DIABETES/METABOLISM RESEARCH AND REVIEWS
Diabetes Metab Res Rev 2011; **27:** 94–101.
Published online 5 November 2010 in Wiley Online Library (wileyonlinelibrary.com) **DOI:** 10.1002/dmrr.1156

RESEARCH ARTICLE

Acute hypoxia and exercise improve insulin sensitivity (S_I^{2*}) in individuals with type 2 diabetes

Richard Mackenzie[1]*
Neil Maxwell[2]
Paul Castle[3]
Gary Brickley[2]
Peter Watt[2]

[1]*School of Life Sciences, Department of Human and Health Sciences, University of Westminster, London, United Kingdom*

[2]*Chelsea School Research Centre, University of Brighton, Eastbourne, United Kingdom*

[3]*Department of Sport and Exercise Science, University of Bedfordshire, Polhill Avenue, Bedford, United Kingdom*

*Correspondence to:
Richard Mackenzie, School of Bioscience, University of Westminster, 115 New Cavendish Street, London W1W 6UW, United Kingdom.
E-mail:
r.mackenzie@westminster.ac.uk

Received : 6 August 2010
Revised: 14 October 2010
Accepted: 28 October 2010

Abstract

Background Hypoxia has been shown to increase glucose uptake in skeletal muscle using the contraction-stimulated pathway, independent of the actions of insulin. Yet, the same stress has also been linked with causing insulin resistance and hyperglycaemia. The aim of this study was to examine the effects of acute hypoxia with and without exercise on insulin sensitivity (S_I^{2*}) in individuals with type 2 diabetes.

Methods Eight type 2 diabetic patients completed 60 min of the following: (1) normoxic rest; (2) hypoxic rest [$O_2 = 14.6$ (0.4)%]; (3) normoxic exercise and (4) hypoxic exercise [$O_2 = 14.6$ (0.4)%]. Exercise trials were set at 90% of lactate threshold. Each condition was followed by a labelled intravenous glucose tolerance test to provide estimations of insulin sensitivity (S_I^{2*}) and β-cell function.

Results Two-compartmental analysis showed that insulin sensitivity (S_I^{2*}) was higher following hypoxic rest compared with normoxic rest ($p = 0.047$). Insulin sensitivity (S_I^{2*}) was also higher following hypoxic exercise [4.37 (0.48) $\times 10^{-4}$/min (μU/mL)] compared with normoxic exercise [3.24 (0.51) $\times 10^{-4}$/min (μU/mL)] ($p = 0.048$). Acute insulin response to glucose was reduced following hypoxic rest *versus* normoxic rest ($p = 0.014$).

Conclusions This study demonstrated that (1) hypoxic-induced improvements in glucose tolerance in the 4 h following exposure can be attributed to improvements in peripheral insulin sensitivity (S_I^{2*}) and (2) exercise and hypoxia have an additive effect on insulin sensitivity (S_I^{2*}) in type 2 diabetic patients. Acute hypoxia may therefore improve short-term glycaemic control in individuals with type 2 diabetes. The application of these findings in the clinic will require further investigation. Copyright © 2010 John Wiley & Sons, Ltd.

Keywords exercise; hypoxia; type 2 diabetes; insulin sensitivity

Abbreviations: AIR_g – acute insulin response to glucose; AUC – under the curve; AUC_{Glu} – under the curve glucose; AUC_{Ins} – under the curve insulin; DI – disposition index; EGP – endogenous glucose production; Hy Ex – hypoxic exercise; Hy Rest – hypoxic rest; IVGTT – intravenous glucose tolerance test; Nor Ex – normoxic exercise; Nor Rest – normoxic rest; TMS – trimethylsilylation; TMCS – trimethylchorosilane; S_I^{2*} – insulin sensitivity.

Introduction

Type 2 diabetes is a multifactorial metabolic disease characterized by defects in β-cell function, insulin sensitivity (S_I^{2*}), glucose effectiveness (S_G^{2*})

and endogenous glucose production (EGP) [1]. It is widely accepted that insulin and exercise [2] are potent stimuli for glucose transport. Acute exercise is known to promote glucose uptake in skeletal muscle via an intact contraction-stimulated mechanism [3], while improvements post-exercise are due to increased activity of insulin-dependent mechanisms [4].

In skeletal muscle, the majority, but not all, research has concluded that muscle contraction and hypoxia stimulate glucose transport activity via the same signalling pathway, independent of the action of insulin [5]. The ability of hypoxia to encourage glucose disposal, independent of contractile activity has been documented in animal models [6,7] and in *vitro* work using isolated human skeletal muscle [3]. The work on humans has also demonstrated an increased dependence on blood glucose during exposure to high altitude (4300 m; ~12% O_2) [8]. Elsewhere, hypoxia is suggested to cause insulin resistance via a sympathoadrenal-induced epinephrine release, causing increased EGP and a reduction in glucose disposal [9]. In more recent work, Louis and Punjabi [10] demonstrated a decline in insulin sensitivity ($S_I{}^{2*}$) and $S_G{}^{2*}$ in response to intermittent hypoxia. The reason for these disparities is not clear. Furthermore, it remains to be determined whether these findings extend to whole body glucose tolerance in type 2 diabetic humans.

Despite the onset of peripheral insulin resistance in obese individuals, euglycaemia can normally be preserved via compensatory increases in β-cell insulin secretion [11]. Insulin resistance and insulin secretion are subject to a hyperbolic relationship with a decrease in insulin sensitivity ($S_I{}^{2*}$) being accompanied by elevated insulin secretion [1]. This relationship, represented by the disposition index (DI), can be calculated using the modelled parameters of insulin sensitivity ($S_I{}^{2*}$) and acute insulin response to glucose (AIR_g), is a physiological representation of the β cells' compensatory response to changes in insulin sensitivity ($S_I{}^{2*}$) [12]. There is a gap in the research assessing the effects of exercise on β-cell function. Surprisingly, the effect of prior hypoxia on β-cell responsiveness and function (AIR_g and DI) has also received little attention. The primary aim of the current investigation was to assess the effects of prior hypoxia, with and without exercise, on insulin sensitivity ($S_I{}^{2*}$) and $S_G{}^{2*}$ in individuals with type 2 diabetes.

Materials and methods

Eight sedentary males, diagnosed with type 2 diabetes within the last 5 years by a local general practitioner, were recruited for this investigation. The clinical characteristics of the subjects are presented in Table 1. Ethical approval was granted by the East Sussex Local Research Ethics Committee (UK). Exclusion criteria included any signs of diabetes-related complications (i.e. neuropathy, peripheral vascular and cardiovascular disease), a current smoker or treatment with insulin. Three subjects were diet treated, and therefore free from glycaemic-altering drugs. The remaining three subjects were treated with metformin (2×500 mg/day). Three individuals were also being treated for hypertension, taking moderate doses of calcium channel blockers (5–10 mg twice daily). Subjects requiring metformin were asked to abstain from medication in the 48 h prior to experimental trials. Metformin has been shown to have a plasma-specific half-life of ~6.2 h and whole blood half-life of ~17.6 h [13].

Study protocol

The experimental protocol was based on five laboratory visits separated by a minimum of 7 and a maximum of 14 days. The first visit acted as a familiarization and allowed for determination of lactate thresholds. Following an overnight fast (~12 h), subjects completed a further four trials; 60 min of (1) normoxic rest (Nor Rest); (2) hypoxic rest (Hy Rest); (3) normoxic exercise (Nor Ex) and (4) hypoxic exercise (Hy Ex). Dietary intake was controlled in the 48 h prior to each condition and analysed for total calorie and carbohydrate intake using nutritional software Comp-Eat (version 6; Visual Information Systems Ltd, UK).

Preliminary testing

Percentage of body fat was estimated using Bio Electrical Impedance analysis (Bodystat, Isle of Man, UK). Venous blood samples (~5 mL) were drawn for the determination of glycosylated haemoglobin (Axis-Shields Diagnostics, UK), fasting blood glucose and plasma insulin concentrations. Fasting blood glucose and plasma insulin concentrations were used to estimate homeostasis model of insulin resistance [fasting insulin (μU/mL) × fasting glucose (mmol/L)/22.5] and homeostasis model of β-cell function [20 × fasting insulin (μU/mL)/fasting glucose – 3.5 (mmol/L)] [14] to determine metabolic status. Lactate threshold was determined on an electronically braked Jaeger cycle ergometer using an incremental exercise protocol starting at 0 W with 10-W increments every 3 min. Cadence remained constant throughout (~60 rpm). Fingertip blood samples were collected at the end of each stage for analysis for [La] (YSI 2300 STAT, Yellow Springs, USA). Lactate threshold was defined as the power output preceding a sudden and sustained increase in lactate concentration (≥1 mmol/L above the previous stage) and confirmed by three experienced and objective exercise physiologists.

Main experimental conditions

Subjects arrived at the laboratory following an overnight fast at ~0800. An 18-gauge cannula was then inserted into a dorsal hand vein and placed in a box heated at ~65 °C to ensure the collection of arterialized-venous blood [15]. A second 18-gauge cannula was inserted into an antecubital vein in the opposite arm for the injection of labelled

DOI: 10.1002/dmrr

Table 1. Subjects clinical, physiological and metabolic characteristics

Age (years)	Body mass index (kg/m^2)	Body fat (%)	Glycosylated haemoglobin (%)	Fasting glucose (mmol/L)	Homeostasis model assessment of insulin resistance	Homeostasis model assessment of β-cell function	Interleukin-6 (pg/mL)
58 (4)	29.2 (6.7)	37.0 (10.7)	7.8 (2.3)	8.4 (1.8)	7.3 (2.8)	69.8 (10.2)	3.31 (0.52)

Values are means (standard error of the mean).

Table 2. The integrated area under the curve for arterialized blood glucose and plasma insulin during a 4-h intravenous glucose tolerance test, following normoxic exercise and hypoxic exercise

	Normoxic rest	Hypoxic rest	*p*-value	Normoxic exercise	Hypoxic exercise	*p*-value
Area under the curve for arterialized blood glucose (mmol/L min)	1867 (340)	1663 (171)	$p = 0.23$	1742 (246)	1622 (154)	$p = 0.30$
Area under the curve for plasma insulin (μU/mL min)	7635 (590)	5774 (918)	$p = 0.03$	5637 (820)	4334 (617)	$p = 0.007$

Values are means (standard error of the mean).

glucose. Baseline blood samples (~10 mL) were collected after a 30-min rest before subjects undertook 60 min of the following conditions in a randomized fashion – (1) Nor Rest; (2) Hy Rest; (3) Nor Ex and (4) Hy Ex. Whole body hypoxia [$O_2 = 14.6$ (0.4)%] was continuously administered using air-processing units (King Size, Air units ~SQ-10; Colorado Altitude Training, Boulder, USA) with a steady flow of nitrogen (N_2; ~40 L/min) into a closed environment [temperature; 20 (0.9) °C, relative humidity; 41 (25)%]. Oxygen concentrations were continuously measured throughout hypoxic exposures using Gasman II (Crowcon, Oxfordshire, UK). Exercise trials used a work load [50 (6) W] equivalent to 90% of normoxic lactate threshold (~50–55% $\dot{V}O_{2max}$) and were performed on the same electronically braked Jaeger cycle ergometer. Heart rate and oxyhaemoglobin saturation (S_pO_2; pulse oximeter Nonin 2500) were collected every 10 min during conditions. Arterialized-venous blood samples were drawn every 10 min with plasma separated and stored at −80 °C for later analysis. A sample of whole blood in duplicate (~20 μL) was used for immediate determination of glucose and lactate concentrations. Symptoms of acute mountain sickness were assessed using the Lake Louise Questionnaire. This was completed at the halfway point (30 min) of the protocol. Subjects displayed no symptoms of acute mountain sickness during hypoxic exposure. Safety levels for S_pO_2 were set at ≥70% for hypoxic exposures. A 4-h labelled intravenous glucose tolerance test (IVGTT) was administered immediately following each exposure (i.e. under normoxic conditions). Glucose bolus contained ~28.4 mg/kg of [6,6^{2}H$_2$]glucose and ~250 mg/kg of unlabelled glucose and prepared under sterile conditions on the morning of each experimental condition. Thereafter, arterialized (~10 mL) samples were drawn at 0, 2, 3, 4, 5, 6, 8, 10, 12, 15, 18, 20, 25, 30, 35, 40, 60, 70, 80, 100, 120, 140, 160, 180, 210 and 240 min for analyses of plasma insulin, endogenous glucose concentrations and [6,6^{2}H$_2$]glucose isotoper ratio.

Analyses

Insulin concentrations were determined using commercially available enzyme-linked immunosorbent assay (DRG Diagnostics, UK). Intra-assay and inter-assay coefficient of variation for insulin enzyme-linked immunosorbent assays was <3.9%. Analysis of enriched [6,6^{2}H$_2$]glucose was carried using ~20 μL of each plasma sample. Samples were deproteinized with 100 μL of ethanol before mixtures were centrifuged at 6000 rpm for 5 min. Supernatants were then centrifuged for a further ~30 min (RNA concentrator) after which hydroxylamine hydrochloride (25 mg) was added with pyridine (1 mL) before 100 μL of the resultant oxime reagent converted samples to oxime-trimethylsilylation (TMS). After a 60-min incubation period (70 °C), 100 μL of 99% bis(trimethyl)trifluoroacetamide: 1% trimethylchorosilane (TMCS) (Sigma-Aldrich, Exeter, UK) was added before a further incubation period (45 min; 70 °C). Using gas chromatography mass spectrometer (Hewlett Packard) glucose derivatives were measured for peaks of 319 (unlabelled glucose; trace) and 321 ([6,6^{2}H$_2$]glucose; tracer). Plasma insulin, endogenous glucose concentrations and [6,6^{2}H$_2$]glucose-enriched values were used to determine the metabolic indices – $S_I{}^{2*}$, $S_G{}^{2*}$, EGP, AIR_g and DI ($S_I{}^{2*} \times AIR_g$), as described previously [11,16]. Modelling of data was carried out using two-compartmental minimal modelling software (SAAMII Institute, Seattle, WA). Insulin sensitivity ($S_I{}^{2*}$) explains the effects of insulin on glucose disposal rates while $S_G{}^{2*}$ quantifies the effects of glucose to cause its own transport via mass action effect independent of insulin. AIR_g is an index of β-cell responsiveness and was determined as the

DOI: 10.1002/dmrr

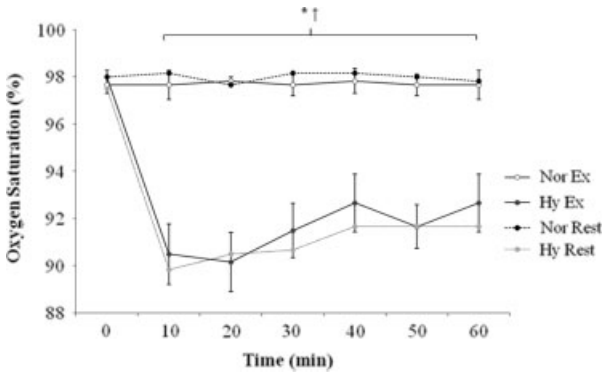

Figure 1. Oxyhaemoglobin saturation response at baseline and during normoxic rest, hypoxic rest, normoxic exercise and hypoxic exercise. *Denotes significant difference between normoxic rest and hypoxic rest ($p = 0.001$). † Denotes significant difference between normoxic exercise and hypoxic exercise ($p = 0.000$). Values are means (standard error of the mean)

area under the curve (AUC) between 0 and 10 min while DI represents an integrated measure of β-cell function with insulin sensitivity ($S_I{}^{2*}$).

Statistical analysis

All results are expressed as mean with standard error of the mean. Statistical significance was set at the level $p < 0.05$. The area under the curve for both glucose (AUC_{Glu}) and insulin (AUC_{Ins}) were calculated using the trapezoidal rule. Differences over time and between conditions were evaluated by two-way repeated measures analysis of variance. Tukey's *post hoc* tests were used when statistical significance was found. All statistical tests were carried out using the statistical software package SPSS (version 15.).

Results

Nutritional intake was not different between conditions for both total calorie ($p = 0.726$) and carbohydrate intake ($p = 0.534$). These data suggest that changes in overall glycaemic control may be largely attributed to the experimental conditions. S_pO_2 decreased on exposure to hypoxia at rest [92 (0)%; $p = 0.006$] and remained lower than the values obtained during Nor Rest [98 (0)%; $p = 0.001$; Figure 1]. Exercise did not alter S_pO_2 from baseline during Nor Ex ($p = 0.996$). During Hy Ex, S_pO_2 decreased over time [92 (1)%; $p = 0.00$] and was statistically lower than Nor Ex ($p = 0.00$). Blood [La] were not different between Nor Rest [1.01 (0.20) mmol/L] and Hy Rest [1.07 (0.38) mmol/L] ($p = 0.786$). Blood [La] increased from baseline during Nor Ex and peaked at 10 min [2.07 (0.45) mmol/L; $p = 0.016$]. Similarly, [La] increased during Hy Ex, peaking at the 30-min point [2.02 (0.44) mmol/L; $p = 0.009$]. Exercising [La] showed no difference between Nor Ex and Hy Ex ($p = 0.094$).

Figure 2 shows changes in blood glucose concentrations for each trial. Hypoxia decreased arterialized blood glucose by −0.74 (0.14) mmol/L (Hy Rest; $p = 0.002$) while the same parameter was unchanged for the normoxic control [Nor Rest; −0.23 (0.16) mmol/L; $p = 0.181$]. Both Nor Ex [−0.91 (0.35) mmol/L] and Hy Ex succeeded in lowering blood glucose. However, this reduction was magnified when exercise was performed in hypoxia [−1.82 (0.64) mmol/L] ($p = 0.031$). Resting hypoxia demonstrated no effect on circulating plasma insulin levels ($p = 0.507$) and was not different from the control condition ($p = 0.591$). Hy Ex caused plasma insulin values to decrease from baseline (Hy Ex; $p = 0.028$), with no difference noted within Nor Ex ($p = 0.208$).

No difference was noted for AUC_{Glu} between Nor Rest [1867 (340) mmol/L min] and Hy Rest [1663 (171) mmol/L min] (Table 2; $p = 0.23$). However, two-way analysis of variance showed arterialized blood glucose concentrations to be lower when main effect comparisons were made between Nor Rest and Hy Rest ($p = 0.001$). Insulin AUC was lower during the 4-h IVGTT period for Hy Rest compared with the normoxic resting control Nor ($p = 0.03$). Hy Ex had no effect on AUC_{Glu} over exercise in normoxia ($p = 0.30$), although AUC_{Ins}

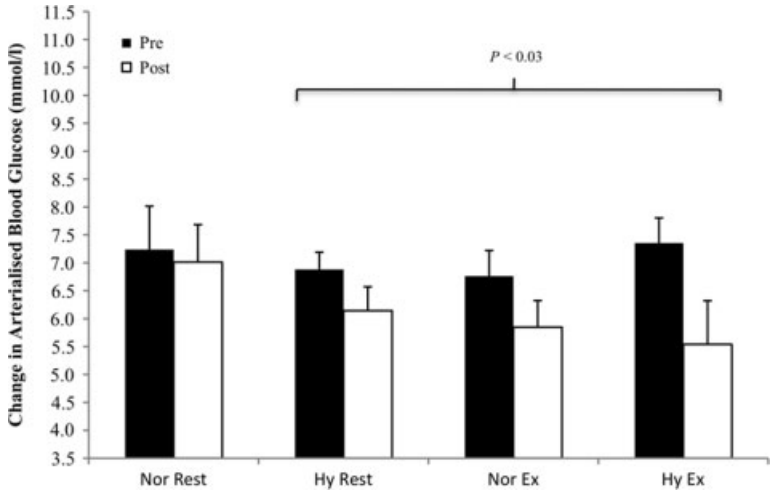

Figure 2. Mean (standard error of the mean) values showing changes in arterialized blood glucose from pre- to post-exercise

DOI: 10.1002/dmrr

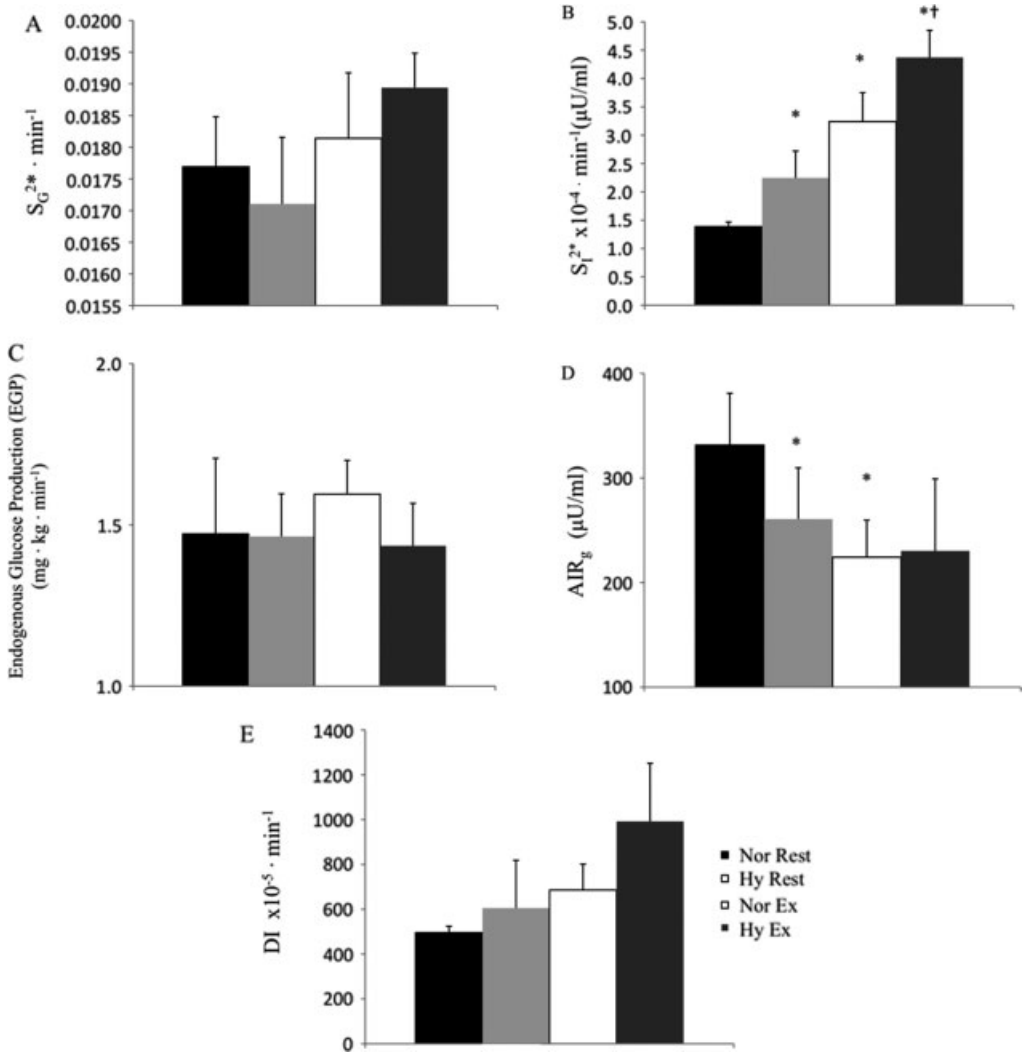

Figure 3. Glucose effectiveness (A), insulin sensitivity (B), endogenous glucose production (C), acute insulin response to intravenous glucose (D) and disposition index (DI) (E) during each 4-h intravenous glucose tolerance test. *Denotes a significant difference from normoxic rest ($p < 0.05$). †Denotes a significant difference from normoxic exercise ($p < 0.05$). ‡Denotes a significant difference from normoxic rest ($p < 0.05$)

was significantly lower when comparisons were made between the same conditions [Hy Ex; 4334 (617) and Nor Ex; 5637 (820) μU/mL min] ($p = 0.007$). Two-way analysis of variances demonstrated that Hy Ex had no effect on blood glucose concentrations in the 4 h following the hypoxic/exercise treatment ($p = 0.268$). Plasma insulin values were, however, significantly lower following intravenous glucose injection in the Hy Ex trial (Hy Ex *versus* Nor Ex; $p = 0.005$).

S_I^{2*}, S_G^{2*} and EGP

Data analysed from two-compartmental minimal model analysis showed there was a main effect difference between conditions for insulin sensitivity (S_I^{2*}) ($p = 0.000$; Figure 3). Improvements in insulin sensitivity (S_I^{2*}) were demonstrated in the 4 h following Hy Rest compared with Nor Rest [2.25 (0.50) and 1.39 (0.08) $\times 10^{-4}$/ min (μU/mL), respectively] ($p = 0.047$) and for Nor Ex [3.24 (0.51) $\times 10^{-4}$/min (μU/mL)] compared with the normoxic control ($p = 0.039$). Insulin sensitivity (S_I^{2*}) was 26% higher [4.37 (0.48) $\times 10^{-4}$/min (μU/mL)] in the 4 h following Hy Ex than exercise in a normobaric environment ($p = 0.048$). Further analysis showed that insulin sensitivity (S_I^{2*}) was significantly higher in the 4 h following the intravenous glucose challenge for Hy Ex when compared to the resting control trial (Nor Rest; $p = 0.005$). The ability of glucose to stimulate its own transport at basal insulin concentrations (S_G^{2*}) was not different between conditions ($p = 0.321$) (Figure 3). Similarly, EGP was not affected by Hy Rest [1.46 (0.30) mg/kg/min], Nor Ex [1.60 (0.10) mg/kg/min] and Hy Ex [1.44 (0.13) mg/kg/min] when compared to the control trial [Nor Rest; 1.47 (0.52) mg/kg/min] ($p = 0.599$).

AIR_g and DI

Acute insulin response to the IVGTT challenge was different between conditions (main effect; $p = 0.012$)

DOI: 10.1002/dmrr

and was higher for Nor Rest [332 (49) (μU/mL)] when compared to Hy Rest [260 (47); $p = 0.034$] and Nor Ex [224 (35) (μU/mL); $p = 0.021$]. Surprisingly, AIR_g was not different following Hy Ex when compared to control [230 (69) (μU/mL); $p = 0.220$] and Nor Ex ($p = 387$). Comparisons for the integrated measure of β-cell function ($DI = S_I^{2*} \times AIR_g$) showed no difference between Nor Rest and Hy Rest (Figure 3) ($p = 0.579$). Despite being higher following Hy Ex [992 (259) $\times$ 10^{-5}/min], DI was not different from Nor Ex [686 (116) $\times$ 10^{-5}/min] ($p = 0.293$) or Nor Rest [496 (28) $\times$ 10^{-5}/min] ($p = 0.077$) (Figure 3). The change in AIR_g demonstrated for Nor Ex (*versus* control trial) did not correspond to an improvement for DI ($p = 0.119$).

Discussion

There is a great deal of literature that has investigated the effects of hypoxia/altitude on glucose metabolism in animals [7], isolated skeletal muscle [3] and humans [8]. In contrast, little is known about the effects of prior hypoxia on glucose metabolism in type 2 diabetic patients. The present study demonstrated that insulin sensitivity (S_I^{2*}) and first-phase insulin secretion (AIR_g) are improved immediately following hypoxic exposure. Additionally, increases in insulin sensitivity (S_I^{2*}) recorded in the 4 h following exercise were enhanced when exercise was combined with hypoxia in individuals with type 2 diabetes.

Acute hypoxia stimulates whole body glucose metabolism

The data presented shows that circulating blood glucose concentrations are reduced during acute exposure to hypoxia. Azevedo *et al*. [3] have previously shown two- to threefold increases in glucose transport rates during hypoxic exposure in isolated insulin-resistant rectus abdominis muscle tissue. Brooks *et al*. [8] reported an increase in glucose R_d during exposure to 4300 m in healthy humans when compared to sea level values [3.59 (0.08) and 1.80 (0.02) mg/kg/min, respectively $p < 0.05$]. The current work is novel as it studied *in vivo* analysis of whole body glucose metabolism during and following acute (O_2 ~14.6%) rather than severe hypoxia (O_2 ~0.0%) [3,6] or altitude [8]. The data from the current work showed no change in insulin concentrations from baseline values, which was aligned with a reduction in blood glucose concentration during hypoxic exposure. This suggests that a pathway, independent of insulin, may have been responsible for the decrease in blood glucose, although the precise mechanisms involved remain elusive.

Metformin has been shown to have a plasma-specific half-life of ~6.2 h [13]. Despite this, it must be acknowledged that the effects of this treatment may have influenced the results found. However, it is reasonable to assume that the moderate hypoxia used in the present study may have up-regulated the whole body glycolytic energy pathways to compensate for the possible reduction in mitochondrial respiration [17]. During acute hypoxic treatment, stimulation of the sympathoadrenal system (i.e. an increase in blood epinephrine concentrations) is associated with up-regulation in glucose disappearance rates, reduced fatty acid uptake and an inhibition of insulin secretion [8,18–21]. Therefore, increased circulating epinephrine levels have been linked to acute increases in glucose dependency with hypoxic exposure. Indeed, epinephrine has been shown to promote glucose transporter 4 translocation and glucose transport rates by 22–48% ($p < 0.05$) with an associated increase in cyclic adenosine monophosphate levels, while inhibiting insulin-dependent glucose uptake in skeletal muscle [22]. Unfortunately, the current work did not measure epinephrine (given that the main purpose of our study was to examine the effects of prior hypoxia on insulin sensitivity (S_I^{2*}), so it is hard to draw firm conclusions.

Hypoxia improves insulin sensitivity (S_I^{2*})

Similar to the effects of exercise, it would appear that hypoxia alters insulin-mediated transport in the hours following exposure, as demonstrated by lower blood glucose concentrations, lower AUC_{Ins} and improved insulin sensitivity (S_I^{2*}) during the labelled IVGTT period of this study. Similar observations have been made in rodents and are thought to be caused by a 'rebound' increase in insulin sensitivity (S_I^{2*}) in response to hypoxia [23]. Aerobic exercise increases insulin action in skeletal muscle in sedentary, obese and type 2 diabetic individuals [24]. The ability of exercise to stimulate glycogen depletion is a key mechanism by which medium-term improvements in insulin sensitivity (S_I^{2*}) are produced [25]. Muscle glycogen concentrations have also been shown to decrease (~66%) during severe hypoxia (O_2 ~0.0%) in detached skeletal muscle [6] and in healthy humans (O_2 ~11.0%) [26]. Using constant infusion of epinephrine (20 min at 1.0 μg/min), Huang *et al*. [27] demonstrated that both liver and muscle glycogen in rats was reduced by 65 and 20%, respectively. Again, the increased epinephrine during hypoxic exposure seen elsewhere in healthy humans [21] may have contributed to glycogen utilization via an increase in cyclic adenosine monophosphate concentrations, potentially leading to post-treatment increases in insulin sensitivity (S_I^{2*}).

Hypoxia and exercise have an additive effect on insulin sensitivity (S_I^{2*})

AUC_{Ins} was lower in Hy Ex when compared to the exercise-only trial. It is not clear whether this was due to an increase in insulin binding and so post-receptor activity (i.e. phosphoinositide-3 kinase and Akt) at the site of peripheral tissue or a down-regulation in insulin release. However, this increase in insulin sensitivity (S_I^{2*}) suggests

DOI: 10.1002/dmrr

that insulin signalling, and so insulin-dependentglucose transport, may have been up-regulated following hypoxic exercise. This conclusion can be drawn given the short effects that both exercise and hypoxia have on glucose transport via the contraction (hypoxic)-stimulated pathway [28] and that insulin sensitivity (S_I^{2*}), derived from the use of labelled glucose, describes the effects of insulin on whole body glucose disappearance [29]. However, the current work would benefit from the analysis of C-peptide to determine if hypoxia inhibits insulin release or encourages peripheral insulin action. During exercise there is an up-regulation in the contraction-stimulated pathway, which is replaced (following exercise) by an up-regulation in insulin-signalling [29]. As glucose concentrations were not different and AUC_{Ins} and insulin sensitivity (S_I^{2*}) were significantly lower and improved, respectively, it would appear reasonable to suggest that the insulin signalling pathway may have been further up-regulated following Hy Ex when compared to the exercise-only trial [30,31]. Supporting data taken from work involving rodents suggests that Hy Ex increases insulin-stimulated glucose transporter 4 membrane content, in the hours following exposure, to a greater extent than exercise alone [7].

The improvement in insulin sensitivity (S_I^{2*}) in Hy Ex *versus* Nor Ex could also be attributed to an increase in the relative exercise intensity experienced in hypoxia. Hypoxia is known to cause a leftward shift in the power/lactate relationship, which may have resulted in the subjects working at an intensity corresponding to ~100% of lactate threshold during hypoxic exposure [32]. However, this methodological limitation was unavoidable as a subsidiary aim to this study was to examine if hypoxic exercise, at an identical Nor Ex intensity (absolute workload), could increase insulin sensitivity (S_I^{2*}) to a similar or a greater extent.

Hypoxia, exercise and endogenous glucose production

The ability of hyperglycaemia to suppress EGP is blunted in type 2 diabetes. This could be, in part, due to decreased glucose-induced flux through glucokinase [33]. However, the reduction in circulating plasma insulin, as common in the latter stages of the disease, may also contribute to elevated EGP, suggesting both hepatic specific-glucose and specific-insulin resistance [1]. The current study was unable to detect any difference in EGP between conditions. In experiments using type 2 diabetic humans, the decline in blood glucose as a result of exercise has been linked to both increased muscle uptake [34,35] and blunted glucose production [36,37]. Others have shown no change in EGP following moderate intensity exercise (45 min and 50%$\dot{V}O_{2max}$) in diabetic patients with moderate levels of hyperglycaemia [7.5 (0.8) mmol/L] [38]. Using isotope methodology Minuk *et al*. [37] found glucose R_a to be inhibited during exercise in type 2 diabetic patients (basal glucose; ~10.6 mmol/L) when compared to healthy controls (~4.7 mmol/L).

Differences in AIR_g within the Hy and Nor Ex conditions suggest that β-cell function was improved. Given this, and given the relationship between insulin secretion and EGP, we might have expected glucose release to be inhibited in the 4 h post-intervention. We suggest that a greater stimulus, such as repeated bouts of exercise and/or hypoxic exposure, may be required to increase hepatic insulin sensitivity (S_I^{2*}). Segal *et al*. [39] demonstrated that exercise training (12 weeks, 4 h/week at 70%$\dot{V}O_{2max}$) significantly decreased EGP in type 2 diabetic subjects. The decrease in EGP noted in both Segal *et al*. [39] and Devlin *et al*. [40] was attributed to a reduction in insulin production, as shown by lower C-peptide levels, and a decline in fasting glucose concentrations following exercise training. It seems likely that exercise training, rather than a single bout of exercise, can decrease basal EGP in type 2 diabetic patients [39] secondary to improvements in insulin sensitivity (S_I^{2*}), although exercise-induced changes in EGP are dependent on the degree of hyperglycaemia and hyperinsulinaemia that accompany the progression of the disease [38].

Finally, despite having no effect on DI ($AIR_g \times S_I^{2*}$), hypoxia caused a decrease in AIR_g, which was also evident with the Nor Ex trial. It is not clear from the results what underlying mechanisms would have caused this response. However, the homeostatic feedback relationship between insulin sensitivity (S_I^{2*}) and insulin secretion suggests that the improvement in insulin sensitivity (S_I^{2*}) would potentially result in a decrease in insulin requirements and insulin release (AIR_g) because of elevated insulin-stimulated glucose clearance. This can be supported by work carried out in exercising humans [41]. In summary, the present investigation has shown that hypoxia increased glucose tolerance both during and in the 4 h following exposure. Furthermore, the glucose-lowering effect of exercise was enhanced by hypoxia, and hypoxic-induced improvements in post-exposure glucose control were largely attributed to changes in peripheral insulin action (S_I^{2*}). Acute hypoxia may therefore improve short-term glycaemic control in individuals with type 2 diabetes. The application of these findings in the clinic will require further investigation.

Conflict of interest

None declared.

References

1. Stumvoll M, Goldstein BJ, van Haeften TW. Type 2 diabetes: principles of pathogenesis and therapy. *The Lancet* 2005; **365:** 1333.
2. Jessen N, Goodyear LJ. Contraction signaling to glucose transport in skeletal

DOI: 10.1002/dmrr

muscle. *J Appl Physiol* 2005; **99:** 330–337.
3. Azevedo JL, Carey JO, Pories WJ, Morris PG, Dohm GL. Hypoxia stimulates glucose transport in insulin-resistant human skeletal muscle. *Diabetes* 1995; **44:** 695–698.
4. Koval J, Maezono K, Pattime ME, Pendergrass MD, Defronzo RA, Mandarino LJ. Effects of exercise and insulin on insulin signaling proteins in human skeletal muscle. *Med Sci Sports Exerc* 1999; **31:** 998–1004.
5. Fisher JS, Gao J, Han D-H, Holloszy JO, Nolte LA. Activation of AMP kinase enhances sensitivity of muscle glucose transport to insulin. *Am J Physiol Endocrinol Metab* 2002; **282:** E18–E23.
6. Cartee GD, Douen AG, Ramlal T, Klip A, Holloszy JO. Stimulation of glucose transport in skeletal muscle by hypoxia. *J Appl Physiol* 1991; **70:** 1593–1600.
7. Chiu LL, Chou SW, Cho YM, *et al*. Effect of prolonged intermittent hypoxia and exercise training on glucose tolerance and muscle GLUT4 protein expression in rats. *J Biomed Sci* 2004; **11:** 838–846.
8. Brooks GA, Butterfield GE, Wolfe RR, *et al*. Increased dependence on blood glucose after acclimatization to 4,300 m. *J Appl Physiol* 1991; **70:** 919–927.
9. Oltmanns KM, Gehring H, Rudolf S, *et al*. Hypoxia causes glucose intolerance in humans. *Am J Respir Crit Care Med* 2004; **169:** 1231–1237.
10. Louis M, Punjabi NM. Effects of acute intermittent hypoxia on glucose metabolism in awake healthy volunteers. *J Appl Physiol* 2009; **106:** 1538–1544.
11. Utzschneider KM, Prigeon RL, Faulenbach MV, *et al*. Oral disposition index predicts the development of future diabetes above and beyond fasting and 2-h glucose levels. *Diabetes Care* 2009; **32:** 335–341.
12. Bergman RN. Lilly lecture. Toward physiological understanding of glucose tolerance. Minimal-model approach. *Diabetes* 1989; **38:** 1512–1527.
13. Heller JB. Metformin overdose in dogs and cats. *Veterinary Med* 2007; **231:** 231–233.
14. Uwaifo GI, Fallon EM, Chin J, Elberg J, Parikh SJ, Yanovski JA. Indices of insulin action, disposal, and secretion derived from fasting samples and clamps in normal glucose-tolerant Black and White children. *Diabetes Care* 2002; **25:** 2081–2087.
15. Mcguire EA, Helderman JH, Tobin JD, Anders R, Berman M. Effects of arterial versus venous sampling on analysis of glucose kinetics in man. *J Appl Physiol* 1976; **41:** 565–573.
16. Vicini P, Caumo A, Cobelli C. The hot IVGTT two-compartment minimal model: indexes of glucose effectiveness and insulin sensitivity. *Am J Physiol Endocrinol Metab* 1997; **273:** E1024–E1032.
17. Brooks G. Increased glucose dependency in circulatory compensated hypoxia. In Hypoxia and Mountain Medicine. Queen City: Burlington, VA, 1992; 213–226.
18. Mazzeo RS, Bender PR, Brooks GA, *et al*. Arterial catecholamine responses during exercise with acute and chronic high-altitude exposure. *Am J Physiol Endocrinol Metab* 1991; **261:** E419–E424.
19. Roberts AC, Reeves JT, Butterfield GE, *et al*. Altitude and beta-blockade augment glucose utilization during submaximal exercise. *J Appl Physiol* 1996; **80:** 605–615.
20. Mazzeo RS, Child A, Butterfield GE, *et al*. Sympathoadrenal responses to submaximal exercise in women after acclimatization to 4,300 meters. *Metab* 2000; **49:** 1036–1042.
21. Kelly KR, Williamson DL, Fealy CE, *et al*. Acute altitude-induced hypoxia suppresses plasma glucose and leptin in healthy humans. *Metab* 2010; **59:** 200–205.
22. Han X-X, Bonen A. Epinephrine translocates GLUT-4 but inhibits insulin-stimulated glucose transport in rat muscle. *Am J Physiol Endocrinol Metab* 1998; **274:** E700–E707.
23. Yokoe T, Alonso LC, Romano LC, *et al*. Intermittent hypoxia reverses the diurnal glucose rhythm and causes pancreatic β-cell replication in mice. *J Physiol* 2008; **586:** 899–911.
24. Larsen J, Dela F, Kjaer M, Galbo H. The effect of moderate exercise on postprandial glucose homeostasis in NIDDM patients. *Diabetologia* 1997; **40:** 447–453.
25. Richter EA, Garetto LP, Goodman MN, Ruderman NB. Muscle glucose metabolism following exercise in the rat. Increased sensitivity to insulin. *J Clin Invest* 1982; **69:** 785–793.
26. Parolin ML, Spriet LL, Hultman E, Hollidge-Horvat MG, Jones NL, Heigenhauser GF. Regulation of glycogen phosphorylase and PDH during exercise in human skeletal muscle during hypoxia. *Am J Physiol Endocrinol Metab* 2000; **278:** E522–E534.
27. Huang M, Lee C, Dobson GP. Epinephrine enhances glycogen turnover and depresses glucose uptake in vivo in rat heart. *FASEB J* 1997; **11:** 973–980.
28. Garetto LP, Richter EA, Goodman MN, Ruderman NB. Enhanced muscle glucose metabolism after exercise in the rat: the two phases. *Am J Physiol Endocrinol Metab* 1984; **246:** E471–E475.
29. Cobelli C, Pacini G, Toffolo G, Sacca L. Estimation of insulin sensitivity and glucose clearance from minimal model: new insights from labeled IVGTT. *Am J Physiol* 1986; **250:** E591–E598.
30. Holloszy JO. Exercise-induced increase in muscle insulin sensitivity. *J Appl Physiol* 2005; **99:** 338–343.
31. Mu J, Brozinick JT, Valladares O, Bucan M, Birnbaum MJ. A role for AMP-activated protein kinase in contraction- and hypoxia-regulated glucose transport in skeletal muscle. *Mol Cell* 2001; **7:** 1085.
32. Hughson RL, Green HJ, Sharratt MT. Gas exchange, blood lactate, and plasma catecholamines during incremental exercise in hypoxia and normoxia. *J Appl Physiol* 1995; **79:** 1134–1141.
33. Hawkins M, Gabriely I, Wozniak R, Vilcu C, Shamoon H, Rossetti L. Fructose improves the ability of hyperglycemia per se to regulate glucose production in type 2 diabetes. *Diabetes* 2002; **51:** 606–614.
34. Colberg SR, Hagberg JM, McCole SD, Zmuda JM, Thompson PD, Kelley DE. Utilization of glycogen but not plasma glucose is reduced in individuals with NIDDM during mild-intensity exercise. *J Appl Physiol* 1996; **81:** 2027–2033.
35. Martin IK, Katz A, Wahren J. Splanchnic and muscle metabolism during exercise in NIDDM patients. *Am J Physiol Endocrinol Metab* 1995; **269:** E583–E590.
36. Kang J, Robertson RJ, Hagberg JM, *et al*. Effect of exercise intensity on glucose and insulin metabolism in obese individuals and obese NIDDM patients. *Diabetes Care* 1996; **19:** 341–349.
37. Minuk HL, Vranic M, Marliss EB, Hanna AK, Albisser AM, Zinman B. Glucoregulatory and metabolic response to exercise in obese noninsulin-dependent diabetes. *Am J Physiol Endocrinol Metab* 1981; **240:** E458–E464.
38. Giacca A, Groenewoud Y, Tsui E, McClean P, Zinman B. Glucose production, utilization, and cycling in response to moderate exercise in obese subjects with type 2 diabetes and mild hyperglycemia. *Diabetes* 1998; **47:** 1763–1770.
39. Segal KR, Edano A, Abalos A, *et al*. Effect of exercise training on insulin sensitivity and glucose metabolism in lean, obese, and diabetic men. *J Appl Physiol* 1991; **71:** 2402–2411.
40. Devlin JT, Hirshman M, Horton ED, Horton ES. Enhanced peripheral and splanchnic insulin sensitivity in NIDDM men after single bout of exercise. *Diabetes* 1987; **36:** 434–439.
41. Bloem CJ, Chang AM. Short-term exercise improves β-cell function and insulin resistance in older people with impaired glucose tolerance. *J Clin Endocrinol & Metab* 2008; **93**(2): 387–392.