Original Article

MOLECULAR METABOLISM

Constant hepatic ATP concentrations during prolonged fasting and absence of effects of Cerbomed Nemos® on parasympathetic tone and hepatic energy metabolism

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ABSTRACT

Objective: Brain insulin-induced improvement in glucose homeostasis has been proposed to be mediated by the parasympathetic nervous system. Non-invasive transcutaneous auricular vagus nerve stimulation (taVNS) activating afferent branches of the vagus nerve may prevent hyperglycemia in diabetes models. We examined the effects of 14-min taVNS vs sham stimulation by Cerbomed Nemos® on glucose metabolism, lipids, and hepatic energy homeostasis in fasted healthy humans ($n = 10$, age 51 ± 6 yrs, BMI 25.5 ± 2.7 kg/m^2).

Methods: Heart rate variability (HRV), reflecting sympathetic and parasympathetic nerve activity, was measured before, during and after taVNS or sham stimulation. Endogenous glucose production was determined using [6,6-$^2H_2$]glucose, and hepatic concentrations of triglycerides (HCL), adenosine triphosphate (ATP), and inorganic phosphate (Pi) were quantified from $^1H/^{31}P$ magnetic resonance spectroscopy at baseline and for 180 min following stimulation.

Results: taVNS did not affect circulating glucose, free fatty acids, insulin, glucagon, or pancreatic polypeptide. Rates of endogenous glucose production ($P = 0.79$), hepatic HCL, ATP, and Pi were also not different ($P = 0.91$, $P = 0.48$ and $P = 0.24$) between taVNS or sham stimulation. Hepatic HCL, ATP, and Pi remained constant during prolonged fasting for 3 h. No changes in heart rate or shift in cardiac autonomic function from HRV towards sympathetic or parasympathetic predominance were detected.

Conclusion: Non-invasive vagus stimulation by Cerbomed Nemos® does not acutely modulate the autonomic tone to the visceral organs and thereby does not affect hepatic glucose and energy metabolism. This technique is therefore unable to mimic brain insulin-mediated effects on peripheral homeostasis in humans.

Keywords Vagus nerve stimulation; Hepatic insulin sensitivity; Hepatic energy metabolism; Liver fat content

1. INTRODUCTION

Insulin signaling in the central nervous system has been identified as an essential regulator of peripheral energy and glucose homeostasis in rodents and dogs [1–3]. Of note, brain insulin effects are abolished by hepatic vagotomy, suggesting that the brain–liver crosstalk is mediated by the vagus nerve [4]. Furthermore, brain insulin-induced suppression of endogenous glucose production (EGP) via hepatic IL-6/STAT3 activation has been suggested to depend on the inhibition of hepatic vagal branches [5]. Although these results indicate that the vagal nerve may be key to controlling glucose homeostasis, its relevance in humans has not been determined.

Studies in humans using intranasal insulin for delivery to the brain provided support of the concept of central regulation of EGP, whole-body glucose uptake, and adipose tissue lipolysis [6–9]. We recently reported that intranasal insulin improves hepatic energy metabolism and reduces liver fat content in lean healthy humans but not in patients with type 2 diabetes (T2D) [10]. However, the mechanism of this brain–liver crosstalk remained unclear. Interestingly, parasympathetic tone, estimated from heart rate variability, was

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Abbreviations: ANOVA, analysis of variance; ATP, adenosine triphosphate; BRS, baroreflex sensitivity; EGP, endogenous glucose production; HCL, liver fat content; HF, high-frequency; HRV, heart rate variability; LF, low-frequency; MRS, magnetic resonance spectroscopy; NTS, nucleus tractus solitarii; Pi, inorganic phosphate; PP, pancreatic polypeptide; taVNS, transcutaneous auricular vagus nerve stimulation

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shown to correlate with the change in whole-body insulin sensitivity after intranasal insulin application [8], suggesting that vagal outputs also mediate brain-derived peripheral insulin sensitization in humans. Transcutaneous auricular vagus nerve stimulation (taVNS) can be applied in the external ear of humans to non-invasively activate the central projections of the auricular branch of the vagus nerve [11]. Thereby, taVNS induces an increase in the nucleus tractus solitarii (NTS) activity measured by functional magnetic resonance imaging [11]. Another human taVNS study found reduction in sympathetic outflow, as assessed using microneurography and increased heart rate variability, suggesting reduced sympathetic tone after vagal stimulation [12]. The mechanism behind this reduction of sympathetic output supposedly included taVNS induction of caudal ventrolateral medulla activity, which, in turn, inhibits rostral ventrolateral medulla and thus lowers sympathetic tone [13], suggesting that taVNS might act on the sympathetic nervous system independent of vagal activation. VNS is currently applied as adjunctive therapy in medically refractory epilepsy [14] but has also recently emerged as a promising treatment option for major depressive disorder [15], Alzheimer's disease [16,17], and inflammatory bowel disease [18] due to its anti-inflammatory potential [19]. The metabolic effects of vagal stimulation are not completely understood. Energy expenditure has been shown to increase [20] and postprandial insulin secretion is reduced after a single session of VNS in humans [21]. Evidence from Zucker diabetic fatty rats highlights the potential of taVNS to prevent hyperglycemia [22,23]. However, whether non-invasive taVNS in humans can modulate hepatic glucose and lipid metabolism is unknown.

Thus, we designed a randomized, controlled, crossover clinical study to assess the effects of taVNS on hepatic insulin sensitivity, lipid, and energy homeostasis in lean healthy humans. We further examined taVNS effects on cardiac autonomic function and circulating pancreatic polypeptide (PP) levels as readouts for vagal activation. We hypothesized that taVNS would increase parasympathetic tone and mimic intranasal insulin effects in healthy humans.

2. MATERIAL AND METHODS

2.1. Participants

Ten healthy humans not taking any medication and without any family history of diabetes were enrolled in this randomized controlled single-blind, cross-over, monocenter study between August 2015 and January 2017 (ClinicalTrial.gov registration no. NCT01479075). Participants exhibited normal glucose tolerance based on a standard 75-grams oral glucose tolerance test. The study was approved by the ethics board of Heinrich-Heine University Düsseldorf and written informed consent was obtained from each person prior to inclusion. Screening procedure included medical history, clinical exam and blood tests. None had clinical or laboratory signs of infection, cardiovascular, neurological, hepatic, renal, or endocrine disease. Patients with cardiac arrhythmia or peripheral neuropathy were excluded from participation. Female participants were postmenopausal. All volunteers refrained from caffeine-containing drinks consumption and exercise from 3 days before the study.

2.2. Study design

The volunteers arrived at 7:00 am at the German Diabetes Center after 10 h overnight fasting and remained fasted until the end of the study day. All participants were studied on two different days spaced by at least 7 days. Both cubital veins were catheterized for blood sampling and infusions. At time point −180 min, the participants received a continuous infusion (0.036 mg*min^{-1}*kg*body weight^{-1}) of D-[6,6-$^{2}H_2$]glucose (99% enriched in ^{2}H glucose; Cambridge Isotope Laboratories, Andover, MA) after a priming bolus of 3.6 mg*kg body weight^{-1}*fasting plasma glucose [mg/dl]/90 [mg/dl] for 5 min [7]. The tracer infusion lasted until +180 min, and blood samples were drawn to measure tracer enrichment, metabolites, and hormones.

At time point zero, taVNS or sham stimulation using Cerbomed NEMOS® (Cerbomed, GmbH, Erlangen, Germany) device were applied for 14 min in the left external ear as described previously [11]. For taVNS, the earpiece of the device was positioned upright with the electrode in the cymba conchae of the left external ear. Sham stimulation (as a control) was conducted by positioning the earpiece upside down with the electrode on the earlobe of the left external ear. The stimulus intensity was adjusted for each participant starting from 0.1 mA and increasing in 0.1 mA until tingling sensation was achieved. Further increases in the intensity leading to pricking or burning sensations were avoided. All participants reported tingling sensation during all taVNS and sham stimulation procedures. The stimulation intensities that were selected in this way were 0.3–1.2 mA for the sham earlobe stimulation condition (0.8 ± 0.1 mA, mean ± SEM) and 0.6–1.4 mA for the taVNS cymba conchae condition (0.9 ± 0.1 mA, mean ± SEM), with no difference between conditions. The non-adjustable parameters of the device were continuous biphasic square pulses with 0.25 ms duration at 25 Hz.

2.3. Metabolites and hormones

Blood samples were chilled and centrifuged, and supernatants were stored at −80 °C until analysis. The glucose oxidase method was used to measure venous blood glucose concentrations with EKF biosen C-Line glucose analyzer (EKF Diagnostic GmbH, Barleben, Germany). Serum triglycerides and plasma free fatty acids (FFA) were quantified enzymatically [10]. Serum C-peptide and insulin were measured chemoluminimetrically (Immulite 2000 Xpi; Siemens, Erlangen, Germany) [24]. Plasma glucagon was measured by radioimmunoassay (Millipore, St. Charles, Miss, USA). Serum PP was measured using an established radioimmunoassay in the Section of Investigative Medicine, Imperial College London [25]. Briefly, 100 µl of sample were added to 600 µl of 0.05 M phosphate buffer with 0.3% bovine serum albumin (BSA) w/v containing antibody (titer 1:860,000). The assay was incubated for 3 days of at 4 °C. Bound and free radiolabeled PP were separated by charcoal adsorption of the free fraction using 4 mg of charcoal/tube suspended in 0.06 M phosphate buffer with gelatine. The samples were centrifuged at 1500 × g at 4 °C for 20 min, bound and free label separated by aspiration, and both pellet and supernatant counted in a gamma-counter (model NE1600, Thermo Electron Corporation). Gas chromatography-mass spectrometry for assessment of atom percent enrichment of ^{2}H was performed on Hewlett–Packard 6890 gas chromatograph equipped with a 25-m CPSil5CB capillary column (0.2-mm inner diameter, 0.12-µm film thickness; Chrompack/Varian, Middelburg, The Netherlands), interfaced to a Hewlett Packard 5975 mass selective detector (Hewlett Packard) as described previously [26]. Atom percent enrichment was calculated as mass ratio, corresponding to the tracer enrichment in plasma glucose.

2.4. ^{1}H/^{31}P magnetic resonance spectroscopy (MRS)

At time point −60 min, the participants entered the 3-Tesla MR scanner (Achieva 3T Philips, Best, The Netherlands) for scans in the supine position at baseline, 30 min after taVNS or sham procedure and at time point +180 min. A 14-cm circular ^{31}P surface transmit-receive coil (Philips Healthcare, Best, The Netherlands) for ^{31}P-MRS and the built-in ^{1}H whole body coil for localization and proton spectroscopy were used as described before [27]. Participants were not allowed to

leave the scanner between measurements. Hepatic phosphorus metabolite concentrations (adenosine triphosphate, ATP, and inorganic phosphate, Pi) were corrected for the volume captured by lipid droplets within hepatocytes [28]. Intra- and inter-observer variability in spectral processing of ^{31}P-MRS was reported previously [27].

2.5. Heart rate variability

Nine of the 10 volunteers underwent heart rate variability monitoring before, during, and after taVNS and sham stimulation on two additional test days. One volunteer did not want to participate further. Participants were again blinded to the random order of conditions. Stimulation intensities were similar to those on test days with MRS (1.0 $\pm$ 0.1 mA and 1.1 $\pm$ 0.1 mA for sham and taVNS conditions, respectively; $P > 0.05$ versus MRS test days for both sham and taVNS conditions). R—R intervals were recorded before and over 120 min after taVNS and sham stimulation using a digital SpiderView Holter with five electrodes to record two-channel ECG with ECG signal sampling rate of 200 Hz (Sorin Group, Munich, Germany). Heart rate variability (HRV) was analyzed using commercially available software (SyneScope version 3.00 analysis system; Sorin Group) as described before [29]. Frequency domain indices included the low frequency (LF) band (0.04—0.15 Hz), reflecting both sympathetic and parasympathetic modulation of heart rate and the high-frequency (HF) band (0.15—0.4 Hz), reflecting parasympathetic modulation of the heart rate. The low-frequency/high-frequency (LF/HF) ratio can be used as an index of cardiac autonomic balance such that a decrease in LF/HF ratio indicates a shift towards parasympathetic predominance. Additionally, R—R intervals were recorded with VariaCardio TF5 system (MIE Medical Research, Leeds, UK) with one-channel ECG over 10 min before, during and after taVNS or sham stimulation. Both VariaCardio and SpiderView were used for HRV measurements in supine position. They differed by the length of ECG recording (10 min and 2 h) thereby allowing us to assess both short- and long-term HRV, which are known not to be comparable [30].

2.6. Baroreflex sensitivity

Continuous plethysmographic arterial pressure and R—R intervals were recorded from the left middle finger before, during, and after each intervention in a supine position using a Finometer MIDI device and BeatScope Easy v02.10 software (Finapres Medical Systems, Enschede, The Netherlands). For the baroreflex sensitivity (BRS) assessment, time-domain and frequency-domain parameters were computed according to the Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology [31], using commercially available software (BeatScope Easy; Nevrokard BRS Analysis v6.3.0, Nevrokard, Izola, Slovenia).

2.7. Calculations

BRS was determined using the sequence method for positive (BRS+/+), negative (BRS−/−) and all (BRS-allSeq) sequences, spectral analysis (low-frequency, high-frequency and the average of the low- and high-frequency components), cross spectral analysis (transfer function BRS), or by dividing the standard deviation of R—R interval by the standard deviation of systolic blood pressure (BRS-SD) [32].

Rates of EGP were calculated by dividing the tracer ([6,6-$^2H_2$]glucose) infusion rate times tracer enrichment by the tracer enrichment in plasma glucose and subtracting the tracer infusion rate [28].

2.8. Statistical analysis

Data are presented as means $\pm$ SEM. Two-way analysis of variance (ANOVA) was performed with the repeated measures factors time and treatment followed by Tukey multiple comparisons test to determine source of differences. Spearman correlations were used to assess associations between changes in metabolite and hormone levels. P values <0.05 were defined to indicate significance of differences. All analyses were performed using Prism version 6.04 (GraphPad, La Jolla, CA) statistical software.

3. RESULTS

This study included healthy normal-to-overweight glucose tolerant individuals (Table 1).

3.1. taVNS does not alter heart rate variability

Data from nine participants were included in the heart rate variability analysis. There was no difference between HRV frequency domains (Figure 1) at baseline of the active taVNS and sham stimulation. No changes in HRV were seen during and after taVNS and sham stimulation (Figure 1). Both measurements from two-channel (SpiderView Holter) and one-channel (VariaCardio) ECG recordings revealed no effect of taVNS on LF/HF ratio (Figure 1A and B) (ANOVA $P = 0.95$ and $P = 0.49$, respectively).

3.2. taVNS does not alter BRS

Data from nine participants were included in the BRS analysis. Heart rate (Figure 2D), blood pressure (Figure 2A, B), and baroreflex sensitivity (Table 2) at baseline of the active taVNS and sham stimulation did not differ. No changes in heart rate, systolic, diastolic, and mean blood pressure were found during or after taVNS and sham stimulation (Figure 2). There were no differences in BRS during or after taVNS and sham stimulation (Table 2).

3.3. taVNS does not affect circulating metabolites, hormones, and EGP, which change during fasting

Data from ten participants were included in this analysis. Baseline concentrations of blood glucose, serum triglycerides, FFA, insulin, c-peptide, glucagon, PP, and baseline EGP rates were comparable between taVNS and sham stimulation (Figure 3). ANOVA revealed no changes in any of these substrates or hormones for the factor treatment and treatment $\times$ time interaction (180 min for blood glucose, serum triglycerides, FFA, insulin, c-peptide, glucagon, and EGP, 120 min for PP). EGP rates remained unaltered (Figure 3B) (ANOVA treatment $P = 0.79$, treatment $\times$ time $P = 0.22$).

Concentrations of glucose, FFA, insulin, c-peptide, and EGP changed over time (ANOVA time $P < 0.001$, $P < 0.001$, $P = 0.02$, $P < 0.001$ and

Table 1 — Participants' characteristics.

Parameter	Mean $\pm$ SD
N (females)	10 (2)
Age (years)	51.1 $\pm$ 6.0
BMI (kg/m^2)	25.5 $\pm$ 2.7
Waist circumference (cm)	89 $\pm$ 10
Glucose (mg/dl)	77 $\pm$ 6
Insulin (µU/ml)	8.2 $\pm$ 5.2
C-peptide (ng/ml)	1.8 $\pm$ 0.6
HbA1c (%)	5.4 $\pm$ 0.3
GOT (U/l)	24 $\pm$ 12
GPT (U/l)	28 $\pm$ 16
Triglycerides (mg/dl)	95 $\pm$ 33
Free fatty acids (µmol/l)	299 $\pm$ 35

Data are mean $\pm$ SD, GOT — glutamate oxaloacetate transaminase, GPT — glutamate pyruvate transaminase.

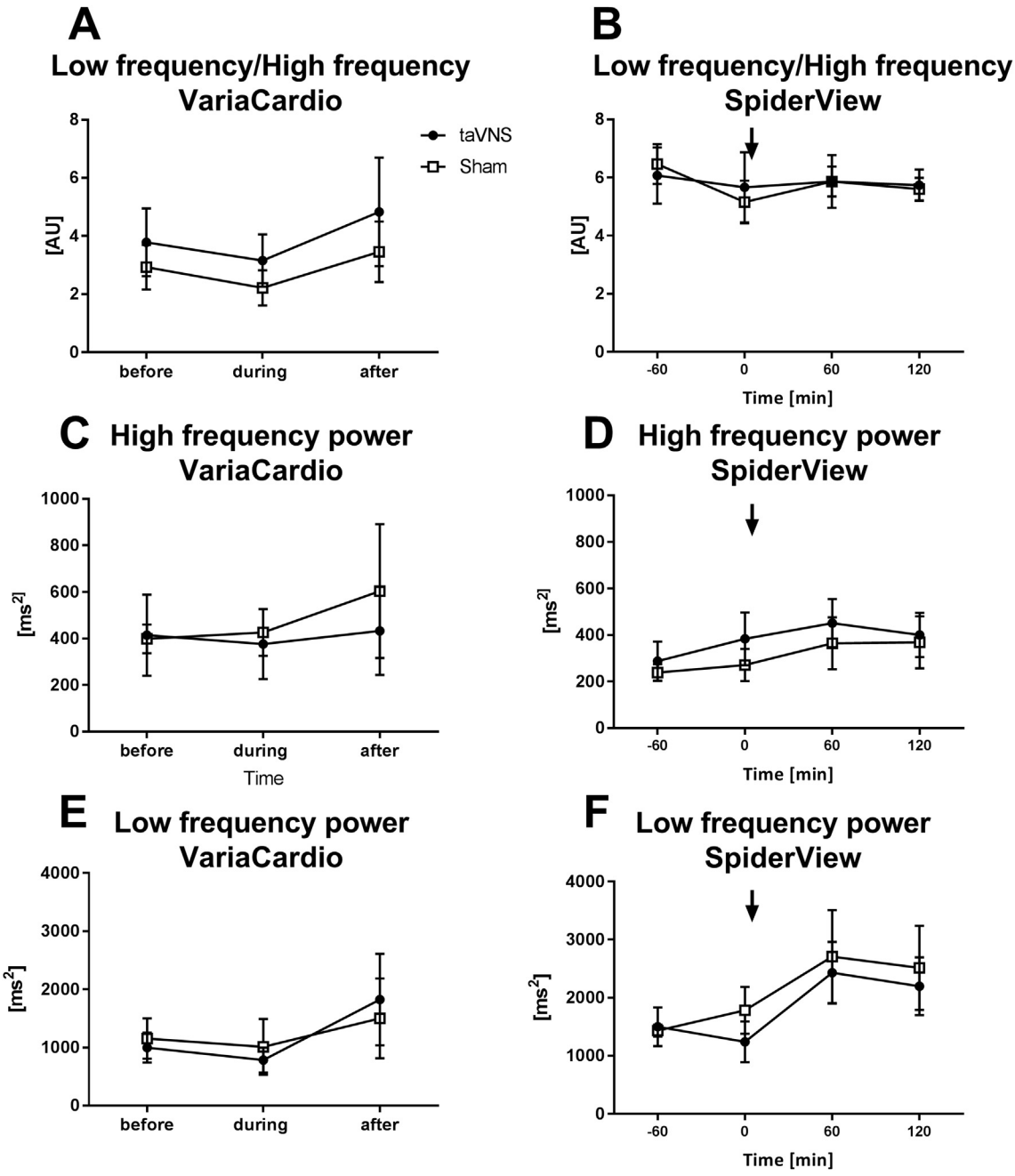

Figure 1: Heart rate variability frequency domains for sham and active taVNS stimulation. Stimulation denoted by an arrow. Mean ± SEM.

$P < 0.001$, respectively). During the 3 h, glucose decreased by 4% after both taVNS and sham procedure, while FFA increased by 40% after taVNS and by 20% after sham stimulation. Serum insulin decreased by 19% and 21% and c-peptide by 17% and 19% during this time period after taVNS and sham stimulation, respectively. Rates of EGP decreased by 19% and 21% after taVNS and sham stimulation, respectively.

3.4. taVNS does not alter hepatic lipid and energy metabolism

Data from ten participants were included in this analysis. Baseline liver fat content and hepatic ATP and Pi concentrations were comparable on taVNS and sham stimulation days at baseline (Figure 4). There was no effect of taVNS on hepatic lipid, ATP and Pi content at 30 and 180 min (Figure 4) (ANOVA treatment × time $P = 0.91$, $P = 0.48$ and $P = 0.24$, respectively).

No changes in hepatic lipid, ATP, and Pi content over time were found (ANOVA time $P = 0.49$, $P = 0.48$, $P = 0.27$, respectively).

3.5. Correlation analyses of hepatic phosphorus compounds with changes in circulating metabolites and hormones

The change in serum insulin between 180 min and baseline related positively to hepatic ATP after taVNS ($P = 0.01$), but not after sham intervention ($P = 0.78$). No associations between changes in FFA, glucose, c-peptide, EGP, and hepatic lipid, ATP and Pi content were found after taNVS and sham stimulation.

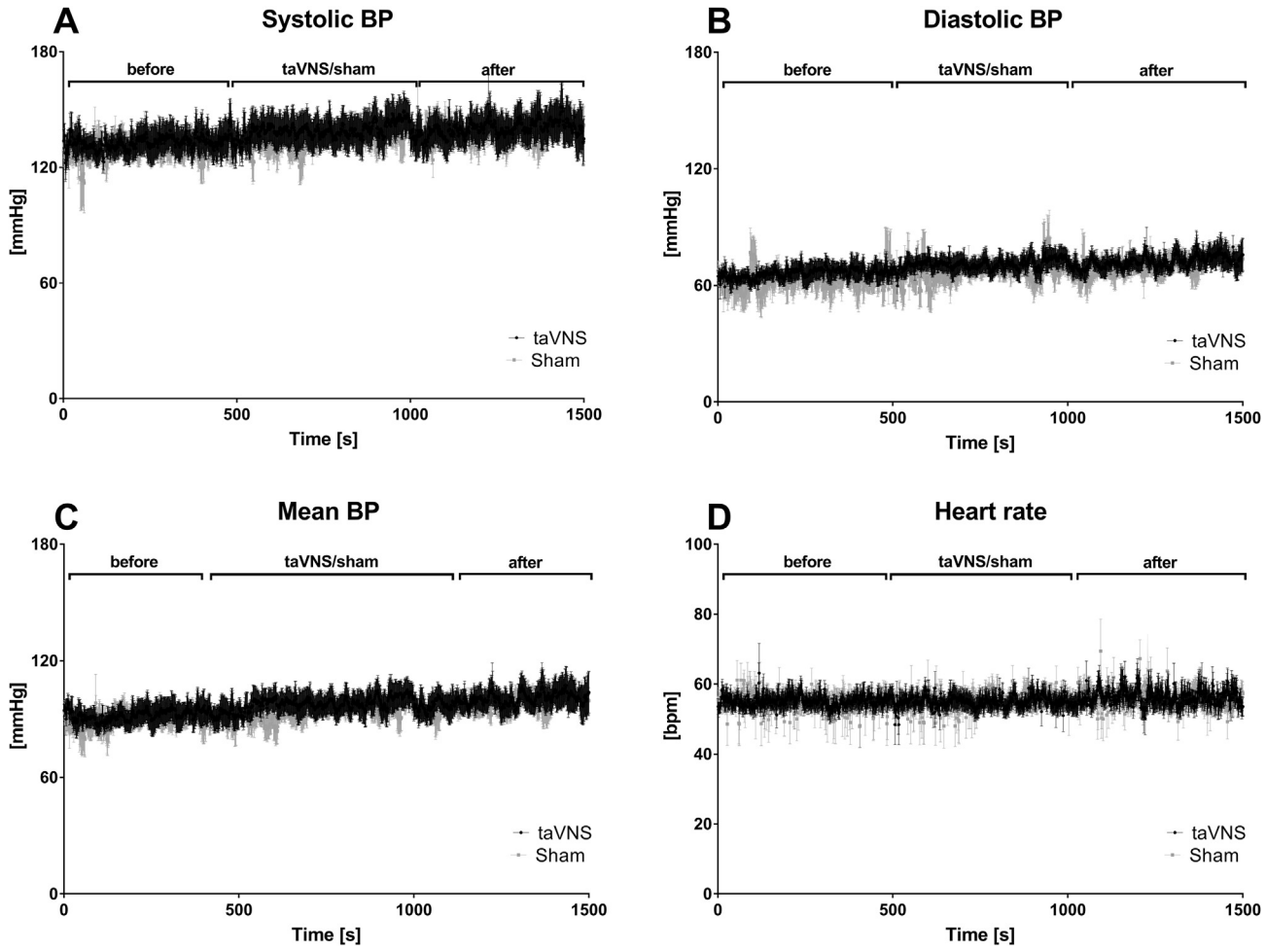

Figure 2: Systolic (A), diastolic (B), mean blood pressure (C), and heart rate (D) before, during and after sham and active taVNS stimulation. Mean ± SEM.

Table 2 — Baroreflex sensitivity (BRS) before, during and after transcutaneous auricular vagus nerve stimulation (taVNS).

Parameter	Condition	Before	During	After
BRS-SD (ms/mmHg)	taVNS	5.6 ± 2.9	5.1 ± 2.5	3.9 ± 2.0
	Sham	4.2 ± 1.5	3.7 ± 1.3	5.3 ± 2.6
BRS +/+ (ms/mmHg)	taVNS	12.3 ± 5.3	13.1 ± 5.7	16.6 ± 5.7
	Sham	11.9 ± 3.7	13.6 ± 5.6	14.5 ± 5.4
BRS −/− (ms/mmHg)	taVNS	18.3 ± 5.5	20.3 ± 10.1	13.9 ± 4.0
	Sham	13.0 ± 2.8	16.2 ± 4.2	17.5 ± 5.8
BRS-allSeq (ms/mmHg)	taVNS	16.7 ± 2.1	15.8 ± 6.2	14.9 ± 4.7
	Sham	15.9 ± 6.5	15.2 ± 4.5	17.8 ± 8.1

Data are mean ± SD; BRS determined using the sequence method for positive (BRS+/+), negative (BRS−/−) and all (BRS-allSeq) sequences or by dividing the standard deviation of R−R interval by the standard deviation of systolic blood pressure (BRS-SD).

4. DISCUSSION

This study found that non-invasive taVNS does not induce a shift in cardiac autonomic function and does not affect pancreatic polypeptide levels, suggesting no change in parasympathetic outflow to the pancreas and the heart in healthy humans. In accord with the lack of effect of taVNS on peripheral vagal tone, it also had no effect on hepatic insulin sensitivity, lipid, and energy metabolism. These data indicate that taVNS is unable to mimic intranasal insulin effects and modulate hepatic glucose, lipid, and energy metabolism in humans, likely because it fails to alter visceral parasympathetic tone. Finally, this study shows that hepatic fat and phosphorous metabolite contents remain constant after prolonged fasting, at least in healthy non-obese humans.

Systemic glucose, insulin, c-peptide, glucagon, FFA, and triglyceride concentrations remained unaltered after taVNS in this study, which is in line with the findings on these substrates and hormones observed upon intranasal insulin under fasting conditions [10]. Previously observed transient reductions in glucose and FFA and the increase in serum insulin after intranasal insulin application in healthy humans are due to spillover of insulin into the circulation rather than to central insulin effects [10]. The lack of taVNS action on substrate and hormone levels in the present study could thus be interpreted as a match between intranasal insulin and vagus stimulation effects. Additionally, no difference in EGP was found after taVNS further supporting this concept. However, intranasal insulin acutely decreases liver lipids and increases hepatic ATP content, while the taVNS affected neither hepatic fat nor ATP concentrations [10]. The lack of taVNS effect on pancreatic polypeptide secretion suggests no changes in parasympathetic outflow to the visceral organs, which possibly explains this inconsistency. Even if intranasal insulin effects on peripheral

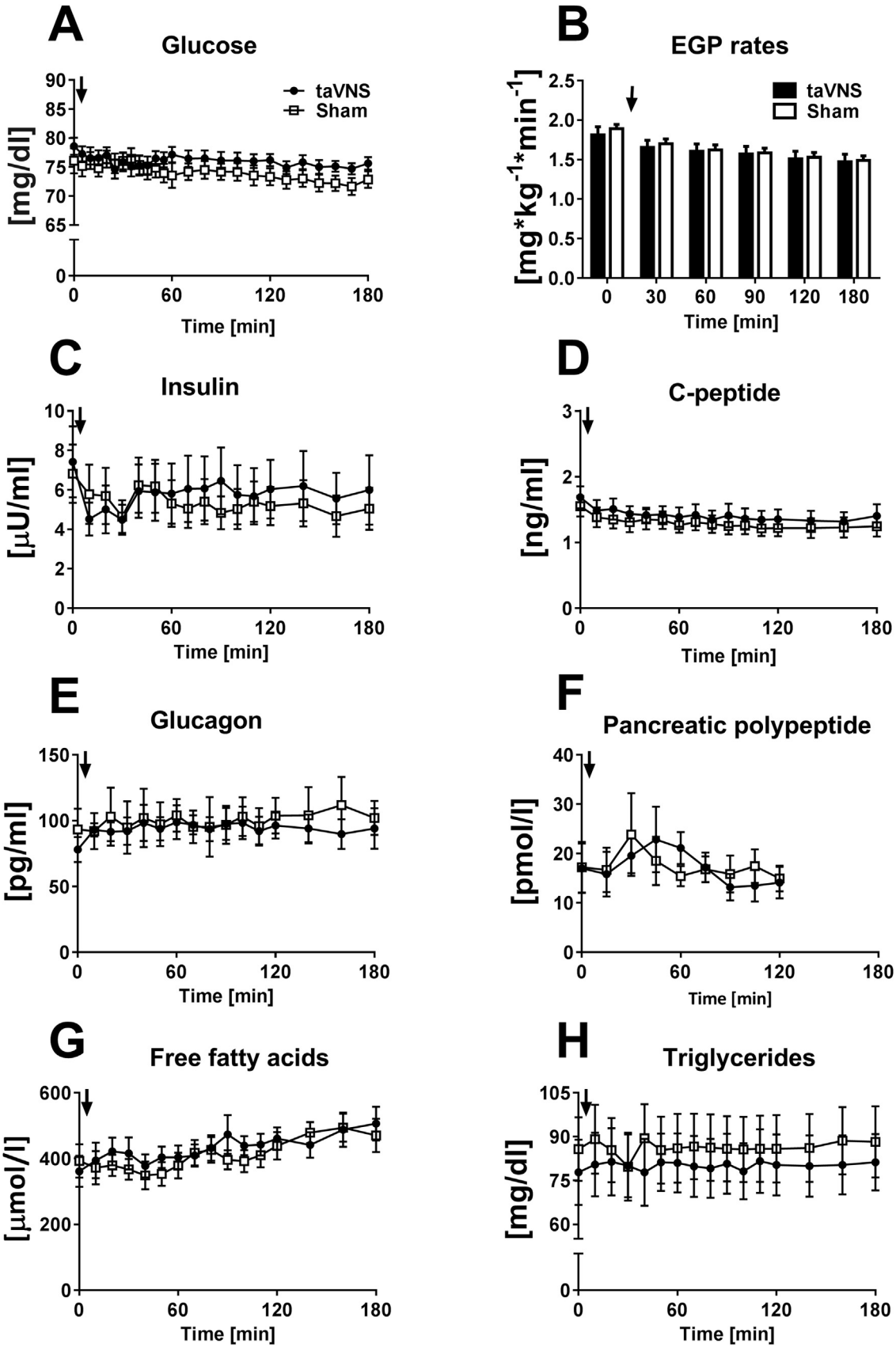

Figure 3: Concentrations of glucose (A), insulin (C), c-peptide (D), glucagon (E), pancreatic polypeptide (F), free fatty acids (G), triglycerides (H), and rates of endogenous glucose production (B) after sham and active taVNS stimulation. Stimulation denoted by an arrow. Mean ± SEM.

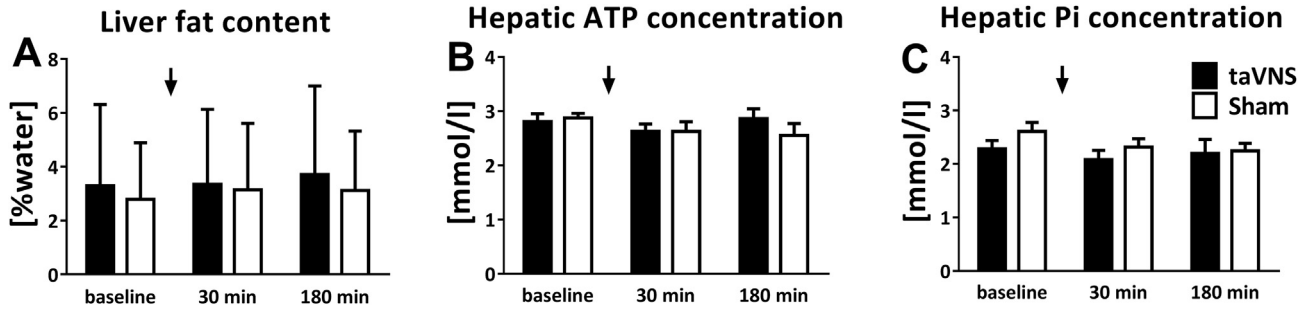

Figure 4: Liver fat content (A), hepatic concentrations of adenosine triphosphate (ATP) (B), and inorganic phosphate (Pi) (C) measured by $^{31}P/^{1}H$ magnetic resonance spectroscopy before and after sham and active taVNS stimulation. Stimulation denoted by an arrow. Mean ± SEM.

metabolism are mediated by the vagus nerve, as suggested previously [8], taVNS is not able to mimic these conditions, as it does not seem to modulate vagal tone to the abdominal viscera.

This study describes stable physiological levels of hepatic ATP and Pi concentrations over 3 h after overnight fasting, which adds to the understanding of the time course of basal ATP and Pi during prolonged fasting of healthy humans. Under these conditions, FFA increase by 20–40%, but do not affect hepatic lipid or energy substrate concentrations. The decrease in EGP rates by about 20% at 3 h is paralleled by a gradual reduction in blood glucose, but does not affect hepatic energy metabolism. These data demonstrate stable concentrations of hepatic phosphorus metabolites with prolonged fasting, in the face of alteration of circulating metabolites and hormones.

One previous report using the same stimulation procedure with NEMOS® device provided evidence for activation of the vagal viscerosensory NTS after taVNS from brain imaging [11]. NTS nuclei are structurally and functionally linked to the most important vagal nucleus for the efferent parasympathetic output to the visceral organs, the dorsal motor nucleus, which receives input from the NTS to directly innervate visceral structures [33]. Thus, NTS activation upon taVNS should translate to changes in vagal outflow to the periphery. Of note, the dorsal vagal complex has been identified as the site of central insulin action accounting for reduction in hepatic glucose production [34].

Furthermore, the role of the parasympathetic nervous system in human glucoregulatory physiology has been studied for over 20 years, providing evidence for direct muscarinic cholinergic inhibition of hepatic glucose production, which is offset by increased glucagon secretion stimulating hepatic glucose output [35]. The lack of any changes in insulin and glucagon secretion in our study point to the fact that taVNS does not elicit any autonomic system effects on the endocrine pancreas. Pancreatic polypeptide is a well-known readout for cholinergic activation [36], which was measured here continuously before, during and after taVNS. No differences were shown (Figure 3F), additionally supporting the concept that taVNS fails to modulate parasympathetic tone in humans.

Interestingly, vagal stimulation has been shown to modulate glucose metabolism in rheumatoid arthritis patients in the postprandial state [21]. Some differences between these and the present results could result from the different experimental settings, such as fasting versus postprandial conditions. Moreover, rheumatoid arthritis patients received invasive vagal stimulation using implanted devices, while participants in the present study underwent non-invasive taVNS in the outer ear. While cutaneous electrical stimulation with NEMOS® does not lead to peripheral metabolic effects, direct electric pulses to the cervical vagus nerve fibers might indeed have the potential to modulate glucose homeostasis.

Of note, vagus nerve blockade has been found to cause no acute changes in hepatic glucose production or in insulin secretion and action in non-diabetic humans [37]. These data indicate that vagal tone modulation in the short-term does not have an effect on glucose metabolism. However, vagus modulation studies in diabetic humans, who are known to have impaired sympathovagal balance [38], have not been performed and remain a question of future investigation.

We further assessed the effects of taVNS on cardiac function and whether parasympathetic outflow to the cardiovascular system is modulated after vagal stimulation. In contrast to a previous report showing reduction in LF/HF ratio [12], this study found neither a change in HRV nor a shift in predominance of the autonomic regulation after taVNS. Of note, Clancy et al. examined two not-matched groups of different size with taVNS and sham stimulation without applying a crossover design. The present study performed simultaneous assessment of heart rate variability using two independent Holter methods with short- and long-term ECG recordings, which both showed no difference in any of the measured frequency domains, pointing to the lack of effect of taVNS on cardiac autonomic function. In line with previous report showing no acute effect of vagal nerve stimulation on cardiovascular autonomic and hemodynamic parameters [39], this study also did not observe alterations in BRS with taVNS (Table 2). Of note, vagus nerve stimulation has been suggested to improve autonomic imbalance in heart failure patients [40] and chronically increases BRS and elevates HF power of HRV [41], but, for this purpose, the stimulation parameters and dipole orientation are different from that used in epilepsy [39] and in the present study. Whether other stimulation parameters or vagus stimulation techniques such as neck vagus stimulation with gammaCore® [42] or VNS Therapy System implantation [20,21,39,40] will be able to affect the parasympathetic tone to the viscera and thereby might alter peripheral metabolism remains to be tested.

In conclusion, taVNS applied non-invasively in the outer ear neither affects hepatic glucose metabolism nor hepatocellular lipid and ATP content in healthy humans. Of note, prolonged fasting does not affect liver lipids and ATP in this cohort. No differences in circulating glucoregulatory hormones and pancreatic polypeptide levels between active and sham stimulation indicate a lack of parasympathetic tone modulation with taVNS, which is confirmed by the absence of alterations in cardiac autonomic function. These data suggest that encouraging results from rodent studies using taVNS on glucose metabolism are not translatable to humans and do not hold promise for the future treatment of T2D patients.

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CONTRIBUTION STATEMENT

S.G. researched, collected and analyzed data and wrote the manuscript. A.B., D.F.M., G.J.B., and K.G.M. collected data, contributed to the interpretation of results, and revised the article. J.L., D.Z., and E.H. contributed to the manuscript. M.R. designed the study, interpreted the data, and wrote the manuscript. M.R. is the guarantor of this work and, as such, had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

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CONFLICT OF INTEREST

The authors have no potential conflicts of interest relevant to this article.

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