Impact of nocturnal hypoxia on glycaemic control, appetite, gut microbiota and inflammation in adults with type 2 diabetes mellitus: A single-blind cross-over trial

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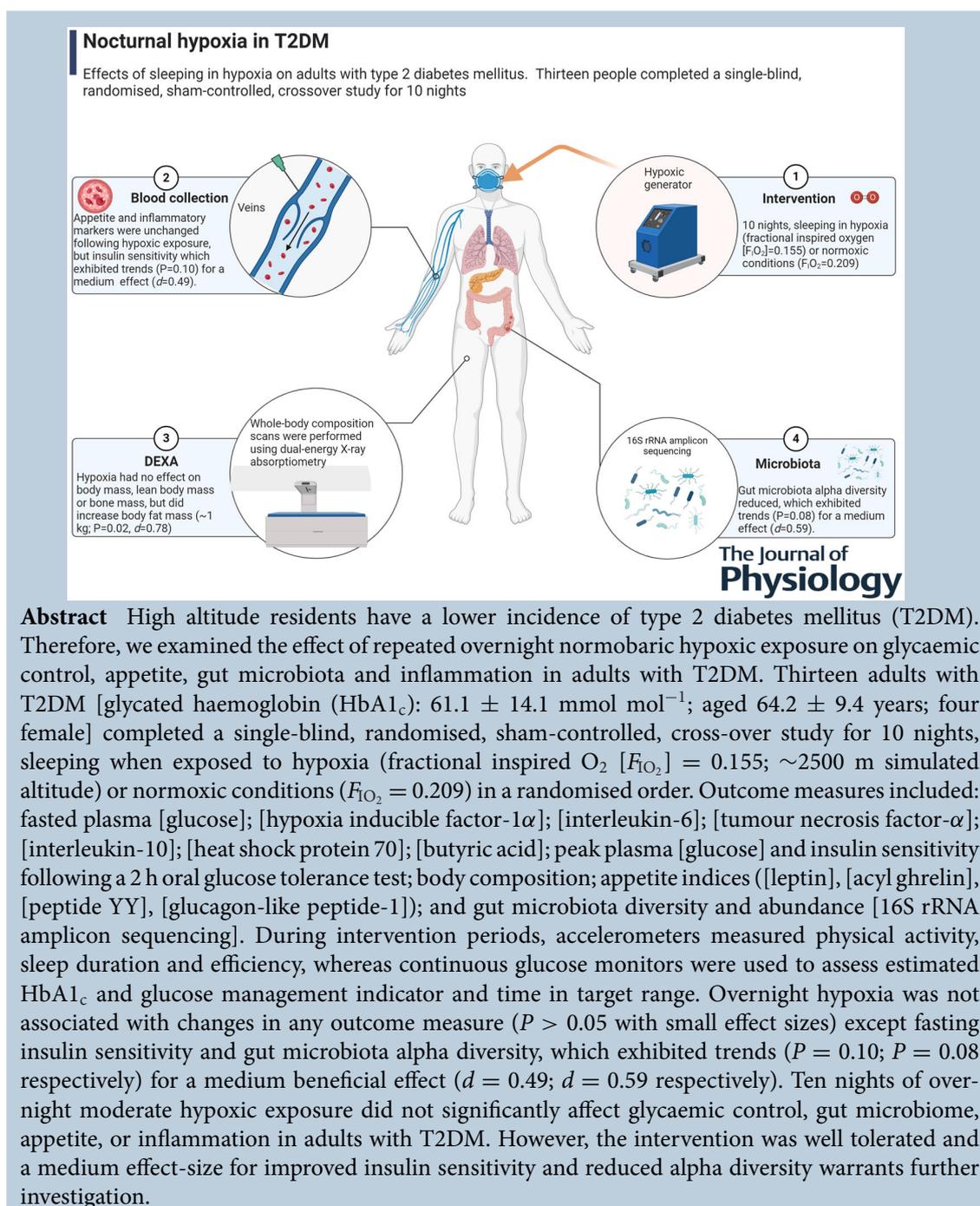
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The Journal of Physiology

Anthony (Ant) Shepherd is an Associate Professor in Clinical Exercise Physiology, based in the Clinical Health and Rehabilitation Team at the University of Portsmouth. Ant's research career focuses on using novel interventions (for example; extreme environments and nutrition) to improve metabolic and cardiovascular health, quality of life and physical function. Ultimately, his research revolves around clinical benefit. He is also interested in understanding the pathophysiology of specific chronic diseases aiming to elucidate any potential therapeutic strategies.

Nocturnal hypoxia in T2DM

Effects of sleeping in hypoxia on adults with type 2 diabetes mellitus. Thirteen people completed a single-blind, randomised, sham-controlled, crossover study for 10 nights

2 — Blood collection   Veins
Appetite and inflammatory markers were unchanged following hypoxic exposure, but insulin sensitivity which exhibited trends (P=0.10) for a medium effect (d=0.49).

Hypoxic generator

1 — Intervention
10 nights, sleeping in hypoxia (fractional inspired oxygen [F_{IO_2}]=0.155) or normoxic conditions (F_{IO_2}=0.209)

Whole-body composition scans were performed using dual-energy X-ray absorptiometry

3 — DEXA
Hypoxia had no effect on body mass, lean body mass or bone mass, but did increase body fat mass (~1 kg; P=0.02, d=0.78)

16S rRNA amplicon sequencing

4 — Microbiota
Gut microbiota alpha diversity reduced, which exhibited trends (P=0.08) for a medium effect (d=0.59).

The Journal of Physiology

Abstract High altitude residents have a lower incidence of type 2 diabetes mellitus (T2DM). Therefore, we examined the effect of repeated overnight normobaric hypoxic exposure on glycaemic control, appetite, gut microbiota and inflammation in adults with T2DM. Thirteen adults with T2DM [glycated haemoglobin (HbA1$_c$): 61.1 ± 14.1 mmol mol^{-1}; aged 64.2 ± 9.4 years; four female] completed a single-blind, randomised, sham-controlled, cross-over study for 10 nights, sleeping when exposed to hypoxia (fractional inspired O$_2$ [F_{IO_2}] = 0.155; ~2500 m simulated altitude) or normoxic conditions (F_{IO_2} = 0.209) in a randomised order. Outcome measures included: fasted plasma [glucose]; [hypoxia inducible factor-1α]; [interleukin-6]; [tumour necrosis factor-α]; [interleukin-10]; [heat shock protein 70]; [butyric acid]; peak plasma [glucose] and insulin sensitivity following a 2 h oral glucose tolerance test; body composition; appetite indices ([leptin], [acyl ghrelin], [peptide YY], [glucagon-like peptide-1]); and gut microbiota diversity and abundance [16S rRNA amplicon sequencing]. During intervention periods, accelerometers measured physical activity, sleep duration and efficiency, whereas continuous glucose monitors were used to assess estimated HbA1$_c$ and glucose management indicator and time in target range. Overnight hypoxia was not associated with changes in any outcome measure ($P > 0.05$ with small effect sizes) except fasting insulin sensitivity and gut microbiota alpha diversity, which exhibited trends ($P = 0.10$; $P = 0.08$ respectively) for a medium beneficial effect ($d = 0.49$; $d = 0.59$ respectively). Ten nights of overnight moderate hypoxic exposure did not significantly affect glycaemic control, gut microbiome, appetite, or inflammation in adults with T2DM. However, the intervention was well tolerated and a medium effect-size for improved insulin sensitivity and reduced alpha diversity warrants further investigation.

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Abstract figure legend Summary of the effects of nocturnal hypoxia on glycaemic control, appetite, gut microbiota and inflammation in adults with type 2 diabetes mellitus (T2DM). Living at altitude lowers the incidence of T2DM. Thirteen adults with T2DM completed a single-blind, randomised, sham-controlled, cross-over study for 10 nights, sleeping when exposed to hypoxia (fractional inspired O$_2$ = 0.155) or normoxic conditions. Appetite and inflammatory markers were unchanged following hypoxic exposure, but an increased insulin sensitivity and reduced gut microbiota alpha diversity were associated with a medium effect-size and statistical trends, which warrant further investigation using a definitive large randomised controlled trial.

Key points

- Living at altitude lowers the incidence of type 2 diabetes mellitus (T2DM). Animal studies suggest that exposure to hypoxia may lead to weight loss and suppressed appetite.
- In a single-blind, randomised sham-controlled, cross-over trial, we assessed the effects of 10 nights of hypoxia (fractional inspired O_2 ∼0.155) on glucose homeostasis, appetite, gut microbiota, inflammatory stress ([interleukin-6]; [tumour necrosis factor-α]; [interleukin-10]) and hypoxic stress ([hypoxia inducible factor 1α]; heat shock protein 70) in 13 adults with T2DM.
- Appetite and inflammatory markers were unchanged following hypoxic exposure, but an increased insulin sensitivity and reduced gut microbiota alpha diversity were associated with a medium effect-size and statistical trends, which warrant further investigation using a definitive large randomised controlled trial.
- Hypoxic exposure may represent a viable therapeutic intervention in people with T2DM and particularly those unable or unwilling to exercise because barriers to uptake and adherence may be lower than for other lifestyle interventions (e.g. diet and exercise).

Introduction

Type 2 diabetes mellitus (T2DM), characterised by progressive insulin resistance and chronic hyperglycaemia (Prospective, 1995), causes multiple comorbidities, shortens life, and is expected to affect 700 million people by 2045 (Saeedi et al., 2019). Weight loss, achieved through exercise and dietary modification, reduces hyperglycaemia (Golay et al., 1985), and thereby cardiovascular disease risk and microvascular complications, in people with T2DM (Stratton et al., 2000). However, diverse psychosocial and logistical factors can impede the initiation of, and/or adherence to, these lifestyle interventions (Ary et al., 1986; Glasgow et al., 1997). Therefore, there is a need to investigate the potential of other cost-effective interventions with therapeutic potential for this growing population.

Exposure to a reduced fractional inspired O_2 (low F_{IO_2}: hypoxia) may represent such an intervention. A single acute hypoxic exposure (60 min; fractional inspired O_2 (F_{IO_2}) = 0.146) can improve glucose tolerance in people with T2DM (Mackenzie et al., 2011), whereas individuals who live exposed to the hypobaric hypoxia of high altitude have reduced serum glucose concentrations ([glucose]) and reduced prevalence of T2DM (Woolcott et al., 2014). Daily hypoxic exposure increases glucose transporter-4 translocation in the skeletal muscle of obese mice (Eid et al., 2018) and returns fasting blood [glucose] to normal levels in healthy (Eid et al., 2018) and diabetic rodents (Wang et al., 2018). Similar effects on glucose homeostasis have been shown in overweight humans exposed to hypoxia (Lecoultre et al., 2013) and in people with T2DM, albeit in a pre-post experimental design rather than a randomised control trial (Marlatt et al., 2020).

The potential mechanisms by which hypoxia might improve glycaemic control are diverse. Hypoxia may function as an 'exercise mimetic', by acting on the same insulin independent signalling pathway for glucose uptake as that stimulated by muscle contraction (Fisher et al., 2002). Hypoxic stress also activates the hypoxia inducible factor (HIF) pathway, which is a major regulator of cellular metabolism (Kim et al., 2006; Park et al., 2020; Zhang et al., 2010), promoting a substrate shift towards glucose metabolism (Kim et al., 2006) and suppresses appetite (Palmer & Clegg, 2014). Reductions in appetite are also probably important given that body mass reduction improves glycaemic control in individuals with T2DM (Wing et al., 1994). HIF also stimulates heat shock protein (HSP) expression, which reduces insulin resistance and improves glucose homeostasis in rodents and humans (Kondo et al., 2016). Finally, gut microbiota have been linked to the pathogenesis and progression of insulin resistance (Lee et al., 2020), with some short-chain fatty acids (SCFA; e.g. butyrate) produced by anaerobic fermentation of dietary fibre in the gut being linked to improved insulin sensitivity (Cui et al., 2022). Indeed, the relative abundance of taxa that synthesise SFCAs, such as *Pseudobutyivibrio*, appears to be increased in individuals living at high altitude (Li et al., 2016; Sturgess & Montgomery, 2021). By reducing F_{IO_2}, it is possible that the gut would become more hypoxic and in turn increase the relative abundance of anaerobes within the gastrointestinal microbiota.

Although utilising hypoxia as a therapeutic intervention in a clinical context is in its infancy, sleeping in hypoxic environments is often used by competitive endurance athletes, who attempt to gain an ergogenic benefit through up-regulating erythropoiesis (Rendell et al., 2017). The delay in implementing hypoxia as an adjunct therapy in a clinical context may, at least partly, be a result of fear of increasing hypoxia, and thus oxidative stress (Siervo et al., 2014) and inflammation (McGarry et al., 2018; Taylor & Colgan, 2017), which can

be pathogenic (Wu et al., 2014). However, acute moderate hypoxia ($F_{IO_2} = 0.155$) does not appear to increase markers of oxidative stress in overweight adults (Corbett et al., 2023) and 10 days of overnight moderate hypoxia causes (diuresis independent) weight loss in athletes (Rendell et al., 2017).

Given the potential for hypoxia to represent a novel, cost-effective, non-invasive therapeutic intervention for people with T2DM, we hypothesised that 10 days of overnight moderate hypoxia would improve: (1) insulin sensitivity, (2) blood glucose and (3) body composition and elicit beneficial changes in (4) appetite hormones and (5) gut microbiota, but would not alter (6) inflammation in adults with T2DM.

Methods

Ethical approval

This single-blind, randomised, cross-over, control trial was granted a favourable ethics opinion by the Berkshire B National Health Service Research Ethics Committee (21/SC/0351) and Health Research Authority. This study also conforms to the standards set by the latest revisions of the Declaration of Helsinki and was registered on the ClinicalTrials.gov database (ID # NCT05147116). Participants were provided with a participant information sheet ≥ 48 h before providing fully informed written consent. Participants were given a full briefing about the study and had the opportunity to ask any questions prior to enrolment.

Participants

Participants were recruited from primary care (i.e. general practitioner practices), local databases and via media releases. Those included were adults (≥ 18 years of age) with a clinical diagnosis of T2DM, as defined by the World Health Organization (Alberti & Zimmet, 1998) and [glycated haemoglobin] (HbA1$_c$) > 48 mmol mol^{-1} who were able to provide fully informed written consent. People were excluded if known to be suffering from haematological conditions (severe anaemia [<80 g L^{-1}]; haemoglobinopathies, including sickle cell anaemia; haematological malignancies; myeloproliferative disease; polycythaemia; and clotting defects) or obstructive sleep apnoea; had a forced vital capacity (FVC) <80% or forced expiratory volume in 1 s (FEV$_1$) <5th percentile relative to appropriate normative reference values (Stanojevic et al., 2022); had any extant cardiac conditions; or suffered any other serious medical condition that might interfere with data quality or participant safety (e.g. heavy smokers).

Participants were randomised (randomizer.org) using concealed allocation to start with either a hypoxic or sham condition. Experimental conditions were separated by a washout period of between 1 and 3 months prior to undertaking the cross-over element (i.e. the remaining condition). Participants were asked to continue their usual lifestyle but were asked not to use air travel during the washout period. Our primary outcome was the delta score for the area under curve (AUC) of venous blood [glucose] during a 2 h oral glucose tolerance test (OGTT). An *a priori* sample size calculation was performed to estimate the required *n*. For 80% power and an alpha of 0.05, with an effect size estimated ~1, 13 people were required to detect a change in [glucose] AUC. A minimum of 15 people were therefore sought to be recruited to account for the 15% dropout rate typically exhibited in our clinical trials.

Pre-experimental tests

Upon recruitment (visit 1), a standard medical history was obtained and clinical examination performed. Venous blood samples were drawn, an electrocardiogram was performed, and stature, body mass and an average of three blood pressure measurements were recorded following 15 min of supine rest. Blood samples were analysed for HbA1$_c$, full blood count and lipid profiles, and to exclude haemoglobinopathies. Lung function was assessed (FVC, FEV$_1$, FEV$_1$/FVC ratio and peak expiratory flow rate). Based on pilot work (Corbett et al., 2023), and in the supervised laboratory environment, participants then inhaled air with an F_{IO_2} ~0.155 (equivalent to a simulated altitude of ~2500 m) for 1 h, with a target of 88–92% peripheral transcutaneous arterial O$_2$ saturation, measured at the fingertip of the index finger (S_{pO_2}; Oxyfit, Getwellue, Newark, CA, USA). A team of independent medical officers determined the eligibility of any potential participants who demonstrated an S_{pO_2} of $\leq 65\%$ or an end-tidal partial pressure of CO$_2$ or of O$_2$ <25 mmHg or <45 mmHg, respectively.

Protocol

Intervention. Participants were asked to sleep at home in hypoxic 'pillow tents' [pillow tent; Sporting Edge, Basingstoke, UK; choice informed by patient and public involvement (PPI) consultations]. A reduced F_{IO_2} was created by a hypoxic generator (Cloud 9; Sporting Edge). Participants breathed either 0.155 or 0.209 F_{IO_2} (sham hypoxia: extracted O$_2$ being remixed with the hypoxic air to create normoxia within the pillow tent) for 10 consecutive nights. Beat-by-beat S_{pO_2} was monitored at the fingertip throughout all intervention nights, with an alarm being triggered if S_{pO_2} fell <80%. During

the intervention and washout periods, participants wore a continuous glucose monitor (CGM) sensor (Abbott Diabetes Care Inc., Alameda, CA, USA) on the anterior portion of the upper arm (worn for at least 24 h before the intervention period), as well as a wrist-worn accelerometer (GENEActiv; Activinsights, Cambridge, UK) to assess habitual physical activity (PA) levels and sleep (Van Hees et al., 2015).

Assessment visits. Visits two to five were identical in nature, with visits two and four occurring prior to the intervention and visit three and five happening 1 day post-completion of the 10-day hypoxic or sham exposure. At each visit, body composition was assessed using dual-energy X-ray absorptiometry (DEXA) (Hologic; Vertec, Reading, UK) and an OGTT was undertaken using venous blood samples. Participants were given instructions on how to collect a stool sample and asked to bring it with them to each assessment visit. During all experimental visits, a CGM sensor and accelerometer were fitted. Participants were studied, in line with clinical guidelines, after an overnight fast, having not taken their hypoglycaemic medications on the morning of testing.

Outcome measures

OGTT. Fasting blood samples were taken for the measurement of plasma [glucose] and [insulin], with these data being used to calculate fasting insulin sensitivity via the quantitative insulin sensitivity check index (Katz et al., 2000). Following this, participants consumed 75 g of glucose (Una Health, Stoke-on-Trent, UK), and blood samples were drawn immediately prior to ($t = 0$) and 30, 60, 90 and 120 min post-glucose ingestion for the assessment of plasma [glucose] and [insulin]. Post-prandial insulin sensitivity was calculated using Stumvoll's insulin sensitivity index (Stumvoll et al., 2001). Plasma [glucose] AUC was calculated using the trapezoidal method and the highest measured value over the OGTT was recorded as peak plasma [glucose].

Body composition. Whole-body composition scans were performed using DEXA (Horizon®; Hologic Inc., Marlborough, MA, USA). Integrated software was used to calculate whole body fat, muscle, and bone mass.

CGM. A CGM (Abbott Diabetes Care Inc., Alameda, CA, USA) was fixed to the anterior portion of the upper arm with a small adhesive patch. The sensor was worn throughout the intervention period to determine free-living glycaemic control from the interstitial fluid. Data are presented for the 10 days of each intervention and for the first 10 days of the respective washout periods. Plasma [glucose] (10 day grand mean) and AUC,

time in range, estimated HbA1$_c$ and glucose management indicator (GMI) (Bergenstal et al., 2018) were calculated. Estimated HbA1$_c$ was calculated automatically by online software (Libreview; Abbott Diabetes Care Inc., Alameda, CA, USA), using methodology outlined by Nathan et al. (2008), who showed a strong correlation with average glucose measurements ($R^2 = 0.84$, $P = 0.0001$) in people with T2DM.

Diet diaries. Participants were asked to record a dietary and physical activity diary (in a standardised format) on paper for all 10 days of the intervention. Dietary macronutrient content was calculated (https://www.myfitnesspal.com) (Evans, 2017).

Gut microbiota. Stool samples were collected within 12 h of each visit (Faeces Catcher; Abbexa, Cambridge, UK) in sterile containers (Faeces tubes; Sarstedt, Nümbrecht, Germany). If participants produced the sample in the evening before the visit, it was stored at −18°C overnight, whereas, if the sample was produced in the morning of the visit, it was brought in ambient conditions and subsequently stored at −80°C until analysed upon study cessation.

Bacterial DNA was extracted from the stool samples using the QIAamp PowerFecal Pro DNA kit (Qiagen, Manchester, UK). DNA/sample quality, purity and quantity were assessed using a DS-11 spectrophotometer (DeNovix Inc., Wilmington, DE, USA) and further DNA-specific quantification was performed using the Qubit fluorometer (Invitrogen, Waltham, MA, USA). The full-length 16S rRNA gene was targeted for amplification by a PCR with primers 27F (5′-AGAGTTTGATCMTGGCTCAG-3′) and 1492R (5′-ACGGYTACCTTGTTACGACTT-3′). Target fragment lengths and DNA integrity were verified using a TapeStation 4150 instrument (Agilent, Santa Clara, CA, USA) in accordance with the manufacturer's instructions. Sequencing libraries were prepared for Oxford Nanopore Technologies (ONT) sequencing using a SQK-LSK114 Ligation Sequencing Kit and EXP-NBD196 Native Barcoding Expansion 96 kit (ONT, Oxford, UK) in accordance with the manufacturer's instructions. Libraries were combined and sequenced for 72 h using two independent R10.4.1 flow cells (ONT) using the GridION X5 platform (ONT). Basecalling, demultiplexing and adapter trimming were performed in real time using *MinKnow*, version 22.12.5 (ONT) and *Guppy*, version 6.4.6 (ONT) with the super-accurate base calling model.

Raw reads were trimmed to remove remaining adapters, reads less than 1.2 kb or greater than 1.8 kb in length, and reads with an average quality score below 15 using *Porechop*, version 0.2.4 (Wick et al., 2017) and *Nanofilt*,

version 2.8.0 (De Coster et al., 2018). In addition, reads were filtered to identify and remove potential host contamination by mapping against the human GRCh38 reference genome using *MiniMap*, version 2.24-r1122 (Li, 2018). Remaining filtered reads were mapped against the SILVA, version138.1 (Pruesse et al., 2007) small sub-unit (i.e. SSU) database (99% identity criteria) of known 16S rRNA sequences for taxonomic classification using Emu, version 3.4.5, with parameters '–type map-ont –min-abundance 0.0001 -N 50 –keep-counts' (Curry et al., 2022). Missing abundance estimates were set to zero, and all abundance estimates were rounded down to the nearest integer prior to analysis. Samples were removed if they had fewer than 20 000 reads ($n = 1$) or if both arms of the trial were not completed ($n = 1$). The pre- and post-treatment samples for remaining individuals ($n = 11$) were rarefied to the lowest read count (37 361 reads) prior to further analysis (see below).

Accelerometry. PA and sleep were assessed using GENEActiv (Activinsights) accelerometers. The GENEActiv monitor has been shown valid and reliable in measuring sleep (Van Hees et al., 2015), physical activity (Eslinger et al., 2011) and sedentary time (Pavey et al., 2016) in adults. The GENEActiv accelerometers measure triaxial movement acceleration in gravity (g) units ($1\ g = 9.81$ m s^2) at a frequency of 100 Hz continuously over a period of 7 days. The Euclidean norm (magnitude) of signals from the three axes minus 1 g (with negative numbers rounded zero) were used to quantify acceleration due to movement in mg ($1\ mg = 0.00981$ m s^2) (Van Hees et al., 2013). Following the measurement periods, data were downloaded using the manufacturer's software and processed in R, version 4.2.2 (R Core Team, Vienna, Austria) using the open source *GGIR* software package (http://cran.r-project.org/package=GGIR). Previously validated acceleration threshold values were used to quantify the time (min day^{-1}) spent on average in each PA intensity category (Fraysse et al., 2021): total PA, and separately for light, and moderate-to-vigorous physical activity (MVPA).

Sleep variables were determined from accelerometer data using an open-source sleep detection algorithm in GGIR software. Sleep metrics derived using this method have demonstrated good levels of agreement with both self-report measures of sleep and poly-somnography (i.e. the gold standard) (Full et al., 2018). The method of accelerometer-based sleep quantification used in the present study has been described in detail elsewhere (Van Hees et al., 2015). Briefly, wrist-worn triaxial accelerometers allow approximation of the angle of orientation of the arm relative to the horizontal plane. Periods of sleep are defined as nocturnal peri-ods characterised by minimal movement frequency and magnitude of changes to the angle of the arm, which does not include daytime sleep. Time in bed was defined as the onset of the first period of sustained inactivity (as measured by changes of less than 5° in a rolling 5 min window) to the end of the last period of inactivity. Sleep duration is the sum of all recorded periods of sleep. Sleep efficiency was then calculated as the sleep duration as a proportion of time in bed.

Periods of accelerometer non-wear were identified using the range and SD of acceleration values at each axis, calculated for rolling 60 min windows. Non-wear was indicated if the SD < 13.0 mg or if the range < 50 mg for two of the three axes. A full explanation of this method is provided elsewhere (Van Hees et al., 2013). To effective assessment of habitual PA and sleep, measurement days when the accelerometer was worn for less than 16 h were excluded from the final analyses (Van Hees et al., 2013). Participants who recorded <4 days with 16 h wear-time were also excluded.

Biochemical analysis

After 10 min of seated rest, fasting venous blood samples were collected via cannulation for plasma [glucose] in fluoride oxalate vacutainers (Becton Dickinson, Wokingham, UK) and circulating [insulin] in ethylenediaminetetraacetic acid (EDTA) vacutainers (Becton Dickinson) at the start and end of an OGTT. Fasting blood samples were also collected for hormonal markers of appetite. For glucagon-like peptide 1 (GLP-1) and peptide YY (PYY), we primed EDTA tubes with 10 μL of DDP-IV inhibitor (DPP4-M; Merck, Gillingham, UK) per mL of whole blood and 100 μL of aprotinin (B02AB01; Nordic Pharma, Reading, UK). The tubes were chilled on ice until used, and remained on ice until they were centrifuged. For measurement of leptin, 100 μL of aprotinin was added to an EDTA tube and for acyl-ghrelin and additional 20 μL of Pefabloc (AEBSF; Roche, Mannheim, Germany) was added prior to collection. Following centrifugation, both ghrelin plasma samples were treated again with Pefabloc (475 μL of plasma to 25 μL of Pefabloc). Blood was also collected into EDTA vacutainers for markers of inflammation [e.g. interleukin-6 (IL-6) and IL-10, tumour necrosis factor (TNF)-α], hypoxic stress (HIF-1α) and extracellular HSP70, as well as butyrate. All venous blood samples were centrifuged at 4500 g at 4°C for 10 min immediately following collection (Heraeus Multifuge 3 S-R; DJB Labcare, Buckinghamshire, UK), with plasma then separated and stored at −80°C.

Plasma [glucose] was analysed in duplicate using an automated analyser (C-Line; Biosen, Nottingham, UK), whereas plasma insulin (DY8056; BioTechne, Abingdon, UK), IL-6 (DY206; BioTechne), IL-10 (DY217B;

BioTechne), TNF-α (DY210; BioTechne), HIF-1α (DYC1935; BioTechne), extracellular HSP70 (eHSP70) (DY1663; BioTechne), active GLP-1 (EGLP-35K; Millipore, Burlington, MA, USA), total peptide-YY (PYY) (EZHPYYT66K; Millipore), leptin (EZHL-80SK; Millipore), active acyl-ghrelin (EZGRA-88K; Millipore) and butyric acid (abx258338; Abbexa) were analysed in duplicate using commercially available enzyme-linked immunosorbent assay kits and analysed using a plate reader (SpectraMax i3x; Molecular Devices, Winnersh, UK).

Statistical analysis

Parametric data are presented as the mean $\pm$ SD and non-parametric data as median [interquartile range (IQR)]. $P < 0.05$ was considered statistically significant. Statistical analyses were performed using SPSS, version 28.0 (IBM Corp., Armonk, NY, USA). All data were tested for normality using the Shapiro–Wilk test and for skewness and kurtosis. For repeated-measures, sphericity was also tested and, if violated, was corrected for using Greenhouse–Geisser (light violation), Huynh-Feldt (moderate violation) or lower-bound (severe violation) methods. Appropriate pairwise tests (paired t test for normally distributed data and Wilcoxon signed-rank test for non-parametric data) were used to assess the delta change between hypoxic and sham-exposure conditions for: blood [glucose] (AUC and peak), insulin sensitivity, IL-10, IL-6, TNF-α, HIF-1α, eHSP70, butyric acid, active GLP-1, total PYY, leptin and active ghrelin. Paired samples t tests were also used to assess differences between intervention periods for S_{pO_2} and macronutrient content of diets. Repeated-measures analysis of variance (ANOVA) or Friedman's test was used for the CGM data for parametric and non-parametric data, respectively. CGM variables included plasma [glucose] (10 day grand mean) and AUC, time in range, estimated HbA1$_c$, and GMI between sham, sham-washout, hypoxia and hypoxia-washout. For parametric paired t test data, effect sizes are reported as Cohen's d (approximating: small = 0.2, medium = 0.5 and large = 0.8) (Cohen, 2013; Lakens, 2013) and, for non-parametric Wilcoxon signed-rank data, Rosenthal's r (small = 0.2, medium = 0.4 and large = 0.6). For parametric repeated-measures ANOVA data, effect sizes were reported as partial eta squared (η^2_p; small = 0.01, medium = 0.06 and large = 0.14) and, for non-parametric Friedman's test data, Kendall's W (small = 0.2, medium = 0.5 and large = 0.8) was used.

Gut microbiota analysis was performed in R, version 4.2.2 (R Core Team). Comparisons of the gut microbiota were made using the delta of alpha diversity levels on the basis of the observed diversity, Chao1 diversity and Shannon diversity metrics. Variability between the cohorts (beta diversity) were identified based via principal co-ordinates (PCoA) analysis. Analysis of alpha and beta diversity was performed using the phyloseq package, version 1.40 (McMurdie & Holmes, 2013). Permutational multivariate analysis of variance (PERMANOVA) analysis was performed with the *adonis2* function from the vegan v2.6-4 package (Oksanen, 2010), using Bray–Curtis dissimilarity and 9999 permutations. The delta Firmicutes:Bacteroidota (F/B) ratio and delta relative abundance of taxa at a phylum and genus taxonomic level were compared between groups. The effect size for the F/B ratio is presented as rank biserial correlation (r_{bc}; small = 0.1, medium = 0.3 and large = 0.5). Comparison of relative abundances were adjusted for multiple testing using Benjamini and Hochberg (BH) correction (Benjamini & Hochberg, 1995). Differential abundance analysis was performed using the DESeq2, version 1.42.0 (Love et al., 2014) and ALDEx2, version 1.34.0 (Fernandes et al., 2014) packages. For DESeq2, the non-rarefied feature tables were passed to the *phyloseq_to_deseq2* function, with model design set to compare group, at the same time as accounting for participant repeated measurements. Test was set to likelihood ratio test 'LRT', and estimation of size factors set to 'poscounts' (Nearing et al., 2022). A fold change threshold of two-fold difference between the groups with an adjusted P value (adjusted for multiple testing using BH correction) <0.05 were obtained. For ALDEx2, a model matrix was created duplicating the DESeq2 model design. The non-rarefied feature tables were passed to the *aldex.clr* function, generating Monte Carlo samples of the Dirichlet distribution for each sample, and converting each instance using a centred log-ratio transform. The output was passed to the *aldex.glm* function, which returns the expected values for each coefficient, as well as the BH adjusted P values. Taxa that were significantly expressed in both packages were considered significant. Additional data processing was performed using TidyVerse, version 2.0.0 (Wickham et al., 2019) and all illustrations were plotted using ggplot2, version 3.4.4 (Wickham et al., 2016).

Results

In total, 22 participants (Table 1) were recruited of whom 13 completed the study (Fig. 1). Failure to complete the study was a result of failing screening ($n = 5$), a change in personal circumstances ($n = 2$), a decline in mental health ($n = 1$) or having an impractical home set-up for the hypoxic pillow tent ($n = 1$). Participants reported excellent anecdotal adherence to the use of the pillow tents. We had one report of excessive condensation (as a result of covered ventilation port) and two participants who could

Table 1. Participant characteristics table

Parameter	Value
Age (years), mean $\pm$ SD	64 $\pm$ 9
Males (n)	9
Females (n)	4
Height (m), mean $\pm$ SD	1.72 $\pm$ 0.09
Weight (kg), mean $\pm$ SD	87.5 $\pm$ 14.6
BMI (kg m^{-2}), mean $\pm$ SD	29.7 $\pm$ 3.9
Clinical information	
HbA$_{1c}$ (mmol mol^{-1}), mean $\pm$ SD	61.1 $\pm$ 14.1
T2DM duration (years), mean $\pm$ SD	9.3 $\pm$ 7.0
SBP (mmHg), mean $\pm$ SD	132 $\pm$ 13
DBP (mmHg), mean $\pm$ SD	80 $\pm$ 11
Medication	
Metformin/yaltormin (n)	7
Insulin (n)	1
SGLT2i (n)	5
Incretin mimetics (n)	2
Sulfonylureas (n)	2
ACEi and ARB (n)	11
Beta blockers (n)	1
Statins (n)	11

Data are presented as the mean $\pm$ SD . ACEi, angiotensin-converting enzyme inhibitor; ARB, angiotensin receptor blocker; BMI, body mass index; DBP, diastolic blood pressure; HbA$_{1c}$, glycated haemoglobin; SBP, systolic blood pressure; SGLT2i, sodium-glucose cotransporter-2 inhibitor; T2DM, type 2 diabetes mellitus. n = 13.

not tolerate the tents for two nights during a heatwave. The full anonymised dataset has been made freely available as supplementary material on our University repository (https://doi.org/10.17029/0567ad32-f374-49a1-b125-62e3f09428b7). For gut microbiota, filtered host-depleted reads were deposited in the NCBI Sequence Read Archive (SRA) under BioProject accession code PRJNA1052613 (https://www.ncbi.nlm.nih.gov/bioproject/1052613). Gut microbiota analysis code is available under a GNU General Public License V3.0 at https://github.com/uopbioinformatics/2024_Hypoxia_T2DM_Nanopore_16S_rRNA.

Oxygen saturation

Hypoxia reduced overnight S_{pO_2} compared to sham [sham: 94 $\pm$ 2% *vs.* hypoxia: 88 $\pm$ 4%; $Z = -2.80$, $P = 0.01$, $r = 0.89$].

Plasma [glucose], plasma [insulin], insulin sensitivity and continuous glucose monitoring

Hypoxia had no effect on Δ plasma [glucose] AUC (sham: 54.9 $\pm$ 226.1 *vs.* hypoxia: -17.7 ± 237.7 mmol L^{-1}·min^{-1}; $t_{11} = 1.33$, $P = 0.21$, $d = 0.39$) (Fig. 2*A*), Δ fasting plasma [glucose] (sham: -0.6 ± 1.1 *vs.* hypoxia: -0.9 ± 1.4 mmol L^{-1}; $t_{12} = 0.47$, $P = 0.65$,
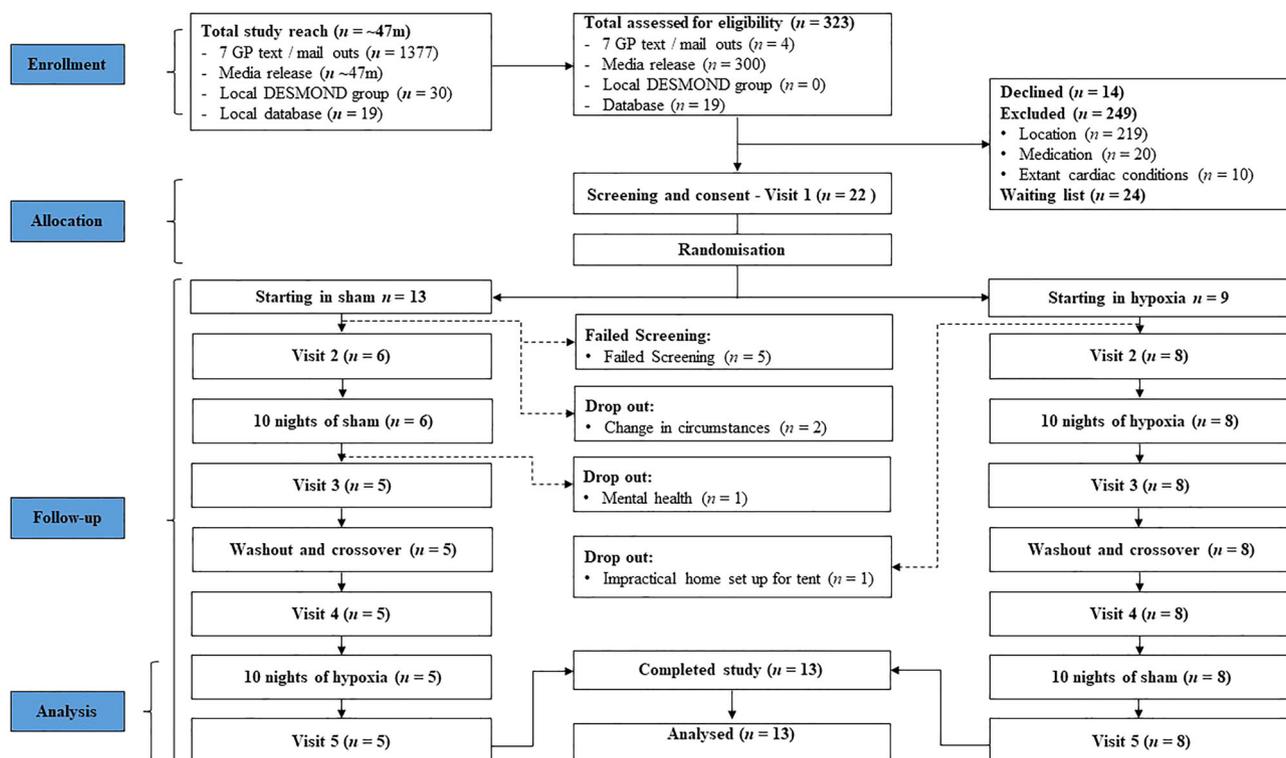
Figure 1. CONSORT flow diagram
Participant flow through the trial. [Colour figure can be viewed at wileyonlinelibrary.com]

$d = 0.13$) (Fig. 2*B*) or Δ peak plasma [glucose] (sham: 1.5 ± 2.8 *vs.* hypoxia: 1.2 ± 3.4 mmol L^{-1}; $t_{11} = 0.40$, $P = 0.70$, $d = 0.12$) (Fig. 2*C*). Hypoxia had no effect on Δ fasting plasma [insulin] (sham: 4.99 ± 19.37 *vs.* hypoxia: -6.13 ± 17.46 pmol L^{-1}; $Z = -1.57$, $P = 0.12$, $r = 0.44$) (Fig. 2*D*), or Δ post-prandial insulin sensitivity (sham: -0.5 ± 1.1

vs. hypoxia: -1.0 ± 1.5 μmol kg^{-1} min^{-1} pmol L^{-1}; $t_{11} = 1.63$, $P = 0.13$, $d = 0.47$) (Fig. 2*E*). Δ fasting insulin sensitivity showed a moderate trend towards improvement following hypoxia, although this was not significant (sham: 0.004 ± 0.025 *vs.* hypoxia: 0.017 ± 0.035 μmol kg^{-1} min^{-1} pmol L^{-1}; $t_{12} = -1.76$, $P = 0.10$, $d = 0.49$) (Fig. 2*F*).
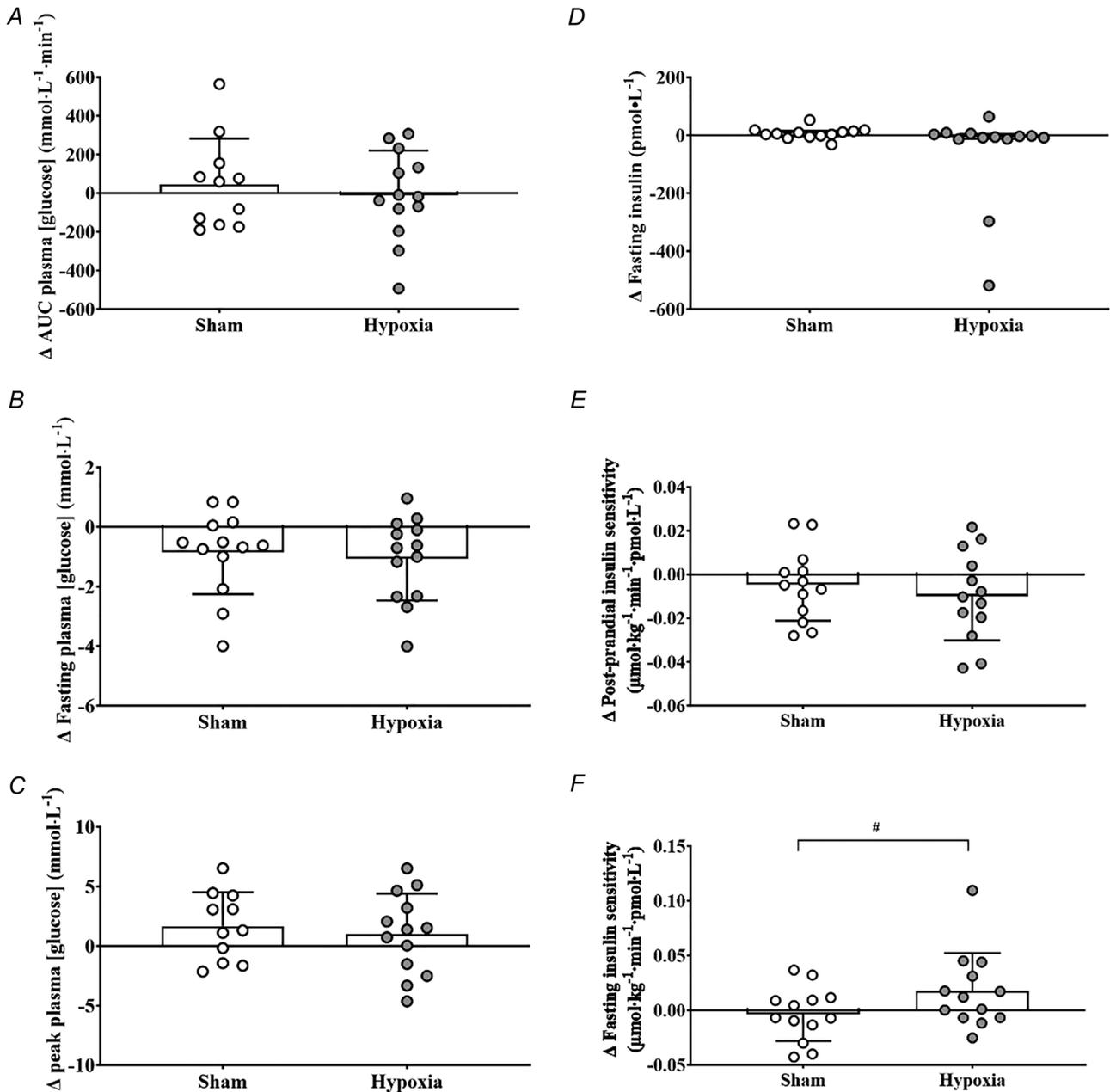
Figure 2. Plasma glucose and insulin indicies
Mean $\pm$ SD (parametric data) or median and interquartile (non-parametric data) are presented for (*A*) plasma [glucose] area under the curve, (*B*) fasting plasma [glucose], (*C*) peak plasma [glucose], (*D*) fasting plasma [insulin], (*E*) post-prandial insulin sensitivity and (*F*) fasting insulin sensitivity following 10 nights of either normoxia ($F_{IO_2} = 0.209$; sham – open circles) or normobaric hypoxia ($F_{IO_2} = 0.155$; hypoxia – closed circles). For parametric data, paired samples *t tests* and, for non-parametric data, Wilcoxon signed-rank test were used to assess differences between conditions ($n = 11$ for *A* and *C* and $n = 13$ for *B*, *D*, *E* and *F*). #$P \leq 0.10$.

Hypoxia had no effect on 10 day grand median [glucose] (sham: 7.65 ± 5.27 *vs.* post-sham: 6.50 ± 4.65 *vs.* hypoxia: 8.25 ± 3.70 *vs.* post-hypoxia: 8.40 ± 5.15 mmol L^{-1}; $\chi^2 = 1.14$, d.f. $= 3$, $P = 0.77$, $w = 0.04$) (Fig. 3*C*). Similarly, hypoxia had no effect on estimated $HbA1_c$ (sham: 58 ± 21 *vs.* post-sham: 53 ± 14 *vs.* hypoxia: 53 ± 11 *vs.* post-hypoxia: 57 ± 16 mmol mol^{-1}; $F_{1,6} = 0.38$, $P = 0.56$, $\eta^2_p = 0.06$), GMI (sham: 57 ± 15 *vs.* post-sham: 53 ± 10 *vs.* hypoxia: 53 ± 8 *vs.* post-hypoxia: 55 ± 11 mmol mol^{-1}; $F_{1.419,8.514} = 0.44$, $P = 0.59$, $\eta^2_p = 0.068$) or time in target range (sham: $10\,640 \pm 3514$ *vs.* post-sham: 9682 ± 4960 *vs.* hypoxia: $10\,007 \pm 3537$ *vs.* post-hypoxia: 9012 ± 4622 min; $F_{3,24} = 1.57$, $P = 0.22$, $\eta^2_p = 0.16$).

Body composition

Hypoxia had no effect on Δ body mass (sham: -0.7 ± 1.0 *vs.* hypoxia: 0.0 ± 0.9 kg; $t_{12} = -1.54$, $P = 0.15$, $d = 0.43$) (Fig. 4*A*) or lean mass (sham: -0.2 ± 0.8 *vs.* hypoxia: -0.3 ± 1.0 kg; $t_{12} = 0.38$, $P = 0.71$, $d = 0.10$) (Fig. 4*B*) but did increase fat mass (sham: -0.6 ± 1.0 *vs.* hypoxia: 0.4 ± 0.7 kg; $t_{12} = -2.80$, $P = 0.02$, $d = 0.78$) (Fig. 4*C*).

Hypoxia had no effect on Δ bone mass (sham: 0.0 ± 0.0 *vs.* hypoxia: 0.0 ± 0.0 kg; $Z = -0.38$, $P = 0.71$, $r = 0.10$) (Fig. 4*D*).

Biomarkers

Hypoxia had no effect on Δ plasma HIF-1α (sham: 0.20 ± 34.19 *vs.* hypoxia: 6.30 ± 64.29 pg mL^{-1}; $Z = -0.71$, $P = 0.48$, $r = 0.20$) (Fig. 5*A*); Δ plasma [eHSP70] (sham: -126.1 ± 824.2 *vs.* hypoxia: -250.6 ± 1332.1 pg mL^{-1}; $t_{11} = 0.27$, $P = 0.79$, $d = 0.08$) (Fig. 5*B*); Δ plasma TNF-α (sham: 0.61 ± 2.36 *vs.* hypoxia: 0.66 ± 2.72 pg mL^{-1}; $Z = -0.31$, $P = 0.75$, $r = 0.09$) (Fig. 5*C*); Δ plasma IL-6 (sham: -0.55 ± 1.53 *vs.* hypoxia: 1.06 ± 2.72 pg mL^{-1}; $Z = -1.18$, $P = 0.24$, $r = 0.34$) (Fig. 5*D*); Δ plasma IL-10 (sham: 0.05 ± 2.17 *vs.* hypoxia: 0.52 ± 1.78 pg mL^{-1}; $Z = -0.71$, $P = 0.48$, $r = 0.20$) (Fig. 5*E*) or plasma butyric acid (sham: -3.18 ± 423.84 *vs.* hypoxia: 4.09 ± 136.46 pg mL^{-1}; $Z = -0.39$, $P = 0.70$, $r = 0.11$) (Fig. 5*F*).

Hypoxia had no effect on Δ plasma leptin (sham: -3.41 ± 11.29 *vs.* hypoxia: 3.37 ± 9.80 pg mL^{-1}; $t_{11} = -1.36$, $P = 0.20$, $d = 0.39$) (Fig. 6*A*); Δ
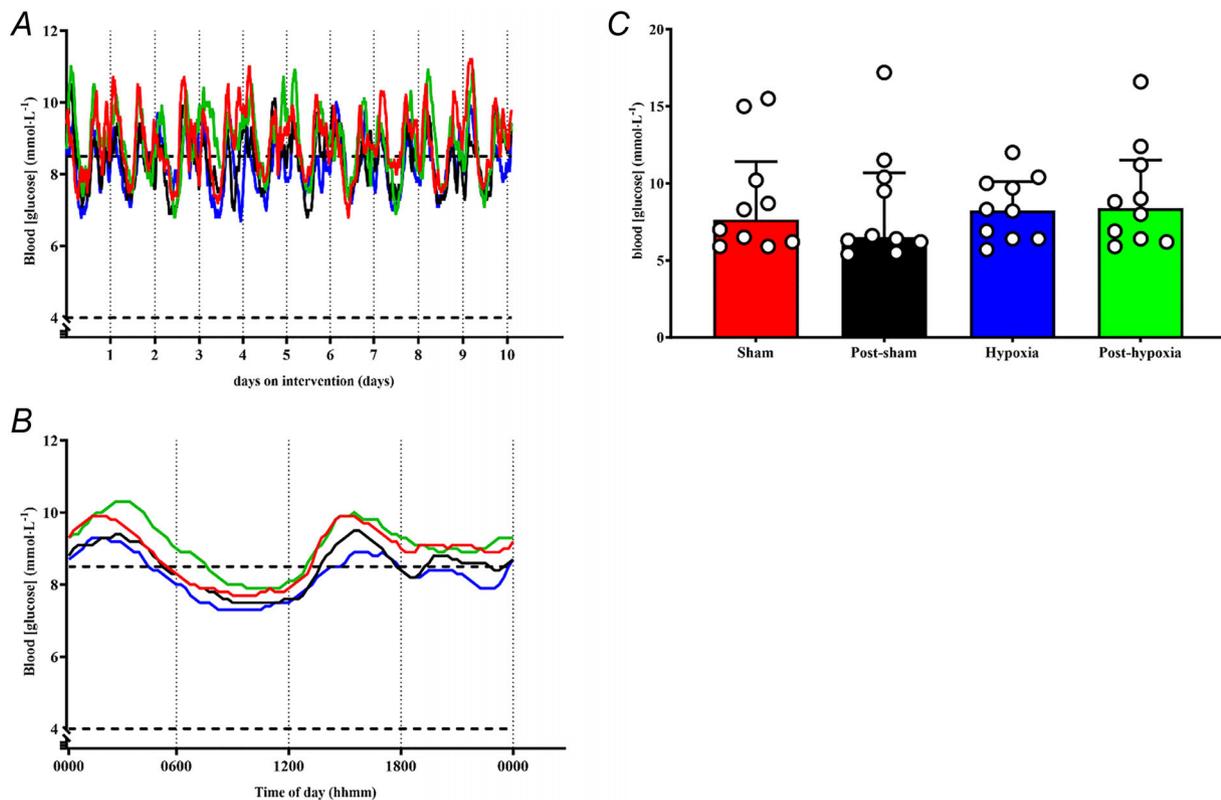

Figure 3. Continous glucose data
Mean continuous [glucose] over 10 days (*A*) and 24 h (*B*), with grand medians (IQR) (*C*), measured via continuous glucose monitors during 10 nights of either normoxia ($F_{IO_2} = 0.209$; red line/box) or normobaric hypoxia ($F_{IO_2} = 0.155$; blue line/box) and the first 10 days of washout for each intervention period (normoxia washout – black line/box; normobaric hypoxia – green line/box). $n = 10$. [Colour figure can be viewed at wileyonlinelibrary.com]

plasma acyl ghrelin (sham: 24.66 ± 40.22 *vs.* hypoxia: 4.61 ± 74.10 pg mL^{-1}; $t_{10} = -0.86$, $P = 0.41$, $d = 0.26$) (Fig. 6*B*); Δ plasma PYY (sham: -15.14 ± 70.43 *vs.* hypoxia: 0.76 ± 60.93 pg mL^{-1}; $t_{11} = -0.57$, $P = 0.58$, $d = 0.16$) (Fig. 6*C*); Δ plasma GLP-1 (sham: -0.05 ± 2.66 *vs.* hypoxia: 0.17 ± 1.86 pmol L^{-1}; $Z = -0.55$, $P = 0.58$, $r = 0.16$) (Fig. 6*D*).

Diet diaries

Hypoxia had no effect on daily energy intake (sham: 1475 ± 330 *vs.* hypoxia: 1506 ± 349 kcal; $t_9 = -0.40$, $P = 0.70$, $d = 0.13$), daily CHO intake (sham: 148 ± 44 *vs.* hypoxia: 164 ± 55 g; $t_9 = -1.45$, $P = 0.09$, $d = 0.46$), daily fat intake: (sham: 49 ± 11 *vs.* hypoxia: 58 ± 12 g; $t_9 = -0.13$, $P = 0.45$, $d = 0.04$) or daily protein intake (sham: 58 ± 8 *vs.* hypoxia: 59 ± 14 g; $t_9 = -0.23$, $P = 0.41$, $d = 0.07$).

Gut microbiota

Delta pairwise comparisons for alpha diversity in each condition were conducted across all samples ($n = 11$). Δ Observed (sham: 5 ± 14 *vs.* hypoxia: -10 ± 15; $t_{10} = 1.96$, $P = 0.08$, $d = 0.59$), Δ Chao1 (sham: 4 ± 15 *vs.* hypoxia: -10 ± 15; $t_{10} = 1.70$, $P = 0.12$, $d = 0.51$) and Δ Shannon (sham: 0.10 ± 0.28 *vs.* hypoxia: -0.18 ± 0.40; $t_{10} = 1.95$, $P = 0.08$, $d = 0.59$) were not significantly different between sham and hypo-

xia (Fig. 7*A*). PERMANOVA revealed no significant differences in the overall distribution of taxa abundance between sham and hypoxia ($F_{3,40} = 0.36$, $P = 1.00$), as further demonstrated through assessment of the PCoA beta diversity analysis (Fig. 7*B*). Reads corresponding to seven and eight phyla (seven in common, one unique to post) and 170 and 165 genera (152 in common, 18 unique to pre, 13 unique to post) were detected in pre- and post-sham samples, respectively. Similarly, seven and seven phyla (seven in common) and 164 and 146 genera (138 in common, 26 unique to pre, eight unique to post) were detected in pre- and post-hypoxia samples, respectively. Furthermore, no significant difference was identified in the Δ F/B ratio between sham and hypoxia (sham: -2.65 ± 34.45 *vs.* -0.22 ± 28.55; $w = 26$, $P = 0.58$, $r_{bc} = -0.21$) (Fig. 7*E*). Individual participant data showing the distribution of taxa at the phylum and genus taxonomic level are shown in Fig. 7*C* and Fig. 7*F*, respectively. No significant differences were detected in the relative abundance of any taxa at a phylum (Fig. 7*D*) or genus (Fig. 7*G*) taxonomic level. DESeq2 and ALDEx2 analysis identified no significantly differentially expressed taxa.

Sleep and physical activity

Hypoxia had no effect on sleep efficiency (sham: $86 \pm 8\%$ *vs.* hypoxia: $88 \pm 9\%$; $Z = -0.46$, $P = 0.65$, $r = 0.15$) or sleep duration (sham: 421.51 ± 57.63 *vs.* hypoxia:
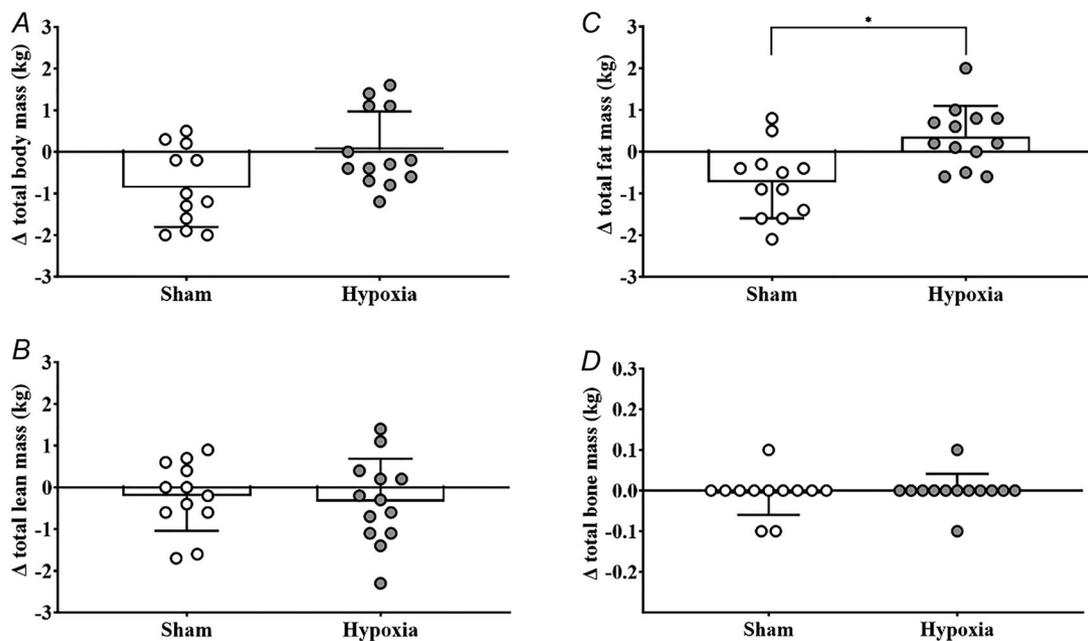

Figure 4. Body composition
Mean $\pm$ SD for (*A*) total body mass, (*B*) lean body mass, (*C*) body fat mass and (*D*) total bone mass following 10 nights of either normoxia ($F_{IO_2} = 0.209$; sham – open circles) or normobaric hypoxia ($F_{IO_2} = 0.155$; hypoxia – closed circles). Paired samples *t* tests were used to assess differences between conditions ($n = 12$ in sham and $n = 13$ for hypoxia). *$P < 0.05$ significant difference between conditions.

378.39 ± 71.78 min; $t_9 = 1.82$, $P = 0.10$, $d = 0.57$), but did reduce time in bed (sham: 487 ± 57 *vs.* hypoxia: 439 ± 70 min; $t_9 = 2.53$, $P = 0.03$, $d = 0.80$). Hypoxia had no effect on total PA (sham: 183 ± 67 *vs.* hypoxia: 170 ± 67 min; $Z = -0.66$, $P = 0.51$, $r = 0.21$), light-intensity PA (sham: 106.87 ± 52.77 *vs.* hypoxia: 104.68 ± 44.34 min; $t_9 = 0.41$, $P = 0.69$, $d = 0.13$), MVPA (sham: 82.26 ± 47.74 *vs.* hypoxia: 74.71 ± 36.74 min; $t_9 = 1.13$, $P = 0.29$, $d = 0.36$) but did increase total inactivity (sham: 773.45 ± 57.43 *vs.* hypoxia: 819.97 ± 62.50 min; $t_9 = -4.12$, $P < 0.01$, $d = 1.30$). For all accelerometery variables, $n = 10$.

Discussion

The present study is the first to assess the effects of normobaric hypoxic exposure on free-living, fasted and post-prandial glucose control, appetite, body composition, gut microbiota, and inflammation in people with T2DM. The principal novel findings were that 10 nights of moderate hypoxia did not improve glucose tolerance, although there was a trend towards a moderate increase in fasting insulin sensitivity and reduced gut microbiota alpha diversity in adults with T2DM. However, 10 nights of moderate nocturnal hypoxia does not appear to significantly alter body mass, appetite or inflammation
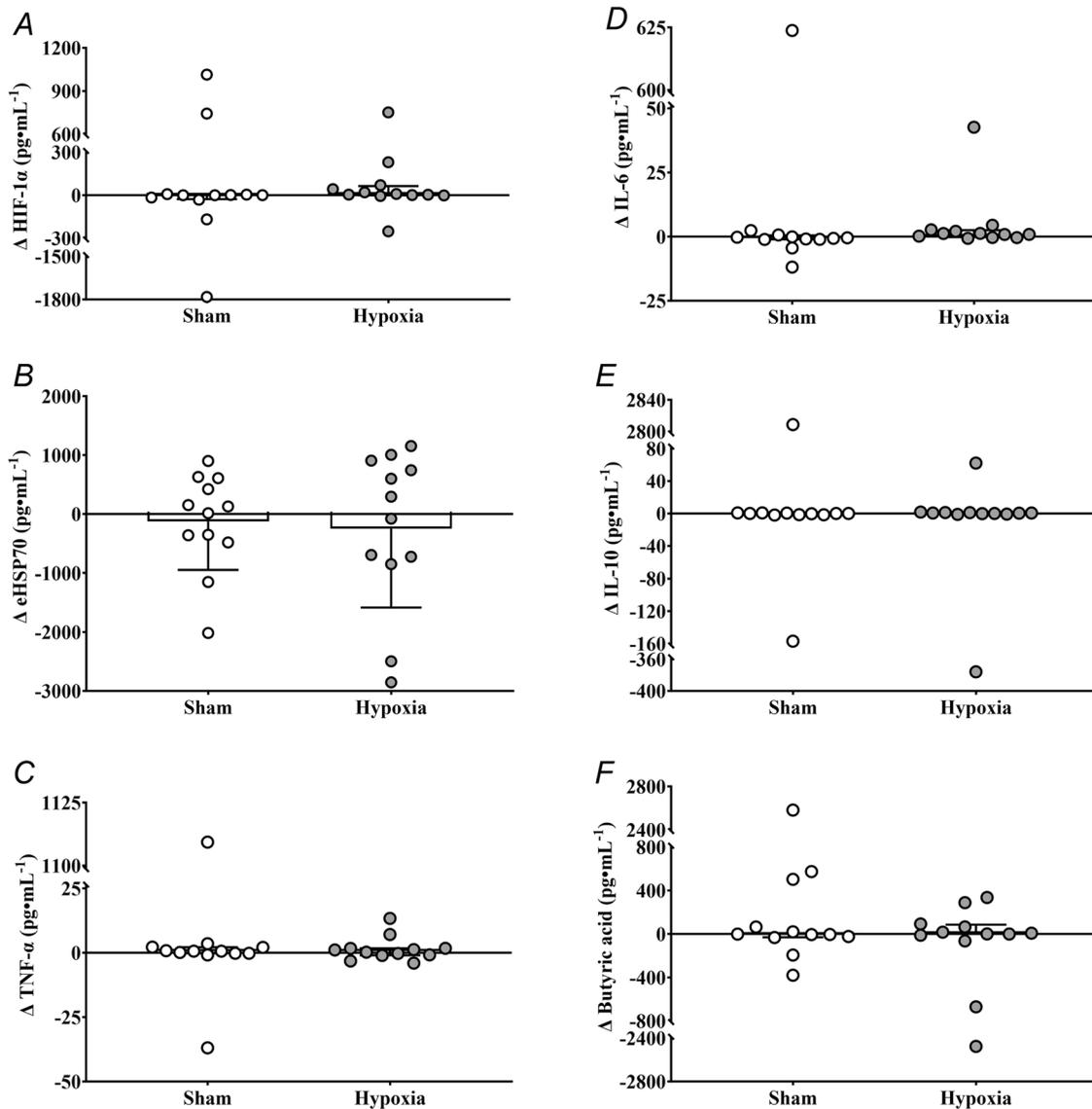
Figure 5. Inflammation and hypoxic stress markers
Mean ± SD (parametric data) or median and interquartile (non-parametric data) are presented for (*A*) [HIF-1α], (*B*) [eHSP70], (*C*) [TNF-α], (*D*) [IL-6], (*E*) [IL-10] and (*F*) [butyric acid] following 10 nights of either normoxia ($F_{\text{IO}_2} = 0.209$; sham – open circles) or normobaric hypoxia ($F_{\text{IO}_2} = 0.155$; hypoxia – closed circles). For parametric data, paired samples *t* tests and, for non-parametric data, Wilcoxon signed-rank tests were used to assess differences between conditions, whereas effect sizes were estimated with Cohen's *d* and Rosenthal's *r*, respectively ($n = 12$).

in adults with T2DM. Importantly this intervention appears to be well tolerated and did not increase markers of systemic inflammation.

Plasma [glucose], plasma [insulin], insulin sensitivity and CGM

For the first time in a randomised control trial, we show that 10 nights of normobaric hypoxia has no effect on our primary outcome, AUC [glucose]. Similarly, we also showed no effect on free-living, fasted or post-prandial blood [glucose]. This is contrary to the majority of literature in this emerging field, where acute hypoxic exposure in individuals with T2DM (Mackenzie et al., 2011) and observational studies of individuals residing at high altitudes (Woolcott et al., 2014) have reported reduced [glucose] and reduced prevalence of T2DM. This is also the case in pre-clinical models, such as rodents (Eid et al., 2018; Wang et al., 2018), in people who are overweight (Lecoultre et al., 2013), insulin resistant humans and in those people with T2DM (Marlatt et al., 2020).

It is noteworthy that a non-significant trend towards a moderate effect size for improvement in fasting insulin sensitivity ($P = 0.10$, $d = 0.49$) following 10 nights of normobaric hypoxia was found. Although this is not significant, it is pertinent to note the relatively small sample size and therefore future larger, and thus higher-powered studies, are required. Indeed, our data suggest that a definitive trial (with insulin sensitivity as the primary outcome) would require 28 people (effect size; 0.49 and power at 0.8). This is in line with previous studies in a rodent model (Wang et al., 2018), obese humans (Lecoultre et al., 2013) and T2DM humans (with a pre-post design) (Marlatt et al., 2020), which report improvements in [insulin] and/or insulin resistance following hypoxic exposure. One study, using a mouse model, did report impairments in insulin sensitivity following hypoxia (Eid et al., 2018), although this exposure was at the highest relative altitude ($F_{IO_2} = 0.10$) and for the longest exposure (3 months), suggesting that the degree and duration of hypoxia exposure are key factors that influence any effects on insulin sensitivity. Specifically, prolonged exposure to a more significant degree of hypoxia (4 weeks; $F_{IO_2} = 0.104$) results in substantial metabolic remodelling in skeletal muscle, including loss of oxidative metabolism (O'Brien et al., 2019) and atrophy (Murray & Montgomery, 2014), both of which would limit the capacity to clear glucose post-prandially. Interestingly, the shift in insulin sensitivity following hypoxia previously reported in people with T2DM appears to be driven by the change in [glucose] (Marlatt et al., 2020), whereas, in the present study, it appears to be driven by a change in [insulin].
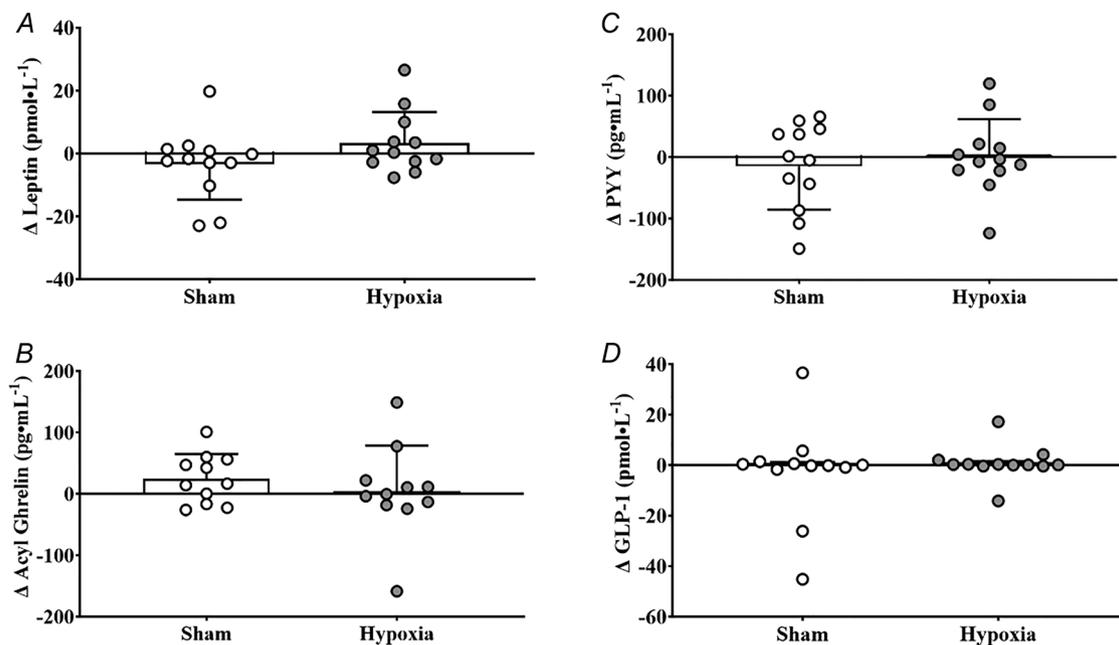

Figure 6. Gut hormone markers
Mean ± SD (parametric data) or median and interquartile (non-parametric data) are presented for (*A*) [leptin], (*B*) [acyl ghrelin], (*C*) [PYY] and (*D*) [GLP-1] following 10 nights of either normoxia ($F_{IO_2} = 0.209$; sham – open circles) or normobaric hypoxia ($F_{IO_2} = 0.155$; hypoxia – closed circles). For parametric data, paired samples *t* tests and, for non-parametric data, Wilcoxon signed-rank tests were used to assess differences between conditions; whilst effect sizes were estimated with Cohen's *D* and Rosenthal's *r*, respectively (*n* = 12 for *A*, *C* and *D*, as well as *B* sham, whereas *n* = 11, hypoxia, in *B*).
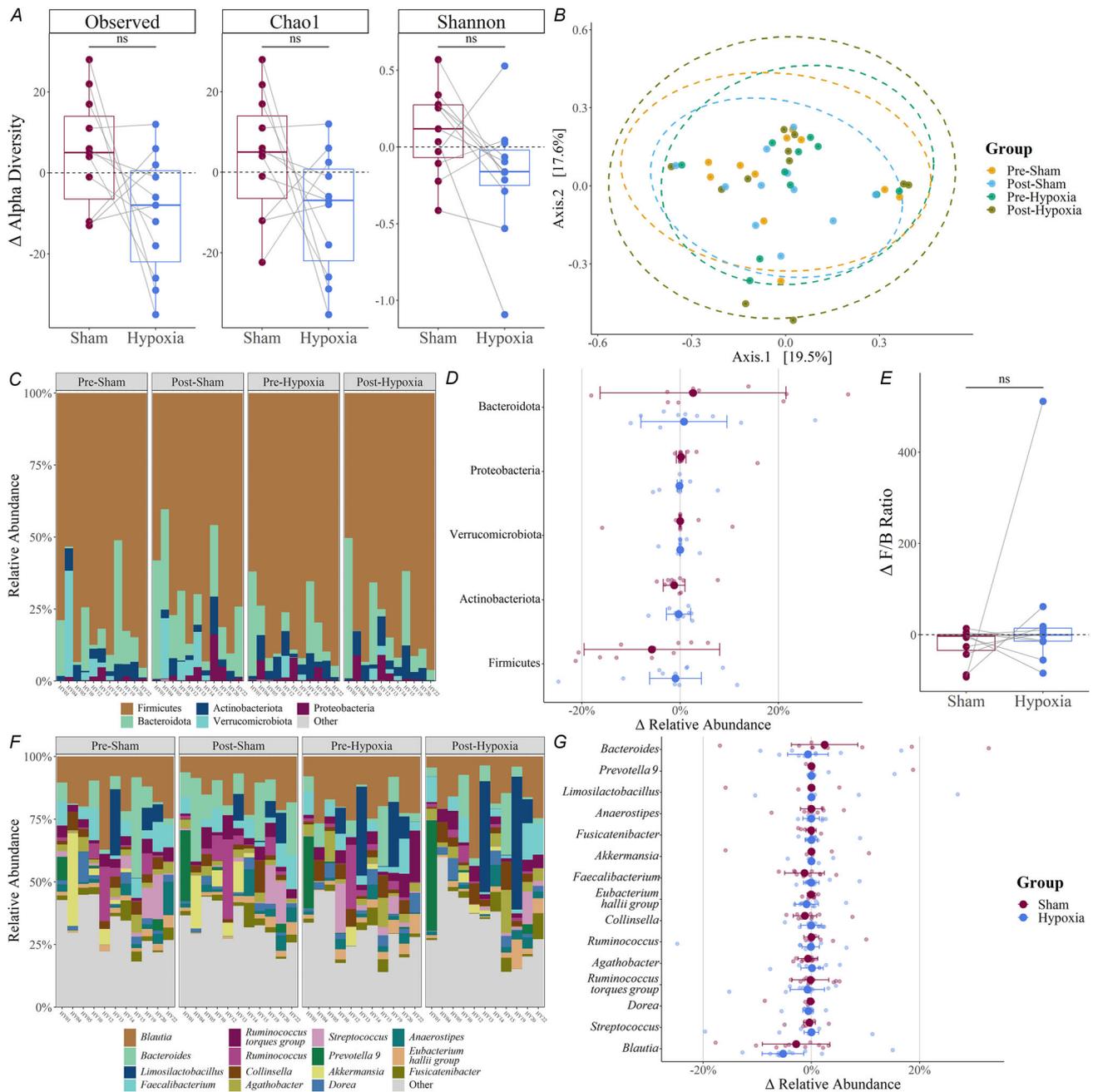
Figure 7. Comparison of gut microbiota in adults with T2DM sleeping at hypoxia compared with sham

A, Delta (Δ) alpha diversity; from left to right: observed, Chao1 and Shannon. Test of difference: paired samples *t* test; ns, not significant; $P > 0.05$. *B*, PCoA based on Bray–Curtis dissimilarity measure. Groups compared by PERMANOVA using the *adonis2* function. *C*, top five phyla of individual participants. *D*, Δ relative abundance of phyla compared between groups. *E*, F/B ratio compared between groups. Test of difference: Wilcoxon signed-rank test. *F*, top 15 genera of individual participants. *G*, Δ relative abundance of genera compared between groups. Microbial Δ relative abundance data presented as median (IQR) and differences compared using Wilcoxon signed-rank tests, and corrected for multiple comparisons using Benjamini and Hochberg false discovery rate correction. No significant differences ($P_{adj} < 0.05$) were present in any taxa at a phylum or genus taxonomic level, nor at any taxonomic level. [Colour figure can be viewed at wileyonlinelibrary.com]

Hypoxia may therefore work via separate mechanisms; one that alters glucose uptake independently of insulin and another that improves insulin secretion. However, this previous work conducted in people with T2DM (Marlatt et al., 2020) had no control group and implemented a pre–post experimental design, which may also explain some of the differences.

We report no statistical change in estimated $HbA1_c$ following hypoxic exposure, although we note that the reduction observed (sham *vs.* hypoxia; -3.7 mmol mol^{-1}) was larger than a clinically meaningful change in $HbA1_c$ (~3 mmol·mol^{-1}) (Lind et al., 2008, 2010). Given the time-course kinetics of changes in $HbA1_c$ (i.e. 3 months), we might expect to see larger reductions over a longer intervention period. However, the $HbA1_c$ time kinetics also make interpreting these findings difficult. These data, although interesting, need to be interpreted with caution given the lack of statistical differences, coupled with the fact that similar differences were also seen in the post-sham condition. However, controlling perturbations in plasma [glucose] for protection against an increased cardiovascular risk, as well as the associated microvascular complications (Stratton et al., 2000), would be essential. Alongside current evidence, our novel findings (identification of a moderate trend towards improved fasting insulin sensitivity, and clinically meaningful, albeit non-significant, changes in $HbA1_c$) indicate that future multisite randomised control trials are warranted, with longer intervention periods and $HbA1_c$ as the primary outcome. Our data indicate that a definitive trial, with $HbA1_c$ as the primary outcome, an effect size of 0.2 and power at 0.8, would require 199 participants.

Body composition and diet diaries

Hypoxia had no effect on body mass, lean body mass or bone mass, but did increase body fat mass (~1 kg). The coefficient of variation for the measurement of fat mass using DEXA is $\sim1.6\%$ (Moreira et al., 2018), similar to the increase in fat mass found. We therefore cannot preclude that this is a spurious finding. Other studies have examined the effect of hypoxia on weight loss, for instance within our own laboratory, where we reported a ~0.8 kg (Rendell et al., 2017) reduction in healthy athletes (10 nights; $F_{IO_2} = 0.156$), whereas, in people with insulin resistance, the weight loss is ~1.2 kg (Serebrovska et al., 2017). These changes (at least in our laboratory) do not appear to be a result of diuresis. Possible explanations for these differences in findings could be a lack of control of diet. However, we report no change in self-reported macronutrient intake. We hypothesised that hypoxic exposure would suppress appetite given the rodent models that had shown positive results (Palmer & Clegg, 2014). Our data suggest that this is not the case

for hunger hormones, incretins or gut hormones. Given these were secondary outcomes and that we were not powered to see these changes, caution should be taken in their interpretation. Hypoxia also had no effect on total PA, light-intensity PA or MVPA, but did increase sedentary time. Given the reduction in energy expenditure observed during hypoxic exposure (Corbett et al., 2023) it is promising that it does not translate into reduced PA in people with T2DM.

Inflammation or oxidative stress

Hypoxia had no effect on markers of inflammation or oxidative stress compared to sham conditions. This is in line with our recent work in overweight males showing that transient exposure (60 min) to moderate hypoxia ($F_{IO_2} = 0.15$) does not alter redox balance (Corbett et al., 2023). Therefore, it does not appear that short- or longer-term exposure effects markers of redox balance (at least at moderate hypoxia). Interestingly, other research has shown that lower partial pressures of inspired O_2 (i.e. greater altitude) are associated with oxidative stress (Hartmann et al., 2000; Ribon et al., 2016; Siervo et al., 2014). This is critical because higher levels of oxidative stress are hallmarks of many chronic disease (Wu et al., 2014). In combination, this suggests that higher simulated altitudes may not be feasible and efficacious as a treatment, whereas moderate levels of overnight hypoxia for 10 days, at a minimum, do no harm.

Gut microbiota

Cross-sectional studies demonstrate that the relative abundance of taxa which synthesise SFCAs, such as *Pseudobutyivibrio* are increased in individuals living at high altitude in a dose-dependent effect (Li et al., 2016; Sturgess & Montgomery, 2021) and these SCFAs have been linked to improved insulin sensitivity (Cui et al., 2022). For the first time, we examined the effect of sleeping in a hypoxia tent on the gut microbiota. Hypoxia had no significant effect on microbial diversity and relative abundance or indeed [butyric acid] compared to sham. We did note that there was a non-significant but medium effect size for a greater reduction in Δ alpha diversity in hypoxia, but the present study may be underpowered to detect such an effect. There was also one participant with a considerable increase in Δ F/B ratio in hypoxia. There are many variables that may have influenced this, such as diet (Singh et al., 2017). Moreover, we cannot rule out that a longer intervention or greater hypoxic stimulus (i.e. $F_{IO_2} < 0.15$) may elicit favourable changes to the composition of the gut microbiota, such as increases in taxa capable of butyrate production, as documented in individuals living at a high altitude (Li

et al., 2016), potentially resulting in increased insulin sensitivity (Puddu et al., 2014). However, the tolerance to lower F_{IO_2} in people with T2DM has yet to be examined and this greater hypoxic stimulus would need to be balanced against the potential for adverse effects (as detailed above). Given the increased risk of sleep apnoea, using lower F_{IO_2} would probably limit the number of people who could benefit from this intervention. Caution should be taken when interpreting the gut microbiota findings given the modest sample size.

Adherence and tolerance

Hypoxia, as expected, significantly reduced S_{pO_2} compared to sham. This reduction is in line with other studies at similar simulated altitudes (Corbett et al., 2023) and also to that experienced during commercial air flight (Humphreys et al., 2005), suggesting the stimulus was moderate and tolerable. In the sham condition, S_{pO_2} was 1% lower than expected, perhaps becaude of the effects of obesity on lung mechanics and S_{pO_2} (Vishesh et al., 2013). We had no reported adverse events related to hypoxic exposure, which suggests this is a feasible intervention in an overweight/obese metabolically compromised cohort.

There was no change in sleep efficiency or sleep time but a slightly reduced time in bed with the hypoxic intervention, although it should be noted that both conditions occurred within a tent and therefore we cannot preclude that there may have been an influence on sleep efficiency in both arms. However, the sleep efficiency observed (86%) was higher than in other groups with long-term conditions previously reported (74%) by our research group (Shepherd et al., 2018).

Strengths, limitations and future directions

The study design (a randomised, balanced, single-blind, cross-over, control trial) is a key strength of the present study, which is the first to assess glucose control in people with T2DM following hypoxic exposure. This, coupled with an excellent adherence to the intervention and sham conditions, enabled key findings to be investigated. Nonetheless, several limitations warrant discussion, including limited statistical power. Specifically, the present study was only powered to detect changes in our primary outcome (AUC [glucose]). However, it is possible that we overestimated the effect size. Estimating effect size from other studies given the novelty of this work was difficult. In rodent models (Wang et al., 2018) the effect of hypoxia appears to be large ($\sim d = 2$) on resting blood [glucose]. However, the intervention in that previous study was longer than ours and continuous. Thus, we would expect a smaller effect. The acute exposure work carried out by Mackenzie et al. (2012) in people with T2DM suggests that

we would see and effect size of $d = 0.7$. In hindsight, $d = 1$ was too large; however, at the time we powered the study, it was our best estimate given the available data.

We also lacked controlled of participants diet. Although this was critical to examine the free-living effects of the intervention (i.e. strong ecological validity), future experimental trials could impose greater controls that may influence the results for body composition and gut microbiota. It is also noteworthy that, to ensure minimal invasiveness to prospective participants, microbiome analyses were conducted based on extractions from faeces. Although a common surrogate for assessing the gut microbiota, there may be differences in microbiota composition between the gut and the faeces (Zhou et al., 2021). We also cannot preclude that participants using insulin may have adapted doses based on glucose changes, which may have affected outcomes. There is also the possibility that the effects of the intervention may have been blunted by the diverse medications being taken. Finally, a larger drop-out of participants was observed in comparison to our trials because a large proportion of consented participants did not meet the stringent inclusion criteria during the hypoxic screening. It is therefore possible that our screening criteria were overly conservative.

Conclusions

The present study is the first to assess the effects of nocturnal hypoxia on free-living glucose control, as well as fasted and post-prandial glucose control, appetite, body composition and inflammation in people with T2DM. Overall, hypoxia was shown not to improve glucose tolerance but there was a trend towards an increase in fasting insulin sensitivity and reduced alpha diversity. Moreover, hypoxia does not appear to alter appetite, inflammation, sleep or PA indices. However, we do report a small increase in fat mass which should be taken into account in future trials.

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Additional information

Open research badges

This article has earned an Open Data badge for making publicly available the digitally-shareable data necessary to reproduce the reported results. The data is available at https://doi.org/10.17029/0567ad32-f374-49a1-b125-62e3f09428b7.

Data availability statement

The full anonymised dataset has been made freely available as supplementary material on our University repository (https://doi.org/10.17029/0567ad32-f374-49a1-b125-62e3f09428b7). For gut microbiota, filtered host-depleted reads were deposited in the NCBI Sequence Read Archive (SRA) under BioProject accession code PRJNA1052613 (https://www.ncbi.nlm.nih.gov/bioproject/1052613). Gut microbiota analysis code is available under a GNU General Public License V3.0 at https://github.com/uopbioinformatics/2024_Hypoxia_T2DM_Nanopore_16S_rRNA.

Competing interests

The authors declare that they have no competing interests.

Author contributions

J.C., M.J.T., H.M., A.J.M., Z.L.S., M.P.W.G., J.R.S. and A.I.S. conceived and designed research. T.J.J., A.A.M.G., H.D., J.M., J.S. and A.I.S. performed experiments. T.J.J., A.A.M.G., S.C.R. and A.I.S. analysed data. J.C., T.J.J., A.A.M.G., H.M., R.N., J.S., M.J.T., H.M., Z.L.S., M.P., H.M., C.S., J.M., A.J.M., M.P.W.G., S.Y.-M., J.R.-S., M.A.M., K.A.M., H.D., S.C.R. and A.I.S. interpreted the results of the experiments. T.J.J., A.A.M.G. and A.I.S. prepared figures. J.C., T.J.J. and A.I.S. drafted the manuscript. J.C., S.C.R. and A.I.S. edited and revised the manuscript J.C., T.J.J., A.A.M.G., H.M., R.N., J.S., M.J.T., H.M., Z.L.S., M.P., H.M., C.S., J.M., A.J.M., M.P.W.G., S.Y.-M., J.R.-S., M.A.M., K.A.M., H.D.,

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Supporting information

Additional supporting information can be found online in the Supporting Information section at the end of the HTML view of the article. Supporting information files available:

Peer Review History

Translational perspective

Importantly, the high adherence rates in the present study suggest that the proposed intervention is feasible in people with T2DM and may overcome a number of barriers to uptake and adherence that are associated with recommended lifestyle interventions, which we anticipate will facilitate higher levels of uptake and compliance. As a consequence, if larger-scale trials demonstrate efficacious effects on insulin sensitivity and/or HbA1$_c$, this intervention has the potential to improve outcomes in individuals with T2DM, and could also deliver significant financial savings in terms of the clinical care costs associated with the treatment of T2DM.