion and exclusion criteria were checked in a standardized telephone interview using the Structured Clinical Interview for both axis I and axis II [22] according to the Diagnostic and Statistical Manual of Mental Disorders [23]. Exclusion criteria included any acute and/or chronic medical illness, medication affecting glucose metabolism (e.g., steroids), heart rate, or central nervous system (e.g., β blockers), history of prediabetes, mental disorders, illicit drug abuse, stressful life-events within the previous 6 months, and a body mass index <18.5 or >27 kg/m². Because of age-related attenuation of slow-paced breathing effects on cardiovascular variability [24], only participants between 18 and 35 years of age were included. Thirty healthy young adults were enrolled (Fig. 1). A detailed description of the participants is given in Table 1.

Study design

The participants were instructed to perform RFB with HRV-BF at home via an ambulatory training system over the course of 4 weeks. They were further instructed that no fundamental change in lifestyle, including diet and physical activity, should be taking place during the intervention period. This was

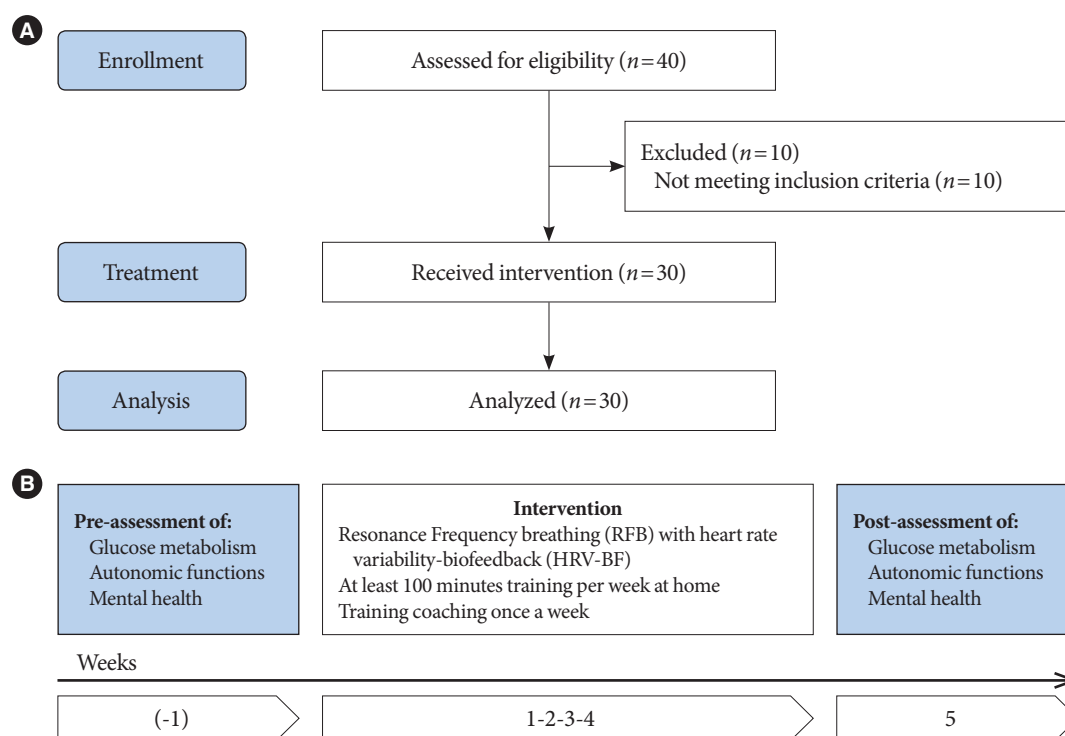


Fig. 1. Study outline. (A) Consolidated Standards of Reporting Trials (CONSORT) flow diagram. (B) Study design of resonance frequency breathing with heart rate variability biofeedback intervention.

Table 1. Characteristics of the participants ($n=30$)

Characteristic	Value
Age, yr	25.8 ± 3.6
Female sex	17 (57)
Smoking	5 (17)
Body mass index in kg/m ²	22.7 ± 3.0
Waist to hip ratio	0.84 ± 0.06
Systolic blood pressure, mm Hg	119 ± 10
Diastolic blood pressure, mm Hg	79 ± 7
BSA-total activity, min/wk	204 ± 141
BSI-18 in sum score	6.80 ± 8.59
PSS in sum score	12.57 ± 3.88
TICS-9 in sum score	10.60 ± 3.61

Values are presented as mean ± standard deviation or number (%). BSA, Physical Activity, Exercise, and Sport Questionnaire; BSI, Brief Symptom Inventory; PSS, Perceived Stress Scale; TICS, Trier Inventory for Chronic Stress.

checked using the questionnaires 'Physical Activity, Exercise, and Sport Questionnaire' (BSA [25]) and 'Food Frequency Questionnaire' (FFQ [26]). The participants were to do RFB-HRV-BF training for at least 100 minutes per week. The assess-

ment of glucose metabolism, autonomic activity and well-being took place before and after the 4-week-training period. A detailed description of the study design is given in Fig. 1. The study protocol was approved by the local Ethics Committee of the Landesärztekammer Rheinland-Pfalz, Germany (Nr. 2022-16489). All study procedures were conducted in accordance with the Principles of the Declaration of Helsinki for studies with human participants. All participants provided written informed consent prior to study initiation.

Resonance frequency breathing with HRV-BF

The mobile system 'eSense Pulse' by Mindfield Biosystems Ltd. (Gronau, Germany) was used for RFB-HRV-BF training. The participants sat in an upright position, started the training via the eSense app on their smartphone, and were instructed to practice breathing at their individually determined resonance frequency. The procedure of the mobile RFB-HRV-BF training is described in Herhaus et al. [27]. The data from each training session were saved in the individual and pseudonymized participant's eSense account. The electrocardiography (ECG) recordings were analyzed with Kubios software (Kubios Oy, Kuopio, Finland).

Outcomes

Glucose metabolism and laboratory measurements

Glucose metabolism was assessed through a frequently-sampled 75 g oral glucose tolerance test (OGTT) starting at 8:00 AM after an overnight fast. Participants received detailed instructions on diet and exercise the day before the test to standardize conditions. An intravenous cannula was inserted 45 minutes before the first blood sample. After basal blood sampling, the participants drank a 75 g glucose solution, with further blood samples taken at 30, 60, 90, and 120 minutes after glucose ingestion. Blood samples were handled on ice, were immediately centrifuged, and stored at -80°C before batch measurements. Plasma glucose concentrations were determined by the hexokinase/glucose-6-phosphate dehydrogenase method, total cholesterol, high-density lipoprotein cholesterol, low-density lipoprotein cholesterol, and triglycerides were quantified using a homogeneous enzymatic color assay and C-reactive protein concentrations were determined using a wide range turbidimetric immunoassay on the Alinity chemistry analyzer (Abbott, Wiesbaden, Germany). Serum insulin and C-peptide concentrations were measured on the Atellica solution immunoassay analyzer (Siemens Healthineers, Eschborn, Germany). Glycosylated hemoglobin (HbA1c) was determined using a high-performance liquid chromatography using the Variant-II Hemoglobin Testing-System (Bio-Rad Laboratories GmbH, Feldkirchen, Germany). All of these measurements have been performed in the accredited diagnostic laboratories of the University Hospital Tübingen, Germany and the University Medical Center Mainz, Germany. Measurements of the biosamples were conducted in a blinded fashion in routine diagnostic laboratories.

Areas under the curve were calculated based on the trapezoid method. Glucose stimulated insulin secretion was assessed as oral disposition index and as ratio of the areas under the C-peptide curves and the areas under the glucose curves during the first 30 minutes of the OGTT ($\text{AUC C-peptide}_{0-30 \text{ min}} / \text{AUC glucose}_{0-30 \text{ min}}$) [28]. For the estimation of insulin sensitivity, homeostasis model assessment-insulin resistance (HOMA-IR) was used for the fasting state and Matsuda index [29] for the post-glucose load situation. Insulin clearance was estimated by the ratio of the areas under the C-peptide and insulin curves during the OGTT ($\text{AUC C-peptide}_{0-120} / \text{AUC insulin}_{0-120}$).

Heart rate variability measurements

For the assessment of the ANS activity before and after the

training period of 4 weeks, the short-term measurement (5 minutes) of sitting in an upright position was carried out. In addition, the individual RFB was determined using the protocol by Lehrer et al. [30]. For this purpose, the participants performed various breathing frequencies (6.5, 6.0, 5.5, 5.0, and 4.5 breaths per minute) for 2 minutes each while sitting. The breathing frequency that maximized the HRV parameter root mean square successive differences (RMSSD) the most, was identified as the individual RFB. The HRV system HRVScanner™ (BioSign, Ottenhofen, Germany) was used for the measurement of a single-channel ECG with a sample rate of 500/sec. All recorded ECGs were analyzed using the software Kubios Premium (Kubios Oy) with the automatic beat correction algorithm [31] and the smoothness priors method of trend component rejection [32]. The rate of corrected beats was $<0.25\%$. HRV was quantified by the following metrics: RMSSD, standard deviation of all NN-intervals (SDNN), power in the low-frequency band 0.04–0.15 Hz (HRV-LF), and power in the high-frequency range 0.15–0.4 Hz (HRV-HF). The evaluator analyzing the ECG recordings was blinded and did not know the allocation of the recording into the pre- or post-measurement.

Mental health

The degree of stressful situations in one's life during the previous month and perceived chronic stress were captured by the German version of the Perceived Stress Scale (PSS [33]) and the German short version of the Trier Inventory for Chronic Stress (TICS-9 [34]). The Brief Symptom Inventory 18 (BSI-18 [35]) measured physical and psychological complaints of the previous 7 days. The FFQ [26] was used to estimate the consumption of foods and beverages during the 4 weeks' intervention. The amount of total activity and sport/exercise activity during the 4 weeks' intervention was assessed by the BSA [25].

Statistical analysis

The software programs used to analyze and visualize the data were SPSS Statistics version 26 (IBM Co., Armonk, NY, USA) and Prism v.8.3.0 (GraphPad Software). For the specification of the glucose, insulin, C-peptide and cortisol response in the OGTT, the AUC was calculated. The effects of the RFB-HRV-BF intervention on whole-body glucose metabolism, lipids, inflammation, and hematological parameters, HRV parameters, and psychological measures were analyzed by a dependent *t*-test. The HRV values were transformed by logarithm natu-

ralis+1 to obtain approximately normal distributions. In addition, regression was calculated to predict the influence of baseline Matsuda index and baseline fasting glucose level on changes in glycemia after the 4 weeks' intervention.

RESULTS

Effects of RFB-HRV-BF training on autonomic nervous system tone

Over the 4 intervention weeks, the participants trained on

average $\pm$ standard deviation 97 ± 11 minutes/week. There was a significant increase in the HRV parameters SDNN and RMSSD over the course of 4 weeks RFB-HRV-BF training ($P \leq 0.001$) (Fig. 2). Concerning the 5-minute resting sitting position, a significant increase in HRV parameters SDNN, RMSSD, power in the HRV-LF, and power in the high-frequency range 0.15 to 0.4 Hz (HRV-HF) could be observed after the 4 weeks' intervention (Fig. 3). There were no changes in the reported diet or the physical activity during the intervention period (FFQ: $t(29) = 1.649$, $P = 0.11$; BSA: $t(29) = -1.464$, $P = 0.15$).

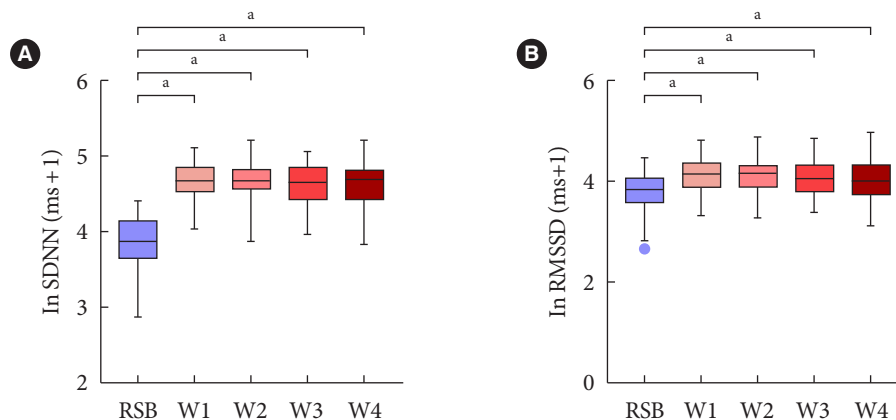


Fig. 2. Changes in autonomic function showing in heart rate variability-parameters standard deviation of all NN-intervals (SDNN) and root mean square successive differences (RMSSD) during baseline and 4 weeks of intervention with resonance frequency breathing with heart rate variability biofeedback in healthy adults ($n = 30$). Data presented as mean $\pm$ standard deviation. ln, logarithm naturalis+1; ms, millisecond; RSB, resting sitting position with spontaneous breathing; W1, intervention week 1; W2, intervention week 2; W3, intervention week 3; W4, intervention week 4. ^a $P \leq 0.001$, significant difference between resting sitting position with spontaneous breathing and intervention week.

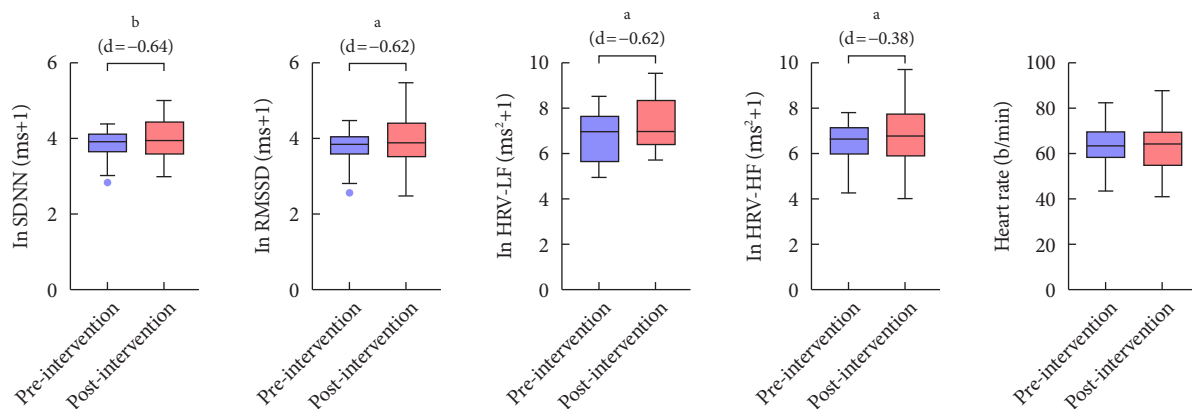


Fig. 3. Box-and-Whisker plots of heart rate and heart rate variability (HRV)-parameters before and after 4 weeks intervention of resonance frequency breathing with heart rate variability biofeedback in healthy adults ($n = 30$). Data presented as mean $\pm$ standard deviation. ln, logarithm naturalis+1; ms, millisecond; SDNN, standard deviation of all NN-intervals; RMSSD, root mean square successive differences; LF, power in low-frequency range 0.04–0.15 Hz; HF, power in high-frequency range 0.15–0.4 Hz. ^a $P \leq 0.05$, significant difference between pre- and post-intervention value, ^b $P \leq 0.01$, significant difference between pre- and post-intervention value.

Table 2. Systemic metabolism before and after 4 weeks resonance frequency breathing with heart rate variability biofeedback

Parameter	RFB with HRV-BF 4-week intervention			<i>t</i>	<i>P</i> value
	Pre-intervention	Post-intervention	Δ Post–Pre-intervention ^a		
Glucose metabolism					
Fasting glucose, mmol/L	4.35±0.49	4.39±0.30	0.03±0.4	−0.381	0.71
2-hr glucose, mmol/L	4.45±1.10	4.41±0.94	−0.04±1.19	0.205	0.84
Area under the glucose curve 0–120 min, mmol/L	10.70±2.20	10.86±1.49	0.15±1.86	−0.450	0.66
HbA1c, mmol/mol	31.89±3.21	30.26±5.91	−1.63±5.32	1.677	0.10
Fasting insulin, pmol/L	36.73±15.95	38.80±23.03	2.07±21.54	−0.525	0.60
Area under the insulin curve 0–120 min, pmol/L	566±226	624±273	58±201	−1.589	0.12
Fasting C-peptide, pmol/L	390±109	382±119	−8±10	0.402	0.69
Area under the C-peptide curve 0–120 min, pmol/L	3,019±760	3,093±748	74±578	−0.701	0.49
HOMA-IR, AU	1.03±0.46	1.10±0.67	0.07±0.60	−0.649	0.52
Matsuda index (OGTT-derived), AU	7.53±2.64	6.97±2.68	−0.56±2.79	1.106	0.28
Disposition index, AU	1,408±2,764	1,440±1,307	32±2,606	−0.069	0.95
AUC C-peptide _{0–30 min} /AUC glucose _{0–30 min} , AU	187±57	175±53	−12±38	1.706	0.10
Insulin clearance, AU	5.64±1.04	5.38±1.29	−0.26±0.90	1.587	0.12
Fasting cortisol, nmol/L	414±159	402±165	−12±27	0.509	0.61
Area under the cortisol curve 0–120 min, nmol/L	650±211	683±198	33±189	−0.952	0.35
Lipids					
Total cholesterol, mg/dL	158±23	156±22	−2±20	0.593	0.56
HDL-cholesterol, mg/dL	55±10	55±9	<1±6	−0.151	0.88
LDL-cholesterol, mg/dL	89±21	88±20	−1±16	0.389	0.70
Triglycerides, mg/dL	70±31	63±23	−6±24	1.455	0.16
Inflammation					
C-reactive protein, mg/L	1.49±2.67	1.13±1.66	−0.36±2.56	0.771	0.45

Values are presented as mean ± standard deviation.

RFB, resonance frequency breathing; HRV-BF, heart rate variability biofeedback; HbA1c, glycosylated hemoglobin; HOMA-IR, homeostasis model assessment-insulin resistance; AU, arbitrary unit; OGTT, oral glucose tolerance test; AUC, area under the curve; HDL, high-density lipoprotein; LDL, low-density lipoprotein.

^aΔPost–Pre-intervention, delta of after (post) and before (pre) intervention value (positive value represented an increase; negative value represented a decrease).

Effects of RFB-HRV-BF on glucose metabolism

After the 4 weeks training intervention, there were neither significant changes in fasting glucose, nor in 2-hour-post-load glucose (all $P \geq 0.71$) (Table 2, Fig. 4). In line, there were no significant changes in HbA1c ($t(29) = 1.667$, $P = 0.10$). Furthermore, insulin sensitivity (HOMA-IR $P = 0.52$, Matsuda index $P = 0.28$) and insulin secretion adjusted for insulin sensitivity ($F_{(1,52)} = 1.245$, $P = 0.27$) remained unchanged.

Baseline insulin sensitivity (assessed through the Matsuda index) and baseline fasting glucose were significant predictors of changes in glycemia after the 4 weeks' intervention ($P \leq 0.05$).

Based on these results, subgroups of baseline Matsuda index and baseline fasting glucose were formed. Exploratory analyses revealed a significantly stronger reduction in glycemia during the intervention in the more insulin resistant half of the study participants (for fasting glucose [$t(29) = -2.421$, $P \leq 0.05$, $d = -0.88$]; for 2-hour-post-load glucose [$t(29) = -2.303$, $P \leq 0.05$, $d = -0.84$]; and for AUC glucose [$t(29) = -2.401$, $P \leq 0.05$, $d = -0.88$]) (Supplementary Table 1, Supplementary Fig. 1). There were also significant interaction effects in two-way analysis of variance (ANOVA) testing time (pre- and post-measurement) × group (low/high baseline Matsuda index) interactions on fast-

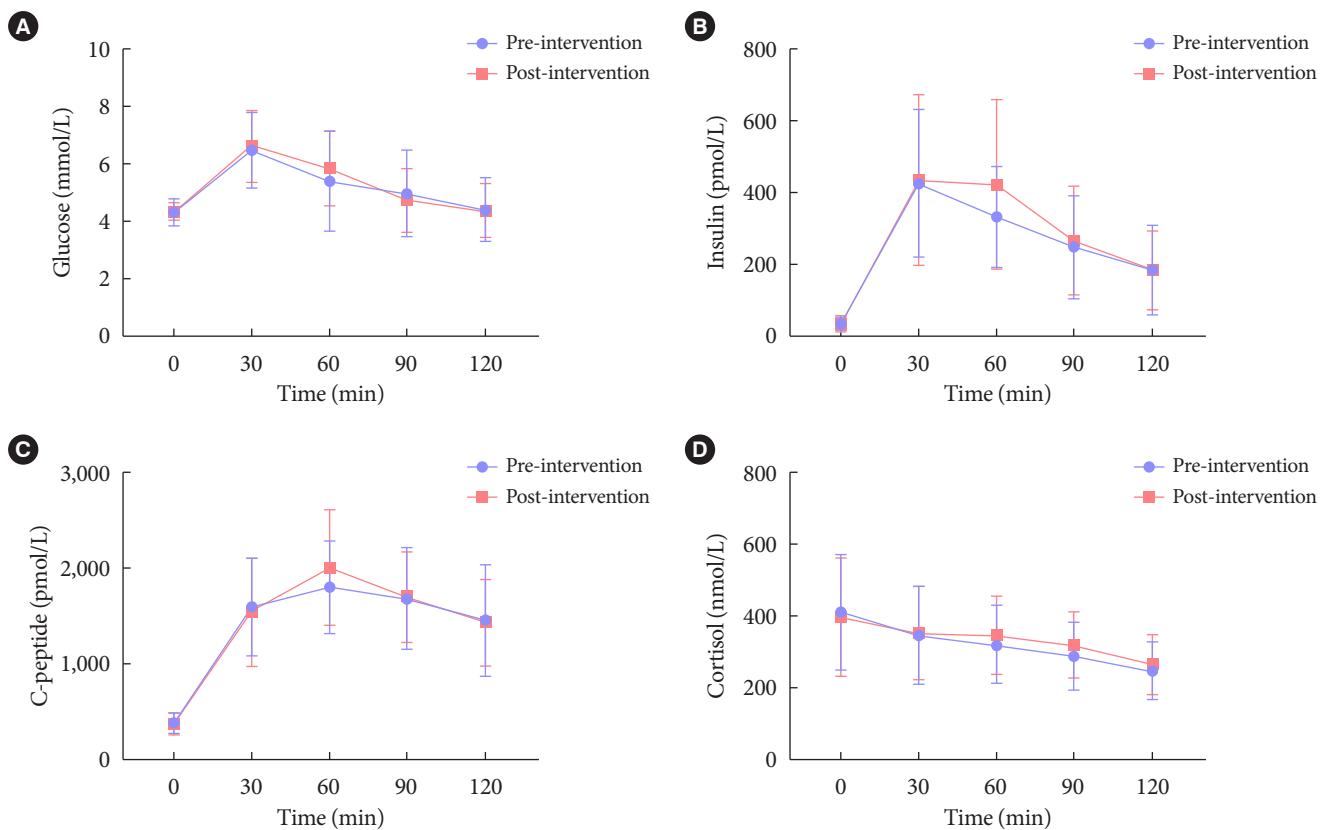


Fig. 4. Changes in (A) blood glucose, (B) insulin, (C) C-peptide, and (D) cortisol after oral glucose tolerance test before and after 4 weeks of intervention with resonance frequency breathing with heart rate variability biofeedback in healthy adults ($n=30$). Data presented as mean $\pm$ standard deviation.

ing glucose ($F_{(1,28)}=5.862$, $P\leq 0.05$, $\eta^2=0.173$), on 2-hour-post-load glucose ($F_{(1,28)}=5.304$, $P\leq 0.05$, $\eta^2=0.159$), and on AUC glucose ($F_{(1,28)}=5.764$, $P\leq 0.05$, $\eta^2=0.171$). Comparable results were obtained when comparing those with higher versus lower fasting glucose prior to intervention with significantly stronger reduction in glycemia during training in those with higher glucose ($P\leq 0.001$) (Supplementary Table 2, Supplementary Fig. 2). There were also significant interaction effects in two-way ANOVAs testing time (pre- and post-measurement) $\times$ group (low/high baseline fasting glucose) interactions on fasting glucose ($F_{(1,28)}=8.293$, $P\leq 0.01$, $\eta^2=0.229$), on 2-hour-post-load glucose ($F_{(1,28)}=8.741$, $P\leq 0.01$, $\eta^2=0.238$), and on AUC glucose ($F_{(1,28)}=6.723$, $P\leq 0.05$, $\eta^2=0.194$).

Effect of RFB-HRV-BF on lipids and subclinical inflammation

During the intervention, there were no significant changes in lipids, cortisol, or C-reactive protein (all $P\geq 0.05$) (Table 2).

Effect of RFB-HRV-BF on mental health

The symptoms of somatization, depression, and anxiety demonstrated significantly lower total scores after the 4 weeks' intervention ($t(29)=2.586$, $P\leq 0.05$, $d=0.41$). With regard to the perceived stress, there was no significant changes ($P=0.22$).

DISCUSSION

We here tested the effect of modulating ANS tone through RFB-HRV-BF on glucose metabolism in healthy adults. Our intervention was successful in modulating the ANS, but had no significant impact on metabolism when analyzing the entire cohort. However, the more insulin resistant half of our participants experienced improvements in glucose metabolism in response to the intervention. In addition, the training improved somatic and depressive symptoms but not perceived stress without differences between more and less insulin resistant persons.

Our findings are well in line with previous studies that demonstrated the potential of HRV-BF interventions in improving HRV and alleviate symptoms in patients with various medical conditions [21]. Interestingly, RFB-HRV-BF not only strengthens the modulation of the ANS and reduces the symptom severity of diseases, but there is also initial evidence of positive effects on immunological [36,37] and neurobiological processes [38]. A cross-over study did not find acute effects of short-term slow-paced breathing on glycemia in healthy men but reported potential links between heart rate and insulin secretion during the intervention [39].

The lack of effects on glucose metabolism after our current 4-week-intervention of RFB-HRV-BF despite an increase in vagal activity might be due to the fact that we included healthy persons who already had excellent glucose control and might therefore not be improvable. The most interesting finding of our current work is the possible impact of baseline glycemia and insulin sensitivity on the glycemic effects of our intervention. Those with a slightly higher glucose and those who were more insulin resistant showed improvement of glucose metabolism after the 4 weeks' intervention. This suggests the possibility that RFB-HRV-BF intervention can enhance glucose metabolism in individuals with impaired glucose regulation. This effect likely involves both, the parasympathetic activation via maximized respiratory sinus arrhythmia [11] and the stimulation of the vagal nerve with its anti-inflammatory pathway [15] with potential impact on both insulin secretion and insulin sensitivity [40]. In addition to these potential benefits, RFB-HRV-BF might lead to additional improvements in cardiovascular risk in people with prediabetes or diabetes [10].

Several studies have also demonstrated positive effects on mental health through RFB-HRV-BF [21,41]. The afferent vagus nerves project to brain areas which mediate mood and emotional regulation [42]. Our data provide further support for a possible effect by means of lower somatization, depression, and anxiety symptoms after 4 weeks of intervention.

The strengths of this study are the determination of the individual RFB, the high ECG data quality with a low rate of corrected beats ($<0.25\%$), the use of a standardized assessment of glucose metabolism through frequently-sampled OGTTs, and control of the confounders diet and physical activity during the intervention period. Although the RFB-HRV-BF system used in this study is proprietary, similar positive effects have been demonstrated with slow-paced breathing techniques [37,43], which can be performed using freely accessible mobile applica-

tions. This highlights the potential for broader adoption and accessibility of the intervention beyond the proprietary system used in the current study. However, there are limitations of the current study. Our study only included healthy volunteers. The sample size was furthermore too small and the intervention duration was too short to explore potential underlying mechanisms (e.g., impact of systemic inflammation) and to observe long-term metabolic changes (e.g., HbA1c). It must be considered that use of a median split has some limitations [44]. Although the study controlled lifestyle changes through self-reported questionnaires, objective measures, such as wearable activity trackers or diet logs, would enhance reliability. This study hypothesizes potential mechanisms, such as vagal activation and anti-inflammatory effects, but does not include direct measurements (e.g., cytokines or vagal nerve activity), which limits the ability to establish causal relationships.

In conclusion, our RFB-HRV-BF intervention was able to increase vagal activity in healthy individuals and may improve glucose metabolism in those who are more insulin resistant before the intervention. As brain-periphery communication through the ANS is crucial in modulating both insulin secretion and insulin sensitivity, further mechanistic studies are needed to clarify the precise mechanisms by which RFB-HRV-BF affects metabolism. Given the known increased sympathetic tone in prediabetes and diabetes, RFB-HRV-BF training may be a non-pharmacological option to restore ANS tone, with potential benefits for glucose metabolism. Future studies specifically targeting individuals with prediabetes or diabetes are needed to explore its potential benefits for improving impaired glucose metabolism.

SUPPLEMENTARY MATERIALS

Supplementary materials related to this article can be found online at <https://doi.org/10.4093/dmj.2024.0647>.

CONFLICTS OF INTEREST

Outside of the current work, Martin Heni reports lecture fees from Chiesi/Amryt, AstraZeneca, Bayer, Boehringer Ingelheim, Lilly, Novartis, Novo Nordisk and Sanofi. He also served on advisory boards for Chiesi/Amryt, Boehringer Ingelheim, and Sanofi. Benedict Herhaus, Andreas Peter, Julia Hummel, Thomas Kubiak, and Katja Petrowski report no conflicts of interest.

AUTHOR CONTRIBUTIONS

Conception or design: B.H., J.H., M.H., K.P.

Acquisition, analysis, or interpretation of data: B.H., A.P., J.H., M.H., K.P.

Drafting the work or revising: all authors.

Final approval of the manuscript: all authors.

ORCID

Benedict Herhaus <https://orcid.org/0000-0002-3456-9735>

Martin Heni <https://orcid.org/0000-0002-8462-3832>

FUNDING

This study was funded by the Internal University Research Funding of the University Medical Center of the Johannes Gutenberg University Mainz & by the DFG-project 'Effects of autonomic nervous system modulation by HRV-BF training with resonant frequency breathing on glucose metabolism in individuals with prediabetes' (Project-number: 540546352).

ACKNOWLEDGMENTS

We thank the medical doctoral students Cécile Gobin, Caroline Rech, Sarah Rüther, and Josephine Spiegel (all University of Mainz) for assisting with data collection.

REFERENCES

1. World Health Organization. Diabetes. Available from: <https://www.who.int/news-room/fact-sheets/detail/diabetes> (cited 2025 Mar 12).
2. Gregg EW, Li Y, Wang J, Burrows NR, Ali MK, Rolka D, et al. Changes in diabetes-related complications in the United States, 1990-2010. *N Engl J Med* 2014;370:1514-23.
3. Roden M, Shulman GI. The integrative biology of type 2 diabetes. *Nature* 2019;576:51-60.
4. Heni M. The insulin resistant brain: impact on whole-body metabolism and body fat distribution. *Diabetologia* 2024;67:1181-91.
5. Hansen CS, Færch K, Jorgensen ME, Malik M, Witte DR, Brunner EJ, et al. Heart rate, autonomic function, and future changes in glucose metabolism in individuals without diabetes: the Whitehall II Cohort Study. *Diabetes Care* 2019;42:867-74.
6. Faber CL, Deem JD, Campos CA, Taborsky GJ Jr, Morton GJ. CNS control of the endocrine pancreas. *Diabetologia* 2020;63:2086-94.
7. Mirzadeh Z, Faber CL, Schwartz MW. Central nervous system control of glucose homeostasis: a therapeutic target for type 2 diabetes? *Annu Rev Pharmacol Toxicol* 2022;62:55-84.
8. Teff KL. Visceral nerves: vagal and sympathetic innervation. *JPEN J Parenter Enteral Nutr* 2008;32:569-71.
9. Kullmann S, Kleinridders A, Small DM, Fritsche A, Haring HU, Preissl H, et al. Central nervous pathways of insulin action in the control of metabolism and food intake. *Lancet Diabetes Endocrinol* 2020;8:524-34.
10. Coopmans C, Zhou TL, Henry RM, Heijman J, Schaper NC, Koster A, et al. Both prediabetes and type 2 diabetes are associated with lower heart rate variability: the Maastricht Study. *Diabetes Care* 2020;43:1126-33.
11. Lehrer PM, Gevirtz R. Heart rate variability biofeedback: how and why does it work? *Front Psychol* 2014;5:756.
12. Yasuma F, Hayano J. Respiratory sinus arrhythmia: why does the heartbeat synchronize with respiratory rhythm? *Chest* 2004;125:683-90.
13. Heni M, Wagner R, Kullmann S, Veit R, Mat Husin H, Linder K, et al. Central insulin administration improves whole-body insulin sensitivity via hypothalamus and parasympathetic outputs in men. *Diabetes* 2014;63:4083-8.
14. Berntson GG, Bigger JT Jr, Eckberg DL, Grossman P, Kaufmann PG, Malik M, et al. Heart rate variability: origins, methods, and interpretive caveats. *Psychophysiology* 1997;34:623-48.
15. Pavlov VA, Tracey KJ. Neural regulation of immunity: molecular mechanisms and clinical translation. *Nat Neurosci* 2017;20:156-66.
16. Tracey KJ. The inflammatory reflex. *Nature* 2002;420:853-9.
17. Wang H, Yu M, Ochani M, Amella CA, Tanovic M, Susarla S, et al. Nicotinic acetylcholine receptor alpha7 subunit is an essential regulator of inflammation. *Nature* 2003;421:384-8.
18. Pavlov VA, Tracey KJ. The cholinergic anti-inflammatory pathway. *Brain Behav Immun* 2005;19:493-9.
19. Rehman K, Akash MS. Mechanisms of inflammatory responses and development of insulin resistance: how are they interlinked? *J Biomed Sci* 2016;23:87.
20. Vaschillo EG, Vaschillo B, Lehrer PM. Characteristics of resonance in heart rate variability stimulated by biofeedback. *Appl Psychophysiol Biofeedback* 2006;31:129-42.
21. Lehrer P, Kaur K, Sharma A, Shah K, Huseby R, Bhavsar J, et al. Heart rate variability biofeedback improves emotional and

- physical health and performance: a systematic review and meta analysis. *Appl Psychophysiol Biofeedback* 2020;45:109-29.
22. Wittchen HU, Zaudig M, Fydrich T. Structured clinical interview for DSM-IV (Strukturiertes Klinisches Interview für DSM-IV). Göttingen: Hogrefe; 1997.
23. American Psychiatric Association. Diagnostic and statistical manual of mental disorders: DSM-IV-TR. 4th ed. Washington DC: APA; 2000.
24. Lehrer P, Vaschillo E, Lu SE, Eckberg D, Vaschillo B, Scardella A, et al. Heart rate variability biofeedback: effects of age on heart rate variability, baroreflex gain, and asthma. *Chest* 2006; 129:278-84.
25. Fuchs R, Klaperski S, Gerber M, Seelig H. Measurement of Physical Activity and Sport Activity with the BSA Questionnaire (Messung der bewegungs- und sportaktivität mit dem BSA-Fragebogen). *Z Gesundh Psychol* 2015;23:60-76.
26. Haftenberger M, Heuer T, Heidemann C, Kube F, Krems C, Mensink GB. Relative validation of a food frequency questionnaire for national health and nutrition monitoring. *Nutr J* 2010;9:36.
27. Herhaus B, Kalin A, Gouveris H, Petrowski K. Mobile heart rate variability biofeedback improves autonomic activation and subjective sleep quality of healthy adults: a pilot study. *Front Physiol* 2022;13:821741.
28. Hudak S, Huber P, Lamprinou A, Fritsche L, Stefan N, Peter A, et al. Reproducibility and discrimination of different indices of insulin sensitivity and insulin secretion. *PLoS One* 2021;16: e0258476.
29. Matsuda M, DeFronzo RA. Insulin sensitivity indices obtained from oral glucose tolerance testing: comparison with the euglycemic insulin clamp. *Diabetes Care* 1999;22:1462-70.
30. Lehrer PM, Vaschillo E, Vaschillo B. Resonant frequency biofeedback training to increase cardiac variability: rationale and manual for training. *Appl Psychophysiol Biofeedback* 2000;25: 177-91.
31. Lipponen JA, Tarvainen MP. A robust algorithm for heart rate variability time series artefact correction using novel beat classification. *J Med Eng Technol* 2019;43:173-81.
32. Tarvainen MP, Ranta-Aho PO, Karjalainen PA. An advanced detrending method with application to HRV analysis. *IEEE Trans Biomed Eng* 2002;49:172-5.
33. Schneider EE, Schonfelder S, Domke-Wolf M, Wessa M. Measuring stress in clinical and nonclinical subjects using a German adaptation of the Perceived Stress Scale. *Int J Clin Health Psychol* 2020;20:173-81.
34. Petrowski K, Kliem S, Albani C, Hinz A, Brahler E. Norm values and psychometric properties of the short version of the Trier Inventory for Chronic Stress (TICS) in a representative German sample. *PLoS One* 2019;14:e0222277.
35. Franke GH. BSI. Brief symptom inventory: Deutsche version. Manual. Göttingen: Beltz; 2000.
36. Herhaus B, Conrad R, Petrowski K. Effect of a slow-paced breathing with heart rate variability biofeedback intervention on pro-inflammatory cytokines in individuals with panic disorder: a randomized controlled trial. *J Affect Disord* 2023;326: 132-8.
37. Balint EM, Gruner B, Haase S, Kaw-Geppert M, Thayer JF, Gundel H, et al. A randomized clinical trial to stimulate the cholinergic anti-inflammatory pathway in patients with moderate COVID-19-pneumonia using a slow-paced breathing technique. *Front Immunol* 2022;13:928979.
38. Schumann A, de la Cruz F, Kohler S, Brotte L, Bar KJ. The influence of heart rate variability biofeedback on cardiac regulation and functional brain connectivity. *Front Neurosci* 2021;15: 691988.
39. Vosseler A, Zhao D, Hummel J, Gholamrezaei A, Hudak S, Kantartzis K, et al. Slow deep breathing modulates cardiac vagal activity but does not affect peripheral glucose metabolism in healthy men. *Sci Rep* 2021;11:20306.
40. Kiely A, McClenaghan NH, Flatt PR, Newsholme P. Pro-inflammatory cytokines increase glucose, alanine and triacylglycerol utilization but inhibit insulin secretion in a clonal pancreatic beta-cell line. *J Endocrinol* 2007;195:113-23.
41. Pizzoli SF, Marzorati C, Gatti D, Monzani D, Mazzocco K, Pravettoni G. A meta-analysis on heart rate variability biofeedback and depressive symptoms. *Sci Rep* 2021;11:6650.
42. Grundy D. Neuroanatomy of visceral nociception: vagal and splanchnic afferent. *Gut* 2002;51(Suppl 1):i2-5.
43. Laborde S, Allen MS, Borges U, Iskra M, Zammit N, You M, et al. Psychophysiological effects of slow-paced breathing at six cycles per minute with or without heart rate variability biofeedback. *Psychophysiology* 2022;59:e13952.
44. Maxwell SE, Delaney HD. Bivariate median splits and spurious statistical significance. *Psychol Bull* 1993;113:181-90.

Supplementary Table 1. Glucose metabolism before and after 4 weeks resonance frequency breathing with heart rate variability biofeedback in low and high baseline Matsuda index group

Parameter	Low baseline Matsuda index (<i>n</i> =15)			High baseline Matsuda index (<i>n</i> =15)			<i>t</i>	<i>P</i> value
	Pre-intervention	Post-intervention	Δ Post-Pre-intervention ^a	Pre-intervention	Post-intervention	Δ Post-Pre-intervention ^a		
Glucose metabolism								
Fasting glucose, mmol/L	4.50±0.52	4.36±0.28	−0.14±0.43	4.21±0.41	4.41±0.33	0.20±0.35	−2.421	≤0.05
2-hr glucose, mmol/L	4.98±1.23	4.47±0.07	−0.51±0.98	3.93±0.64	4.36±0.94	0.42±1.22	−2.303	≤0.05
Area under the glucose curve 0–120 min, mmol/L	11.80±2.30	11.20±1.42	−0.60±2.02	9.61±1.47	10.51±1.51	0.91±1.37	−2.401	≤0.05
HbA1c, mmol/mol	31.64±3.91	31.80±3.34	0.16±1.48	32.13±2.42	30.63±3.88	−3.42±7.04	1.926	0.06
Fasting insulin, pmol/L	45.80±17.05	42.27±21.57	−3.53±15.53	27.67±7.73	35.33±24.66	7.67±25.55	−1.451	0.16
Area under the insulin curve 0–120 min, pmol/L	689±235	747±325	58±264	444±137	503±129	59±120	−0.010	0.99
Fasting C-peptide, pmol/L	444±119	400±112	−44±81	337±66	366±129	29±115	−2.021	0.05
Area under the C-peptide curve 0–120 min, pmol/L	3431±720	3,452±796	20±714	2,607±561	2,734±499	128±419	−0.502	0.62
HOMA-IR, AU	1.31±0.48	1.20±0.65	−0.12±0.40	0.74±0.20	1.00±0.69	0.26±0.72	−1.756	0.09
Matsuda index (OGTT-derived), AU	5.36±1.27	6.02±2.56	0.66±2.14	9.70±1.65	7.92±2.53	−1.79±2.88	2.640	≤0.05
Disposition index, AU	820±1,016	1,429±1,589	608±1,448	1,995±3,749	1,452±1,005	−543±3,357	1.220	0.23
AUC C-peptide0-30 min/ AUC glucose0-30 min, AU	198±58	189±52	−9±36	175±56	160±53	−15±41	0.469	0.64
Insulin clearance, AU	5.19±0.88	5.16±1.55	−0.03±0.99	6.09±1.01	5.59±0.98	−0.50±0.76	1.457	0.16
Fasting cortisol, nmol/L	467±200	473±194	6±164	361±82	330±86	−32±106	0.748	0.46
Area under the cortisol curve 0–120 min, nmol/L	712±261	731±243	19±235	588±125	635±132	47±135	−0.406	0.69
Lipids								
Total cholesterol, mg/dL	152±22	154±21	2±23	163±23	157±23	−6±16	1.117	0.27
HDL-cholesterol, mg/dL	53±12	54±11	1±6	56±9	55±8	−1±6	1.056	0.30
LDL-cholesterol, mg/dL	85±19	87±16	2±16	93±22	89±24	−4±15	1.025	0.31
Triglycerides, mg/dL	71±25	64±23	−7±24	69±37	23±25	−6±25	−0.111	0.91
Inflammation								
C-reactive protein, mg/L	1.88±3.61	1.11±1.66	−0.77±3.12	1.11±1.23	1.16±1.72	0.06±1.87	−0.886	0.38

Values are presented as mean ± standard deviation.

HbA1c, glycosylated hemoglobin; HOMA-IR, homeostasis model assessment-insulin resistance; AU, arbitrary units; OGTT, oral glucose tolerance test; AUC, area under the curve; HDL, high-density lipoprotein; LDL, low-density lipoprotein.

^aΔPost–Pre-intervention, delta of after (post) and before (pre) intervention value (positive value represented an increase; negative value represented a decrease).

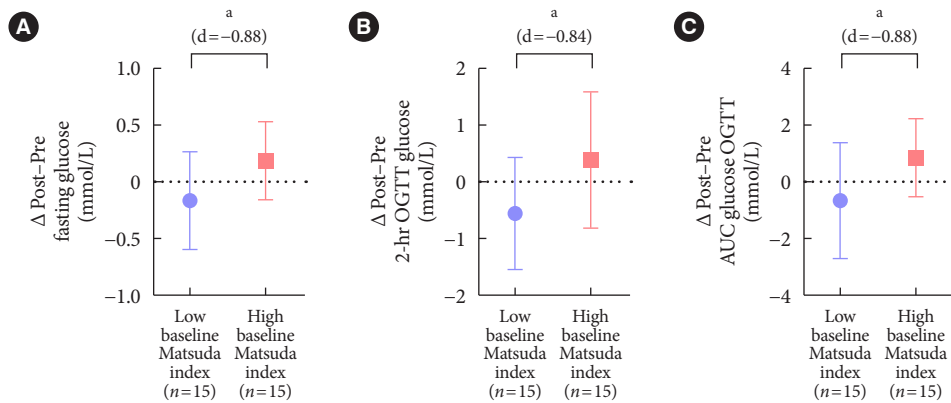
Supplementary Table 2. Glucose metabolism before and after 4 weeks resonance frequency breathing with heart rate variability biofeedback in low and high baseline fasting glucose group

Parameter	Low baseline fasting glucose (<i>n</i> = 15)			High baseline fasting glucose (<i>n</i> = 15)			<i>t</i>	<i>P</i> value
	Pre-intervention	Post-intervention	Δ Post-Pre-intervention ^a	Pre-intervention	Post-intervention	Δ Post-Pre-intervention ^a		
Glucose metabolism								
Fasting glucose, mmol/L	4.01 ± 0.19	4.24 ± 0.28	0.23 ± 0.35	4.70 ± 0.45	4.53 ± 0.26	−0.17 ± 0.41	2.880	≤ 0.01
2-hr glucose, mmol/L	4.04 ± 0.96	4.57 ± 1.06	0.53 ± 1.09	4.87 ± 1.10	4.25 ± 0.82	−0.61 ± 1.01	2.957	≤ 0.01
Area under the glucose curve 0–120 min, mmol/L	9.42 ± 1.76	10.38 ± 1.84	0.96 ± 1.43	11.98 ± 1.85	11.33 ± 0.83	−0.65 ± 1.93	2.593	≤ 0.05
HbA1c, mmol/mol	31.33 ± 2.92	29.83 ± 3.49	−1.50 ± 2.24	32.44 ± 3.48	32.60 ± 3.27	−1.75 ± 7.28	0.127	0.90
Fasting insulin, pmol/L	36.20 ± 15.85	40.73 ± 24.38	4.53 ± 27.64	37.27 ± 16.58	36.87 ± 22.28	−0.40 ± 13.57	0.620	0.54
Area under the insulin curve 0–120 min, pmol/L	568 ± 223	665 ± 280	96 ± 164	564 ± 236	585 ± 268	21 ± 232	1.030	0.31
Fasting C-peptide, pmol/L	378 ± 94	393 ± 123	14 ± 129	402 ± 124	373 ± 120	−30 ± 70	1.159	0.26
Area under the C-peptide curve 0–120 min, pmol/L	2991 ± 810	3,096 ± 820	105 ± 446	3,047 ± 735	3,090 ± 697	43 ± 701	0.288	0.78
HOMA-IR, AU	0.94 ± 0.44	1.12 ± 0.69	0.18 ± 0.77	1.11 ± 0.48	1.07 ± 0.66	−0.04 ± 0.36	1.017	0.32
Matsuda index (OGTT-derived), AU	8.47 ± 2.63	6.76 ± 2.34	−1.72 ± 2.89	6.59 ± 2.37	7.19 ± 3.05	0.59 ± 2.21	−2.463	≤ 0.05
Disposition index, AU	2,069 ± 3,728	18,199 ± 1,707	−250 ± 3,600	746 ± 993	1,062 ± 563	−316 ± 972	−0.588	0.56
AUC C-peptide _{0–30 min} /AUC glucose _{0–30 min} , AU	206 ± 49	185 ± 55	−20 ± 42	167 ± 59	164 ± 51	−3 ± 32	−1.287	0.21
Insulin clearance, AU	5.53 ± 1.02	4.91 ± 0.84	−0.63 ± 0.76	5.74 ± 1.09	5.85 ± 1.51	0.11 ± 0.90	−2.411	≤ 0.05
Fasting cortisol, nmol/L	405 ± 153	421 ± 151	16 ± 123	424 ± 170	382 ± 180	−42 ± 149	1.154	0.26
Area under the cortisol curve 0–120 min, nmol/L	626 ± 213	700 ± 164	74 ± 169	674 ± 213	665 ± 232	−9 ± 204	1.214	0.24
Lipids								
Total cholesterol, mg/dL	151 ± 24	150 ± 24	−1 ± 19	164 ± 21	161 ± 19	−3 ± 21	0.201	0.84
HDL-cholesterol, mg/dL	52 ± 10	52 ± 9	0 ± 7	57 ± 10	58 ± 9	> 0 ± 6	−0.148	0.88
LDL-cholesterol, mg/dL	87 ± 22	86 ± 23	−2 ± 15	91 ± 20	90 ± 16	−1 ± 15	−0.174	0.87
Triglycerides, mg/dL	62 ± 27	62 ± 23	1 ± 23	78 ± 33	65 ± 25	−14 ± 24	1.722	0.10
Inflammation								
C-reactive protein, mg/L	1.20 ± 1.45	1.23 ± 2.09	0.03 ± 1.88	1.79 ± 3.54	1.04 ± 1.16	−0.75 ± 3.12	0.832	0.41

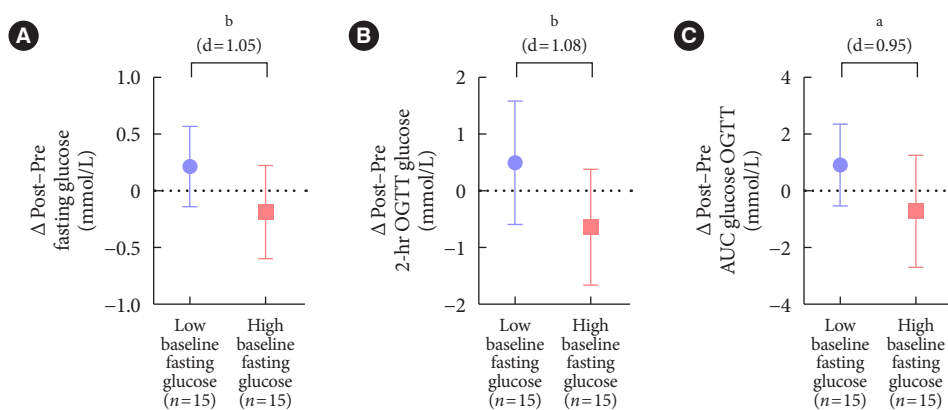
Values are presented as mean±standard deviation.

HbA1c, glycosylated hemoglobin; HOMA-IR, homeostasis model assessment-insulin resistance; AU, arbitrary units; OGTT, oral glucose tolerance test; AUC, area under the curve; HDL, high-density lipoprotein; LDL, low-density lipoprotein.

^aΔPost-Pre-intervention, delta of after (post) and before (pre) intervention value (positive value represented an increase; negative value represented a decrease).



Supplementary Fig. 1. Comparison of Δ post-pre-intervention of (A) fasting glucose, (B) 2-hour-post-load glucose, and (C) area under the curve (AUC) glucose during the oral glucose tolerance test (OGTT) in low and high baseline Matsuda index group. Data presented as mean $\pm$ standard deviation. Δ Post-Pre-intervention, delta of after (post) and before (pre) intervention value (positive value represented an increase; negative value represented a decrease). ^a $P \leq 0.05$ significant difference between low and high baseline Matsuda index group.



Supplementary Fig. 2. Comparison of Δ post–pre-intervention of (A) fasting glucose, (B) 2-hour-post-load glucose, and (C) area under the curve (AUC) glucose during the oral glucose tolerance test (OGTT) in low and high baseline fasting glucose group. Data presented as mean $\pm$ standard deviation. Δ Post–Pre-intervention, delta of after (post) and before (pre) intervention value (positive value represented an increase; negative value represented a decrease). ^a $P \leq 0.05$, significant difference between low and high baseline fasting glucose group, ^b $P \leq 0.01$ significant difference between low and high baseline fasting glucose group.