The effect of hypoxia and work intensity on insulin resistance in type 2 diabetes

Richard W.A. Mackenzie[1]
Bradley Elliott[1]
Neil S. Maxell[2]
Gary Brickley[2]
Peter W. Watt[2]

[1] School of Life Sciences, University of Westminster
[2] Chelsea School Research Centre, University of Brighton

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The Effect of Hypoxia and Work Intensity on Insulin Resistance in Type 2 Diabetes

R. Mackenzie, B. Elliott, N. Maxwell, G. Brickley, and P. Watt

School of Life Sciences (R.M., B.E.), University of Westminster, London W1W 6UW, United Kingdom; and Chelsea School Research Centre (N.M., G.B., P.W.), University of Brighton, Eastbourne BN20 7SR, United Kingdom

Context: Hypoxia and muscle contraction stimulate glucose transport *in vitro*. We have previously demonstrated that exercise and hypoxia have an additive effect on insulin sensitivity in type 2 diabetics.

Objectives: Our objective was to examine the effects of three different hypoxic/exercise (Hy Ex) trials on glucose metabolism and insulin resistance in the 48 h after acute hypoxia in type 2 diabetics.

Design, Participants, and Interventions: Eight male type 2 diabetics completed 60 min of hypoxic [mean (SEM) O_2 = ~14.7 (0.2)%] exercise at 90% of lactate threshold [Hy Ex^{60}; 49 (1) W]. Patients completed an additional two hypoxic trials of equal work, lasting 40 min [Hy Ex^{40}; 70 (1) W] and 20 min [Hy Ex^{20}; 140 (12) W].

Main Outcome Measures: Glucose rate of appearance and rate of disappearance were determined using the one-compartment minimal model. Homeostasis models of insulin resistance ($HOMA_{IR}$), fasting insulin resistance index and β-cell function ($HOMA_{\beta\text{-cell}}$) were calculated at 24 and 48 h after trials.

Results: Peak glucose rate of appearance was highest during Hy Ex^{20} [8.89 (0.56) mg/kg · min, $P < 0.05$]. $HOMA_{IR}$ and fasting insulin resistance index were improved in the 24 and 48 h after Hy Ex^{60} and Hy Ex^{40} ($P < 0.05$). $HOMA_{IR}$ decreased 24 h after Hy Ex^{20} ($P < 0.05$) and returned to baseline values at 48 h.

Conclusions: Moderate-intensity exercise in hypoxia (Hy Ex^{60} and Hy Ex^{40}) stimulates acute- and moderate-term improvements in insulin sensitivity that were less apparent in Hy Ex^{20}. Results suggest that exercise duration and not total work completed has a greater influence on acute and moderate-term glucose control in type 2 diabetics. **(*J Clin Endocrinol Metab* 97: 0000–0000, 2012)**

The current recommendations for general health in individuals with type 2 diabetes are equivalent to 30 min of moderate-intensity exercise daily (1, 2). Adherence to these guidelines remains low (3) with suggestions that 30 min of moderate-intensity exercise [50% of maximal oxygen consumption $\dot{V}O_{2max}$] has little effect on glucose tolerance in type 2 diabetic patients (4). We recently demonstrated that 60 min of exercise at approximately 50% $\dot{V}O_{2max}$ [90% lactate threshold (LT)] improves insulin sensitivity in type 2 diabetics (5). However, 60 min of exercise may not be an obtainable goal for diabetics. The possibility that an equivalent or greater effect (*i.e.* glucose clearance) could occur using shorter exercise durations of similar workload would therefore have clear clinical benefits.

Using identical exercise durations (50 *vs.* 70% $\dot{V}O_{2max}$ for 20 min), Hayashi *et al.* (6) demonstrated that glucose effectiveness was improved after exercise at 70% $\dot{V}O_{2max}$

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Abbreviations: AMPK, AMP-activated protein kinase; FIRI, fasting insulin resistance index; GLUT, glucose transporter; $HOMA_{IR}$, homeostasis model of insulin resistance; Hy Ex, hypoxia/exercise; LT, lactate threshold; MCR, metabolic clearance rate; QUICKI, quantitative insulin sensitivity check index; R_a, rate of appearance; R_d, rate of disappearance; $\dot{V}O_{2max}$, maximal oxygen consumption.

but not at 50% $\dot{V}O_{2max}$, suggesting total work and energy expenditure are key factors in contraction-stimulated improvements in glucose control. Counter to this, Kraniou *et al.* (7) demonstrated that exercise at two different intensities and durations (~40% $\dot{V}O_{2max}$ for 60 min *vs.* approximately 80% $\dot{V}O_{2max}$for ~27 min), but of equal work, increased glucose transporter (GLUT)-4 expression in nondiabetic skeletal muscle to a similar degree, but only high-intensity exercise lowered muscle glycogen content. This latter finding indicates that high-intensity exercise has a greater ability to encourage postexercise glycogen resynthesis, suggesting that total work, and not exercise intensity or duration, is the significant contributing factor in glucose transport activity.

Contractile activity and hypoxia are known to stimulate glucose uptake in skeletal muscle via Ca^{2+}/AMP-activated protein kinase (AMPK) (contraction)-dependent pathway (8–10) that remains largely intact in type 2 diabetes (11). After exercise, improvements in glucose control are largely dependent on insulin-stimulated glucose transport (12). We have shown that moderate-intensity normoxic exercise and resting hypoxic exposure improve insulin sensitivity in type 2 diabetics (5). In addition, this improvement is augmented when exercise and hypoxia are combined (5). Thus, any intervention that has the potential to improve glucose controls warrants further investigation. It now seems logical to examine whether manipulating the exercise/hypoxic stimuli results in further changes in insulin sensitivity. The aim of the present study was therefore to identify whether exercise intensity affects insulin sensitivity after hypoxic exercise in type 2 diabetic individuals. To accomplish this aim, total work completed was kept constant during three hypoxia/exercise (Hy Ex) trials of different duration.

Subjects and Methods

Eight sedentary males, diagnosed with type 2 diabetes within the previous 5 yr by a general practitioner were recruited for this investigation. Subjects' clinical characteristics are presented in Table 1. Ethical approval was granted by East Sussex Local Research Ethics (United Kingdom). Written and verbal explanations of the study design were provided before written informed consent was obtained. Exclusion criteria included diabetic-related complications (*i.e.* neuropathy and peripheral vascular and cardiovascular disease), current smokers, or treatment with insulin. Five subjects were diet treated; the remaining three subjects were treated with metformin (n = 2, metformin 500 mg three times per day; n = 1, metformin 500 mg once per day). Three individuals were also being treated for hypertension [calcium channel blockers (5–10 mg twice daily)]. Subjects requiring metformin were asked to abstain from medication in the 48 h before experimental trials. Metformin has a whole-blood specific half-life of approximately 17.6 h (13).

Experimental design

Study design was based on four visits. The first visit enabled the collection of preliminary data and to obtain individual LT values under normoxic conditions. Thereafter, subjects returned to complete three exercise trials in hypoxia [O_2 ~14.7 (0.2)%] separated by a minimum of 7 d. After each exercise trial (d 1), subjects returned to the laboratory 24 h (d 2) and 48 h (d 3) later for the measurement of glucose kinetics and glycemic control. Subjects refrained from exhaustive exercise and maintained similar lifestyles activities throughout the experimental protocol. Nutritional intake (Compeat version 6, UK) and calorie expenditure (pedometers; Sports-Tech, UK) were recorded over the 3-days of each experimental trial (14). Instructions were given to avoid caffeine and alcohol in the 24 h preceding and during experimental trials.

Preliminary testing

Methodology was as previously described (5). Briefly, percentage of body fat was estimated using bioelectrical impedance analysis (Bodystat, UK). Venous blood samples were drawn for the determination of glycosylated hemoglobin (Axis-Shields Diagnostics, UK), fasting blood glucose (YSI 2300 STAT; YSI, Yellow Springs, OH), and plasma insulin concentrations (ELISA; DRG Diagnostics, UK), for the estimation of homeostasis model of insulin resistance [$HOMA_{IR}$; fasting insulin (microunits per milliliter) × fasting glucose (millimoles per liter)/22.5] and HOMA of β-cell function [$HOMA_{\beta\text{-cell}}$; 20 × fasting insulin (microunits per milliliter)/fasting glucose − 3.5 (millimoles per liter)] (15). LT was determined on an electrically braked Lode cycle ergometer (Lode B.V., The Netherlands) using an incremental 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 of blood lactate concentrations [La] (YSI 2300 STAT). LT was defined as the power output preceding a sudden, sustained increase in lactate concentration (≥1 mmol/liter above previous stage) and confirmed by three objective physiologists.

Determination of exercise intensity

Trial one consisted of continuous exercise for 60 min at 90% of predetermined LT [$Hy\,Ex^{60}$ mean (SEM); 49 (4) W]. Total work completed was calculated and used to determine the intensity and duration for equal work to be completed in the next two

TABLE 1. Subjects' clinical, physiological, and metabolic characteristics

Age (yr)	BMI (kg/m^2)	Waist circumference (cm)	Body fat (%)	HbA_{1c} (%)	Fasting glucose (mmol/liter)	$HOMA_{IR}$	$HOMA_{\beta\text{-cell}}$ (%)
57.5 (2.3)	29.2 (2.9)	113.6 (6.7)	37.2 (3.8)	7.3 (0.3)	7.5 (0.5)	5.0 (1.2)	72.5 (13.7)

Values are means (SEM). BMI, Body mass index; HbA_{1c}, glycosylated hemoglobin.

exercise trials. Subsequently, subjects completed two bouts of exercise lasting 40 min [Hy Ex^{40}; 70 (9) W] and 20 min [Hy Ex^{20}; 140 (12) W] in a randomized order.

Experimental protocol (d 1)

Subjects reported at approximately 0800 h, having fasted for 12 h. Each exercise trial (Hy Ex^{60}, Hy Ex^{40}, and Hy Ex^{20}) acted as d 1. On arrival, one 18-gauge cannula was positioned into a dorsal hand vein for frequent sampling of arterialized blood (~60 C) (5). A second 18-gauge cannula was placed into a contralateral antecubital vein for steady rate [6,6-$^2H_2$]glucose infusion (Cambridge Isotope Laboratories Inc., Andover, MA). Whole-body hypoxia [O_2 ~14.7 (0.2)%] was administered using air-processing units (SQ-10; Colorado Altitude Training, Boulder, CO) with a steady flow of nitrogen (N_2 ~40 liters/min) into a closed environment [temperature of 20 (0.9) C and relative humidity of 41 (25)%].

Exercise/hypoxic trials

Glucose solutions were prepared on the morning of each trial under sterile conditions after which a glucose bolus (40 ml) containing 304 mg [6,6-$^2H_2$]glucose was injected over a 45-sec period using an antecubital vein. A 30-min constant infusion (VP 5000; Medical Systems, Arcomedical Infusions Ltd., Essex, UK) followed at a rate of 5.1 mg/min. Arterialized samples were drawn every 5 min during this period. Subjects were then exposed to hypoxia where they performed exercise trials. Infusion rates were increased to 20 mg/min during exercise (16). Subjects remained in this environment for 60 min during each trial. Arterialized blood samples (~10 ml) were drawn every 10 min. Heart rate (Polar Electro Oy, Kemple, Finland) and oxygen saturation (Nonin 2500; Nonin, Minneapolis, MN), using a pulse oximeter, were measured every 10 min.

Day 2

After a second consecutive overnight fast, subjects arrived 24 h (~1000 h) after hypoxic exercise. Volunteers were cannulated and basal arterialized samples collected at −15 and −30 min before the administration of a primed constant infusion (described above) at a rate of 6 mg/min. Subjects rested while arterialized samples (~10 ml) were drawn at 10-min intervals over 60 min and immediately analyzed in duplicate for blood glucose. Remaining samples were centrifuged and plasma stored at −80 C for later analysis. Day 3 required subjects to arrive at the laboratory after an additional overnight fast for the collection of a 10-ml resting blood sample. Isotope infusion procedures were not repeated for day 3. Control procedures set out for day 1 were repeated in the 24 and 48 h after each trial. Fasting blood glucose and plasma insulin taken at day 1, 2, and 3 were used to calculate $HOMA_{IR}$, $HOMA_{\beta\text{-cell}}$, quantitative insulin sensitivity check index [QUICKI; 1/(log fasting insulin (microunits per milliliter) + log glucose (milligrams per deciliter)] and fasting insulin resistance index [FIRI; fasting glucose (millimoles per liter) × fasting insulin (milliunits per liter)/25] (15). These indices have been validated against the one-compartment model (iv glucose tolerance test; $r = 0.79$; $P < 0.0001$) (18) and the euglycemic-hyperinsulinemic clamp technique (15, 19).

Glucose rate of appearance (R_a), rate of disappearance (R_d), and metabolic clearance rates (MCR)

For [6,6-$^2H_2$]glucose enrichments, approximately 20 μl plasma was deproteinized with 100 μl ethanol and centrifuged (6000 rpm, 5 min). Supernatants were then dried for derivatization, and 100 μl hydroxylamine-pyridine (25 mg/ml) solution was added and incubated for 60 min at 70 C. Subsequently, 100 μl 99% bis(trimethyl)trifluoroacetamide/1% 2,3,5,6-tetrachloro-4-methylsulphonyl (Sigma-Aldrich, Exeter, UK) was added to samples before an additional 45-min incubation period. Glucose was analyzed by gas chromatography mass spectrometer (Clarus 500; PerkinElmer, Waltham, MA) for peaks of 319 (tracee) and 321 ([6,6-$^2H_2$]glucose; tracer). Glucose R_a, R_d, and MCR were estimated using a non-steady-state one-compartment adapted model (20, 21) and were calculated at baseline (d 1), during hypoxic/exercise treatments, and for rest during d 2:

$$C = Cm/(1 + IE) \quad (1)$$

$$R_a(t) = \frac{f(t) - V \cdot C(t) \cdot \frac{dIE(t)}{dt}}{IE(t)} \quad (2)$$

$$R_d = R_a - V\,[(C_2 - C_1)/(t_2 - t_1)]\ (\text{body weight}^{-1}) \quad (3)$$

$$MCR = R_d/[(C_1 + C_2)/2], \quad (4)$$

where f is isotope infusion rate, and V is the volume of distribution assumed to be equal and constant (145 ml/kg for glucose) (21). The concentration (C) of the tracee at time (t) can be calculated from endogenous measured concentration (Cm) of glucose and enrichment at that time. C_1 and C_2; glucose concentrations and time points 1 (t_1) and 2 (t_2) (16). IE is the tracer enrichment expressed as atoms percent excess at sampling times corrected for background enrichment value determined from basal plasma samples (22). To compensate for a known inadequacy of the one-compartment model, the current investigation increased the tracer infusate content of [6,6-$^2H_2$]glucose above that previously used (16). Increasing tracer amounts in exogenous glucose infusates minimizes fluctuations in enriched samples (23).

Statistical analyses

Results are expressed as mean with SEM. Statistical significance was set at the level $P < 0.05$. Differences over time and between

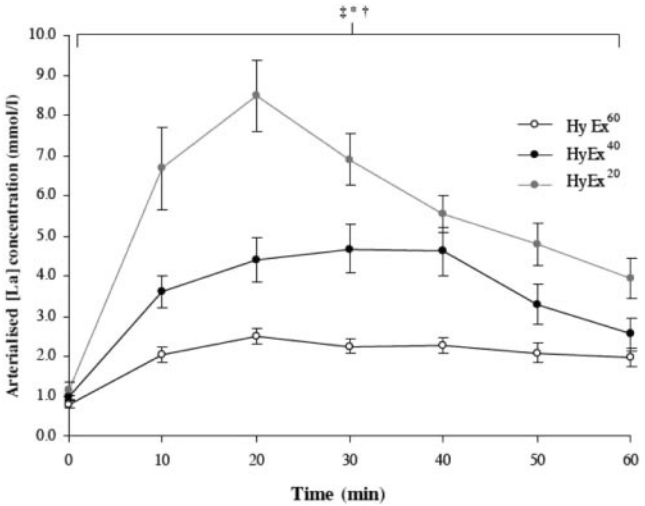

FIG. 1. Arterialized blood lactate concentrations ([La]) during exercise. Main-effects differences are indicated: ‡, Hy Ex^{60} *vs.* Hy Ex^{40}, $P = 0.019$; *, Hy Ex^{60} *vs.* Hy Ex^{20}, $P = 0.002$; †, Hy Ex^{40} *vs.* Hy Ex^{20}, $P = 0.046$. Values are means (SEM).

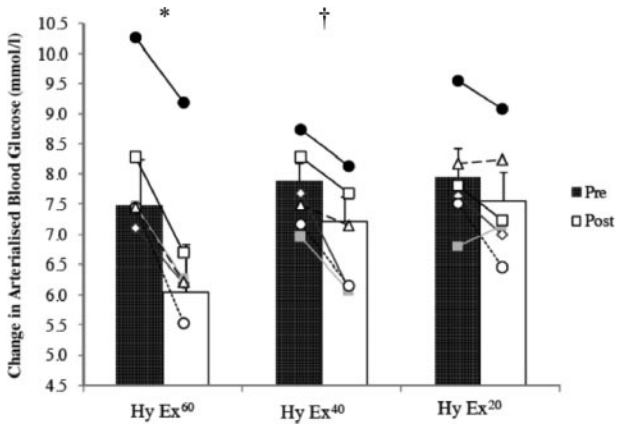

FIG. 2. Mean change in arterialized blood glucose concentrations over the 60 min of each Hy Ex trial. *, Significant difference for Hy Ex^{60} ($P = 0.001$); †, Hy Ex^{40} ($P = 0.005$). *Bars* represent means (SEM). Individual changes are also represented by *lines*.

conditions for all measured and calculated variables were evaluated by two-way repeated-measures ANOVA. Where sphericity of data was broken, *P* values were corrected using the Huynh-Feldt method, and significant differences between data points were identified using Tukey's *post hoc* test (SPSS version 15).

Results

No difference was found for total kilocalorie intake, carbohydrate consumption, or energy expenditure both within and between trials ($P > 0.05$). Main-effect differences for arterialized blood lactate concentrations ([La]) were noted between Hy Ex^{60} and Hy Ex^{40} ($P = 0.019$), Hy Ex^{60} and Hy Ex^{20} ($P = 0.002$), and Hy Ex^{40} and Hy Ex^{20} ($P = 0.046$, Fig. 1). Figure 2 shows mean changes in arterialized blood glucose concentrations from baseline to the end of each trial, representing the combined effects of hypoxia and exercise. Hy Ex^{20} demonstrated no difference in blood glucose concentration (−0.39 mmol/liter) over the 60-min exposure. Both Hy Ex^{60} and Hy Ex^{40} caused reductions in blood glucose of −1.60 and −0.84 mmol/liter ($P = 0.005$), respectively. Time-course data for blood glucose concentrations are presented in Fig. 3. Main effect differences were noted between Hy Ex^{60} and Hy Ex^{40} and between Hy Ex^{60} and Hy Ex^{20} ($P < 0.05$). No difference was found for the comparison between Hy Ex^{40} and Hy Ex^{20}.

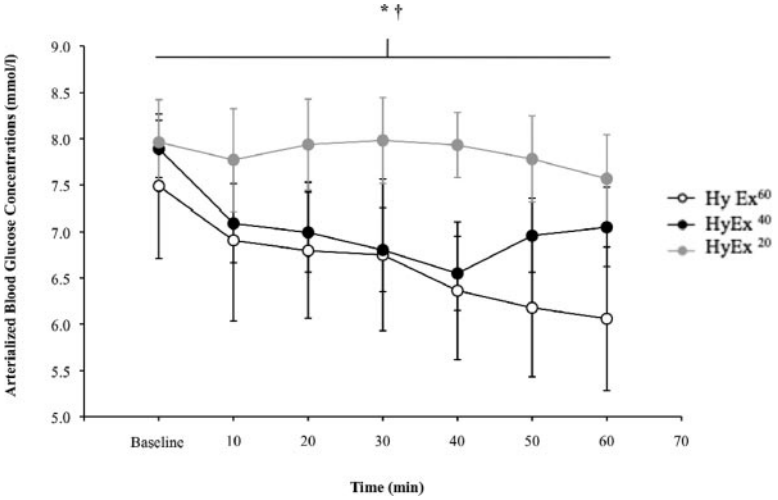

FIG. 3. Arterialized blood glucose concentrations during exercise. Main-effects differences are indicated: *, Hy Ex^{60} *vs.* Hy Ex^{40}; †, Hy Ex^{60} *vs.* Hy Ex^{20}; $P < 0.05$. Values are means (SEM).

Glucose R_a was greater for Hy Ex^{20} when compared with Hy Ex^{60} during exercise at 10, 20, and 30 min ($P = 0.037$). R_a peaked at 8.89 (0.56) mg/kg · min at the 10-min point for Hy Ex^{20} (Fig. 4A). Peak R_a values for Hy Ex^{60} [6.26 (0.30) mg/kg · min] and Hy Ex^{40} [6.66 (0.84) mg/kg · min] occurred at the 20 min point during exercise and were found to be 29.6% ($P = 0.02$) and 25.1% ($P = 0.041$) lower than Hy Ex^{20}, respectively.

The highest R_d for glucose was measured in Hy Ex^{20} during the first 10 min of exercise [8.35 (0.60) mg/kg · min] and remained elevated above both Hy Ex^{60} ($P = 0.001$) and Hy Ex^{40} ($P = 0.040$) until 40 min of exercise/hypoxia (Fig. 4B). R_d peaked within 20 min for both Hy Ex^{60} [6.44 (0.32) mg/kg · min] and Hy Ex^{40} [6.70 (0.79) mg/kg · min] with no difference between conditions. MCR showed no difference between Hy Ex^{60} [4.89 (0.30) ml/kg · min], Hy Ex^{40} [5.00 (0.26) ml/kg · min], and Hy Ex^{20} [5.04 (0.70) ml/kg · min] ($P > 0.05$).

Hypoxia/exercise for 60 min

Fasting blood glucose and plasma insulin concentrations were lower in the 24 and 48 h ($P < 0.05$) after 60 min of moderate-intensity exercise in hypoxia (Hy Ex^{60}). Indices of insulin resistance were also improved after treatment (Table 2). R_a was not different from baseline values taken on d 1 *vs.* d 2 for Hy Ex^{60} [d 1, 1.93 (0.11) mg/kg · min; d 2, 1.87 (0.10) mg/kg · min]. R_d was higher in Hy Ex^{60} after exercise but did not reach significance [d 1, 1.80 (0.11) mg/kg · min; d 2, 1.89 (0.12) mg/kg · min]. MCR demonstrated a similar pattern [d 1, 1.49 (0.06) ml/kg · min; d 2, 1.51 (0.11) ml/kg · min] ($P > 0.05$) to that of R_d.

Hypoxia/exercise for 40 min

Fasting blood glucose was lower 24 and 48 h ($P = 0.03$) after Hy Ex^{40} (Table 3). $HOMA_{IR}$ ($P = 0.049$) and FIRI ($P = 0.04$) were reduced, whereas QUICKI increased ($P = 0.037$) in the 48 h after Hy Ex^{40}. Glucose R_a was not altered from baseline values after Hy Ex^{40} [d 1, 2.08 (0.14) mg/kg · min; d 2, 2.01 (0.13) mg/kg · min]. Although higher, R_d was not significantly different from values recorded on d 1 within the same trial [d 1, 1.99 (0.07) mg/kg · min; d 2, 2.10 (0.25) mg/kg · min].

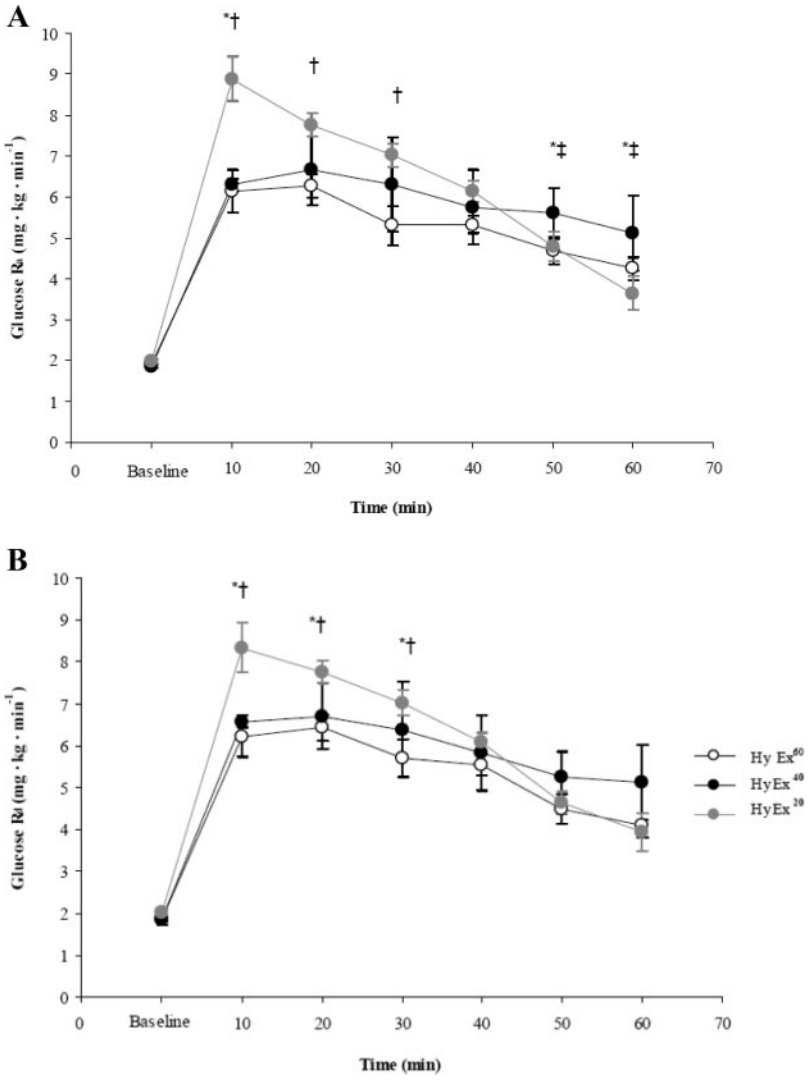

FIG. 4. R_a (A) and R_d (B) during hypoxic exercise. ‡, Significant difference between Hy Ex^{60} *vs.* Hy Ex^{40} ($P < 0.05$); †, Hy Ex^{60} *vs.* Hy Ex^{20} ($P < 0.05$); *, Hy Ex^{40} *vs.* Hy Ex^{20} ($P < 0.05$). Values are means (SEM).

Similar to Hy Ex^{60}, MCR was unchanged in the 24 h after Hy Ex^{40} [d 1, 1.52 (0.00) ml/kg · min; d 2, 1.55 (0.04) ml/kg · min].

Hypoxia/exercise for 20 min

Improvements were shown in fasting glucose, $HOMA_{IR}$, and FIRI in the 24 h after exercise. Unlike both Hy Ex^{60} and Hy Ex^{40}, these improvements were not apparent on d 3 (Table 4). Glucose R_a [d 1, 1.99 (0.06) mg/kg · min; d 2, 1.91 (0.13) mg/kg · min], R_d [d 1, 1.93 (0.08) mg/kg · min; d 2, 1.91 (0.09) mg/kg · min], and MCR [d 1, 1.78 (0.04) ml/kg · min; d 2, 1.75 (0.10) ml/kg · min] were not changed in the 24 h after Hy Ex^{20}.

In addition to within-trial comparisons, differences between Hy Ex^{60}, Hy Ex^{40}, and Hy Ex^{20} were also tested. Baseline values for indices of insulin sensitivity and fasting glucose and insulin were not different between trials. In addition, fasting blood glucose was similar between Hy Ex^{60} and Hy Ex^{40} at d 1 [7.32 (0.67) *vs.* 7.37 (0.33) mmol/liter] and d 2 [7.38 (0.69) *vs.* 7.66 (0.23) mmol/liter, respectively]. Fasting insulin ($P = 0.028$), $HOMA_{IR}$ ($P = 0.004$), QUICKI ($P = 0.025$), and FIRI ($P = 0.015$) were all significantly improved in Hy Ex^{60} when compared with Hy Ex^{40} at 24 h. $HOMA_{IR}$ was lower in the 48 h after Hy Ex^{60} [3.00 (0.39)] compared with Hy Ex^{40} [4.04 (0.28)] with the other indices of insulin sensitivity being similar between conditions at this time point. $HOMA_{IR}$ was higher for Hy Ex^{20} compared with Hy Ex^{60} in the 24 h ($P = 0.01$) and 48 h ($P = 0.044$) after treatment. All other indices were unaltered with the same comparisons. Between-trial comparisons were also made for Hy Ex^{40} and Hy Ex^{20}. The data show that fasting blood glucose was lower in Hy Ex^{40} at d 2 ($P = 0.011$) with no differences noted elsewhere.

Discussion

The present study was undertaken to investigate the effects of three different hypoxic exercise trials, each of equal work, on insulin resistance in the 48 h after acute hypoxic

TABLE 2. Fasting indices of glucose tolerance, insulin secretion, insulin sensitivity, and insulin resistance for the Hy Ex^{60} trial

	Hy Ex^{60}					
	Glucose (mmol/liter)	Insulin (μU/ml)	$HOMA_{IR}$	$HOMA_{\beta\text{-cell}}$	QUICKI	FIRI
d 1	8.39 (0.39)	14.58 (1.1)	4.96 (0.97)	68.7 (6.5)	0.303 (0.005)	4.73 (0.78)
d 2	7.32 (0.67)[b]	8.57 (1.3)[b]	2.48 (0.26)[a]	49.2 (13.9)	0.334 (0.006)[b]	2.47 (0.30)[b]
d 3	7.38 (0.69)[a]	11.91 (0.9)[a]	3.00 (0.39)[a]	68.6 (14.3)	0.317 (0.005)[b]	3.48 (0.40)[a]

Values are mean (SEM).

[a] Significantly difference from d 1 ($P < 0.05$).

[b] Significantly different from d 1 ($P < 0.01$).

TABLE 3. Fasting indices of glucose tolerance, insulin secretion, insulin sensitivity, and insulin resistance for the Hy Ex^{40} trial

	Hy Ex^{40}					
	Glucose (mmol/liter)	Insulin (μU/ml)	$HOMA_{IR}$	$HOMA_{\beta\text{-Cell}}$	QUICK	FIRI
d 1	8.53 (0.43)	14.4 (1.0)	5.51 (0.67)	57.7 (3.1)	0.300 (0.004)	4.96 (0.60)
d 2	7.37 (0.33)[a]	10.8 (0.5)[a]	3.53 (0.18)[a]	57.6 (6.5)	0.317 (0.002)[a]	3.17 (0.16)[a]
d 3	7.66 (0.23)[a]	12.0 (0.7)	4.04 (0.28)[a]	60.3 (5.2)	0.312 (0.003)[a]	3.63 (0.26)[a]

Values are mean (SEM).

[a] Significantly difference from d 1 ($P < 0.05$).

exposure in type 2 diabetics. The main findings from this study were that exercise in hypoxia for 60 min and 40 min acutely increased glucose disposal. That Hy Ex^{60} improved insulin sensitivity in the 48 h after exercise is consistent with our previous findings (5). In addition, shorter-duration exercise of equal work in hypoxia (Hy Ex^{40}) improved moderate-term insulin resistance. Acute improvements in glucose control were greatest in Hy Ex^{60} when comparisons were made with Hy Ex^{40} and Hy Ex^{20}.

Exercise is known to increase blood glucose clearance via augmented muscle blood flow, augmented GLUT recruitment, increased substrate requirement, and elevated insulin sensitivity (24–26). Blood glucose removal is known to be dependent on fiber type recruitment because type II muscle fiber, with high glycolytic capacity, creates a greater potential for glucose disposal (27). The inability of Hy Ex^{20} to reduce blood glucose may be attributable to an elevation in R_a resulting from high-intensity exercise (28, 29). Hy Ex^{40} caused an acute reduction in blood glucose during exercise but was 47% less than Hy Ex^{60}. Given that R_d and MCR were similar in Hy Ex^{60} and Hy Ex^{40}, the smaller change noted in the latter trial would appear to be due to a higher glucose R_a toward the end of exercise (30). The current study suggests that higher-intensity exercise in hypoxia encourages whole-body glucose transport. However, glucose concentrations remain unchanged, or decrease moderately due to a relative increase in endogenous glucose production (29).

Insulin sensitivity has been shown to increase by 773% after moderate-intensity exercise in type 2 diabetics (31). Using a two-compartmental iv glucose tolerance test, our previous findings have shown insulin sensitivity to be increased after 60 min of resting hypoxia when compared with a normoxic control [1.39 (0.08) *vs.* 2.25 (0.50) $\times$ 10^{-4} min^{-1} (μU/ml), respectively]. In addition, insulin sensitivity was also at its highest after hypoxic exercise when comparisons were made with both normoxic rest and normoxic exercise (5). That hypoxia and hypoxic exercise improve glucose tolerance is supported from work carried out in animal models (9). Data presented by Wadley *et al.* (29) demonstrated that hypoxic exercise causes a greater degree of glycogen depletion over exercise alone [normoxic exercise; 485.0 (51.2) *vs.* hypoxic exercise; 371.1 (40.9) mmol/kg dry weight]. These data suggest that hypoxia and exercise have an additive effect on insulin sensitivity with a greater potential for postexercise glucose uptake and glycogen synthesis and may therefore provide a justification for the use of moderate hypoxia in the treatment of type 2 diabetes.

$HOMA_{IR}$ and FIRI were improved in the 24 h after each trial, showing that the combined stress of short-duration exercise and hypoxia can increase insulin sensitivity. The current study did not measure muscle glycogen content; however, it is reasonable to hypothesize that exercise, hypoxia, and an approximately 12-h fast caused glycogen depletion (8, 29). Furthermore, glycogen synthase activity is increased after 60 min of moderate-intensity exercise (90% LT) (34). This effect appears to be reversed within 48 h of Hy Ex^{20} because variables reflecting glycemic control returned to baseline values. Exercise lasting 40 min in hypoxia also demonstrated improvements in insulin sensitivity extending to 48 h after exercise. The decrease in insulin resistance shown in the Hy Ex^{60} trial was greater when compared with Hy Ex^{40}. Combined, these finding

TABLE 4. Fasting indices of glucose tolerance, insulin secretion, insulin sensitivity, and insulin resistance for the Hy Ex^{20} trial

	Hypoxic Exercise (Hy Ex^{20})					
	Glucose (mmol/liter)	Insulin (μU/ml)	$HOMA_{IR}$	$HOMA_{\beta\text{-Cell}}$	QUICKI	FIRI
d 1	8.44 (0.56)	14.1 (1.8)	5.24 (0.66)	60.5 (10.9)	0.302 (0.005)	4.72 (0.59)
d 2	7.41 (0.55)[a]	11.6 (1.3)	3.76 (0.35)[a]	64.4 (11.0)	0.373 (0.010)	3.39 (0.32)[a]
d 3	7.98 (0.58)	13.9 (1.7)	5.06 (0.63)	63.7 (7.8)	0.305 (0.015)	4.55 (0.84)

Values are mean (SEM).

[a] Significantly difference from d 1 ($P < 0.05$).

propose that exercise duration in hypoxia, and not total work, facilitate greater improvements in moderate-term glucose control.

Interestingly, both glucose R_d and MCR were not different between d 1 and 2 for any of the trials. These data are hard to explain given that improvements in insulin sensitivity and decreased fasting glucose were seen. These results may suggest that the improvements in insulin sensitivity are likely the result of increased systemic insulin action (efficiency) on glucose uptake for a given insulin concentration, because both fasting glucose and insulin concentrations were reduced. In addition, the homeostatic feedback relationship between insulin sensitivity and insulin secretion suggests that the improvement in insulin sensitivity would potentially result in a decrease in insulin requirements and insulin release due to elevated insulin-stimulated glucose clearance. This can be supported elsewhere with hypoxia- and exercise-induced improvements in acute insulin response to an iv glucose challenge (5).

The mechanisms responsible for improvements in postexercise insulin resistance likely include increased GLUT-4 membrane content, elevated Akt/protein kinase B activity and AS160 phosphorylation (35). By completing the same amount of work in a shorter duration, the exercise intensities within the present study progressively increased. Chen *et al.* (28) reported that AMPK2α activity increases in an intensity-dependent manner during exercise. AMPK activity is tightly regulated by a number of factors including blood glucose (36) and insulin concentrations (37), free AMP (17), and muscle glycogen (32). Given the collective findings above, it could be expected that improvements in postexercise insulin sensitivity would have been greater with increasing exercise intensity. The findings from the current work refute this notion, because the total amount of work completed between trials was equal. Treebak *et al.* (35) demonstrated that phospho-AS160 increased during moderate exercise, which was not evident in a shorter high-intensity bout, suggesting exercise duration is the key determinate for stimulating signaling mechanisms involved in GLUT-4 translocation.

One critique of this study is the lack of a control group exercising under normoxic conditions. Comparing intensity of exercise between normoxic and hypoxic conditions is complicated by a shift of about 10% in relative intensity (33). We previously showed in a companion study that Hy Ex^{60} gave a greater positive response in terms of glucose homeostasis than matched workload and duration of exercise in normoxic conditions in the same group of participants and using near-identical methodologies (5). The primary aim of this study was to investigate the effect of exercise intensity, independent of work completed, on insulin homeostasis after exercise in hypoxia. Thus, the aim here was to make comparisons between varying exercise intensities in hypoxia, not between normoxic and hypoxic exercise.

The major findings were that moderate-intensity exercise in hypoxia stimulates acute and moderate-term improvements in insulin sensitivity. It appears that high-intensity exercise of shorter duration and of equal work (Hy Ex^{20}) causes improvements in $HOMA_{IR}$ and FIRI; however, these improvements were more apparent during Hy Ex^{60} and Hy Ex^{40}, showing that exercise duration and not total work has a greater influence on acute and moderate-term glucose control in type 2 diabetics.

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Address all correspondence and requests for reprints to: Dr. R. Mackenzie, School of Life Sciences, University of Westminster, 115 New Cavendish Street, London W1W 6UW, United Kingdom. E-mail: r.mackenzie@westminster.ac.uk.

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