Intermittent Exercise with and without Hypoxia Improves Insulin Sensitivity in Individuals with Type 2 Diabetes

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Context: Hypoxia and muscle contraction stimulate glucose transport activity in vitro. Exercise and hypoxia have additive effects on insulin sensitivity in type 2 diabetics (T2D).

Objective: The objective of the study was to examine the effectiveness of intermittent exercise with and without hypoxia on acute- and moderate-term glucose kinetics and insulin sensitivity in T2D.

Setting: The study was conducted at a university research center.

Design, Participants, and Interventions: Eight male T2D patients completed the following: 1) 60 min of continuous exercise at 90% lactate threshold in hypoxia (HyEx60); 2) intermittent exercise at 120% lactate threshold, separated by periods of passive recovery (5:5 min) in hypoxia [Hy5:5; \( \text{O}_2 \sim 14.7 (0.2)\% \)]; and 3) intermittent exercise (5:5 min) at 120% lactate threshold in normoxia (\( \text{O}_2 \sim 20.93\% \)).

Main Outcome Measures: Glucose appearance and glucose disappearance, using an adapted non-steady-state one-compartment model were measured. Homeostasis models of insulin resistance (HOMAIR), fasting insulin resistance index (FIRI), and \( \beta \)-cell function were calculated 24 and 48 h after exercise conditions.

Results: Glucose disappearance increased from baseline (1.85 mg/kg \( \cdot \text{min}^{-1} \)) compared with 24 h after HyEx60 (\( P = 0.031 \)). No difference was noted for both Hy5:5 (\( P = 0.064 \)) and normoxia (\( P = 0.385 \)). Hy5:5 demonstrated improvements in HOMAIR from baseline [d 1, 6.20 (0.40)] when comparisons were made with d 2 [4.83 (0.41)] (\( P = 0.0013 \)). HOMAIR and FIRI improved in the 24 h (HOMAIR, \( P = 0.002 \); FIRI, \( P = 0.003 \)), remaining reduced 48 h after HyEx60 (HOMAIR, \( P = 0.028 \); and FIRI, \( P = 0.034 \)).

Conclusion: HyEx60 offered the greatest improvements in acute and moderate-term glucose control in T2D. Intermittent exercise stimulated glucose disposal and improved post-exercise insulin resistance, which was enhanced when exercise was combined with hypoxia (Hy5:5). The data suggest a use of hypoxic exercise in treatment of T2D. (J Clin Endocrinol Metab 97: E546–E555, 2012)
Exercise improves acute glycemic control in type 2 diabetics (T2D; 1–4). The literature has focused on continuous aerobic exercise (4–8), with more recent work assessing resistance exercise (9–11). Comparisons have been made between continuous, aerobic, and resistance exercise (12, 13). Surprisingly, little work is available assessing the effects of intermittent exercise on glucose tolerance and insulin sensitivity in T2D. The Surgeon General, the Centers for Disease Control and Prevention, and the American Heart Association recommend that individuals should exercise at moderate intensities for 30 min/d. The same governing bodies also propose that this exercise can be accumulated throughout the day (14). However, little evidence is available to either support or refute the latter recommendation. Intermittent exercise, if proven to be effective in lowering blood glucose concentrations, may provide a more palatable alternative to potentially laborious, time-consuming continuous exercise.

Work by Essen et al. (15) showed the contribution of glucose and lipids to energy expenditure was similar in continuous and intermittent exercise of equal power outputs and oxygen uptake (VO2). Christmass et al. (16) demonstrated that intermittent exercise resulted in a higher rate of carbohydrate oxidation when compared with continuous exercise [264 (5) and 229 (6) μmol/kg · min−1, respectively]. A recent study compared continuous exercise [30 min; 60% maximal VO2 (VO2max)] with multiple short duration bouts (3 × 10 min; 60% VO2max) over 5 wk in T2D (17). These authors demonstrated that glucose concentrations (P = 0.01) and glucose area under the curve, determined using the trapezoidal rule, were lower during an oral glucose challenge after intermittent exercise training (P = 0.04). These variables remained unchanged in the continuous exercise group (17), suggesting intermittent exercise separated with periods of passive recovery stimulates glucose transport to a greater extent than continuous exercise. These conclusions are not supported elsewhere with Baynard et al. (18), showing that repeated bouts of exercise (3 × 10 min; 60% VO2peak) had no effect on glucose area under the curve in T2D.

Insulin and contractile activity stimulate glucose disposal in skeletal muscle using separate signaling pathways (19–21). Hypoxia activate glucose transport via AMP-activated protein kinase/Ca2+-dependent signaling, similar to that of contractile activity (22, 23). The ability of hypoxia to stimulate glucose disposal, independent of contractile activity, has been documented in animal work (22, 24), isolated human muscle tissue (19, 21), and T2D patients (25). This latter work also demonstrated that the effects of exercise (60 min at 90% lactate threshold) on glucose disposal are enhanced by moderate hypoxic exposure (25).

Intermittent exercise may have the potential to increase carbohydrate metabolism over more traditional continuous muscle contraction (15) and also offer a potentially more palatable form of exercise. Furthermore, if intermittent exercise can encourage blood glucose removal, then it would be reasonable to speculate that intermittent exercise in hypoxia may have an additive effect, given that the passive recovery phase in hypoxia may further stimulate glucose uptake. The aim of this study was therefore to assess the effectiveness of intermittent exercise with and without hypoxia on acute- and moderate-term glucose control in T2D.

### Materials and Methods

Eight sedentary males, diagnosed with T2D within the previous 5 yr by a general practitioner, were recruited for this investigation (Table 1). Ethical approval was granted by East Sussex Local Research Ethics Committee (United Kingdom). Details of the study were provided using written and verbal communication before gaining written informed consent. Exclusion criteria included diabetes-related complications (i.e. neuropathy, peripheral vascular and cardiovascular disease), current smokers, or treatment with insulin. Five subjects were diet treated, and the remaining three subjects were treated with metformin (n = 2 metformin 500 mg three times per day and n = 1, metformin 500 mg one time 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 about 17.6 h (26).

### Experimental design

This study was based on a repeated-measures design consisting of four visits. The first visit enabled the collection of preliminary data and to obtain individual lactate threshold (LT) values under normoxic conditions. Thereafter subjects returned to complete three exercise trials in hypoxia [O2 ≈ 14.7 (0.2)%] separated by a minimum of 7 d. Data obtained from a normoxic exercise trial have been published elsewhere (25). After each exercise trial (d 1), the subjects returned to the laboratory 24 h (d 2) and 48 h (d 3) later for the measurement of glucose kinetics and glycemic control (Fig. 1). The subjects refrained from ex-

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

<table>
<thead>
<tr>
<th>Age (yr)</th>
<th>BMI (kg/m²)</th>
<th>Body fat (%)</th>
<th>HbA1c (%)</th>
<th>Fasting glucose (mmol/liter)</th>
<th>HOMA IR</th>
<th>HOMA IR-Cell</th>
<th>TNF-α (pg/ml)</th>
</tr>
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<tr>
<td>58.7 (2.2)</td>
<td>28.3 (2.1)</td>
<td>36.0 (8.8)</td>
<td>7.8 (0.4)</td>
<td>8.1 (0.7)</td>
<td>6.0 (0.8)</td>
<td>74.3 (8.2)</td>
<td>60.0 (7.6)</td>
</tr>
</tbody>
</table>

Values are means (SEM). BMI, Body mass index; HbA1c, glycosylated haemoglobin.
haustive exercise and maintained similar lifestyle activities throughout the experimental protocol. Nutritional intake (Compeat version 6, Visual Information Systems Ltd.) and calorie expenditure, using the pedometer method, were recorded over the 3 d of each experimental trial. Electromechanical pedometers are used to detect human motion (step count) and have been shown to provide reliable estimation of energy expenditure (27). Instructions were given to avoid caffeine and alcohol in the 24 h preceding and in the days during experimental trials.

**Preliminary testing**
Methodology has been previously described (25). Briefly, percentage of body fat was estimated using Bio Electrical Impedance analysis (Bodystat, Isle of Man, UK). Venous blood samples were drawn for the determination of glycosylated hemoglobin (Axis-Shields Diagnostics, Dundee, UK), fasting blood glucose (YSI 2300 STAT, Yellow Springs Instruments, Yellow Springs, OH), and plasma insulin concentrations (ELISA, DRG Diagnostics, Boldon, UK), for the estimation of homeostasis model of insulin resistance ([HOMA_R], fasting insulin (microunits per millilitre) × fasting glucose (millimoles per litre)/22.5) and HOMA of β-cell function ([HOMA_B-Cell] = 20 × fasting insulin microunits per millilitre)/fasting glucose − 3.5 (millimoles per litre)] (29). The LT was determined on an electronically braked cycle ergometer (Lode B.V. Medical Technology, Groningen, 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 ([La] (YSI 2300 STAT). LT was defined as the power output preceding a sudden, sustained increase in lactate concentration (≥1 mmol/liter above the previous stage) (30).

**Experimental trials (d 1)**
Subjects reported to the laboratory at approximately 0800 h, having fasted for 12 h to complete each experimental trial. Exercise trials are represented as d 1. On arrival one 18-gauge cannula was positioned into a dorsal hand vein to allow for sampling of arterialized blood, using a thermostatted hot box (−60 C) (31). A second 18-gauge cannula was placed into a contralateral antecubital vein for steady rate infusion of [6,6^2H2]glucose (98% enrichment; Cambridge Isotope Laboratories Inc., Andover, MA).

**Stable isotope ([6,6^2H2]glucose) preparation and infusion**
[6,6^2H2]glucose (1.9 g) was dissolved in 250 ml saline solution (0.9% NaCl). After basal blood sampling (−30 min), a priming dose was then administered (40 ml; 304 mg [6,6^2H2] glucose) before a 30-min constant infusion immediately followed using syringe pump method (VP 5000; Medical Systems, Acromedical Infusions Ltd., Essex, UK) at a rate of 40 ml/h ([6,6^2H2]glucose infusion rate 5.1 mg/min). Arterialized samples (∼10 ml) were drawn every 5 min during this period.

**Hypoxic/exercise trials**
Immediately after the resting infusion, subjects performed three exercise trials on three separate days. Sixty minutes of continuous exercise at 90% lactate threshold in hypoxia (HyEx60) required exercise at 90% LT continuously for 60 min under hypoxic conditions. Whole-body hypoxia [O2 ∼14.8 (0.4)%] was administered using air-processing units (SQ-10; Boulder, CO) with a steady flow of nitrogen (N2; ∼40 liters/min) into a closed environment [temperature; 20 (0.9) C, relative humidity; 41 (25)%]. The remaining trials were conducted in a randomized order and required subjects to exercise for 5 min at 120% LT, separated by 5 min of passive recovery (5:5 min) for a total of 60 min in both normoxic (Nor5:5) and hypoxic environments. Exercise intensities were set using the data obtained in the preliminary visit. The infusion rate of [6,6^2H2]glucose increased to 160 ml/h (20 mg/min) during exercise (32) and blood samples (∼10 ml) drawn every 10 min. Heart rate (Polar Electro, Kiimele, Finland) and oxygen saturation (SpO2; pulse oximeter; Nonin 2500; Nonin, Minneapolis, MN) were recorded at 10-min intervals.

**Day 2**
After a second consecutive overnight fast, subjects arrived 24 h (∼1000 h) after 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, using a contralateral antecubital vein. Subjects rested while arterialized samples (∼10 ml) were drawn at 10-min intervals over 60 min and immediately analyzed in duplicate for blood glucose concentrations. Remaining samples were centrifuged and plasma stored at −80 C for later analysis. Day 3 required subjects to arrive at the laboratory after a further overnight fast for the collection of a 10-ml resting blood sample. Isotope infusion procedures were not repeated for d 3. Control procedures set out for d 1 were repeated in the 24 and 48 h after each trial.

**Indices of insulin sensitivity, insulin resistance, and β-cell function**
Fasting blood glucose and plasma insulin concentrations taken at d 1, 2, and 3 were used to calculate HOMA_R, HOMA_B-Cell; Quantitative Insulin Sensitivity Check Index [QUICKI; 1/(log fasting insulin (μU/ml)+log glucose (mg/dl)], and fasting insulin resistance index [FIKI; fasting glucose (millimoles per liter) × fasting insulin (milliunits per liter)/25] (29). These indices have been validated against the one-compartment model (iv glucose tolerance test; r = 0.79; P < 0.0001) (33) and the euglycemic-hyperinsulinemic clamp technique (29, 34).

**Analysis of [6,6^2H2]glucose enrichments**
Plasma (20 μl) was deproteinized with 100 μl of ethanol and centrifuged at 6000 rpm for 5 min. Supernatants were then dried for derivatization before 100 μl of hydroxylamine-pyridine (25 mg/ml) was added and incubated for 60 min at 70 C. Subsequently, 100 μl of 99% Bis(trimethyl)trifluoroacetamide: 1%
trimethylchlorosilane (Sigma-Aldrich, Exeter, UK) was added to samples before a further 45-min incubation period. Glucose was analyzed by gas chromatography mass spectrometer (Clarus 500; PerkinElmer, Waltham, MA) for peaks of 319 (unlabeled glucose; tracee) and 321 ([6,6°H2]glucose; tracer). Rates of blood glucose appearance (Rap), disappearance (Rdp), and metabolic clearance rates (MCR) were estimated using a non-steady-state, one-compartment adapted model (35, 36).

\[
C = \frac{Cm}{1 + IE}
\]

\[
I(t) - V \cdot C(t) \cdot \frac{dIE(t)}{dt} = \frac{Rc(t)}{IE(t)}
\]

\[
Rd = Rc - V[(C2 - C1)/(t2 - t1)] \text{(body wt}^{-1})
\]

\[
MCR = \frac{Rd[(C1 + C2)/2]}{t}
\]

where \(f\) is the isotope infusion rate, and \(V\) is the volume of distribution assumed to be equal and constant (145 ml/kg) (36). The concentration \(C\) of the tracee at time \(t\) can be calculated from endogenous measured concentration \(Cm\) of glucose and enrichment at that time. \(C1\) and \(C2\) are the glucose concentrations and time points 1 (\(t1\)) and 2 (\(t2\)) (37). IE is the tracer enrichment expressed as atoms percent excess (APE) at sampling times corrected for background enrichment values (38). To compensate for known inadequacy of the one-compartment model, this investigation increased the tracer infusate content above that previously used (37), which has been shown to minimize the fluctuations in enriched samples (39).

**Statistical analyses**

Results are expressed as mean with SEM. Statistical significance was set at the level \(P < 0.05\). Differences over time and between conditions for all variables were evaluated by two-way, repeated-measures ANOVA. Where sphericity of data were broken, \(P\) values were corrected using the Huynh-Feldt method, and significant differences between data points were identified using Tukey’s post hoc test (version 15; SPSS, Portsmouth, UK).

**Results**

No difference was noted within or between each condition for total calories \((P = 0.254)\) or carbohydrates consumed \((P = 0.102)\). Total energy expenditure, measured using pedometers, showed no significant difference within or between HyEx60, Nor5:5, and hypoxia (Hy5:5; \(P = 0.190\)). Analysis also showed no significant differences between total energy intake and energy expenditure within and between trials \((P = 0.254)\).

Heart rate was not different between trials at 107 (1), 102 (1) and 104 (2) beats/min\(^{-1}\) for HyEx60, Nor5:5, and Hy5:5, respectively (main effect; \(P = 0.168\)). \(S_pO_2\) was lower during HyEx60 when compared with Nor5:5 [90 (1)% and 98 (1)%], respectively; main effect; \(P = 0.023\). Although lower in Hy5:5 [93 (1)]% than during Nor5:5, \(S_pO_2\) was not found to be different. In addition, \(S_pO_2\) was not different between HyEx60 and Hy5:5 \((P = 0.123)\). Arterialized [La] concentrations increased during exercise and were highest within Hy5:5. No differences were noted between HyEx60 [2.09 (0.31) mmol/liter], Nor5:5 [2.16 (0.44) mmol/liter], and Hy5:5 [2.74 (0.50) mmol/liter] \((P = 0.163)\).
R₄ does show a tendency for being higher at d 2 when compared with d 1 for Hy5:5 (P = 0.064). R₄ was significantly higher from baseline [d 1; 1.85 (0.11) mg/kg·min⁻¹] during resting infusion 24 h after HyEx60 [d 2; 2.01 (0.12) mg/kg·min⁻¹] (P = 0.031), although R₄ and MCR were unchanged.

**HOMA Insulin Sensitivity, HOMAI₅, and HOMAI₆-Cell function**

**Hypoxic exercise (HyEx60)**

Fasting arterialized blood glucose was lower 24 h (d 2; P = 0.001) but not 48 h (d 3) after 60 min of continuous exercise in hypoxia (Fig. 4). Plasma insulin was lower from preexercise [d 1, 17.5 (1.6) μU/ml] values for d 2 [16.9 (1.2) μU/ml; P = 0.868] and d 3 [17.7 (2.1) μU/ml; P = 0.627; Fig. 5].

**Normoxic intermittent exercise (Nor5:5)**

Blood glucose concentrations were lower 24 h after Nor5:5 (P = 0.016) and returned to near baseline values at d 3 (P = 0.128). HOMAI₅, FIRI, QUICKI, and HOMAI₆-Cell were unaltered in the 48 h after Nor5:5. Fasting insulin values were unchanged from preexercise [d 1, 17.5 (1.6) μU/ml] values for d 2 [16.9 (1.2) μU/ml; P = 0.868] and d 3 [17.7 (2.1) μU/ml; P = 0.627; Fig. 5].

**Hypoxic intermittent exercise (Hy5:5)**

Intermittent exercise in hypoxia caused a reduction in blood glucose concentration in the 24 h after exercise [−1.00 (0.19) mmol/liter; P = 0.001]. No difference was noted for the same variable 48 h after treatment (P = 0.052). HOMAI₅ was lower 24 h after Hy5:5 (P = 0.013) as was FIRI (P = 0.013). Hy5:5 also demonstrated an increase in QUICKI at the 24-h mark (P = 0.018). β-Cell function, as assessed with HOMAI₆-Cell, was found to be significantly higher 48 h after exercise (P = 0.005; Fig. 6).

Figure 7 shows plasma TNF-α concentrations drawn from fasting blood samples on d 1, d 2, and d 3. Comparisons between trials showed a significant difference in baseline samples drawn on d 1 between Nor 5:5 and Hy5:5 [55.8 (8.8) and 67.1 (7.9) pg/ml, respectively; P = 0.04]. No difference was noted between HyEx60 and Nor5:5 (P = 0.772) and HyEx60 and Hy5:5 (P = 0.064). HyEx60 caused a significant reduction in TNF-α 24 h (d 2; P = 0.016) and 48 h (d 3; P = 0.023) after exercise. No change was noted for Nor5:5 (P = 0.064). Circulating TNF-α concentrations were lower 24 h after Hy5:5 (d 2; P = 0.022), which returned to near baseline values on d 3 (P = 0.08).

HOMAI₅ and FIRI decreased significantly from d 1 after HyEx60 and showed the greatest magnitude of change within the variables used to estimate postexercise glucose tolerance (data presented above). Changes in circulating TNF-α values from d 1 were therefore correlated with HOMAI₅ and FIRI to assess the relationship between these variables. No significant correlations were noted between δ-TNF-α and δ-HOMAI₅ [d 2, r = 0.45, P = 0.308; d 3, r = 0.11, P = 0.810] and δ-FIRI [d 2, r = 0.46, P = 0.304; d 3, r = 0.15, P = 0.906].

**Discussion**

We previously demonstrated that a single bout of moderate-intensity exercise increases glucose metabolism during and after exercise in T2D. In addition, improvements seen in glucose homeostasis were increased when exercise was combined with hypoxic exposure (25). This study sought to extend this work and assess whether intermittent ex-
Exercise with and without hypoxia could alter acute- and moderate-term glucose metabolism in type 2 diabetics. The primary finding was 60 min of moderate-intensity exercise in hypoxia provided the greatest improvements in glucose tolerance when compared with intermittent exercise in either normoxia or hypoxia. Intermittent exercise in normoxia also stimulated acute blood glucose removal in T2D. Improvements in insulin resistance were less apparent in the 48 h after Nor5:5. In contrast to normoxic intermittent exercise, Hy5:5 augmented insulin sensitivity to a greater degree in the 24 and 48 h after exercise.

Similar to our previous work, moderate-intensity exercise in hypoxia increased acute glucose disposal in T2D. It would seem that continuous exercise in hypoxia is a more potent stimulus for glucose disposal than intermittent exercise in either environment. The present data showed that continuous exercise (HyEx60) caused a 19.5% decline in blood glucose concentrations. This observation is supported by the difference between Ra and Rd during HyEx60 (−0.20 (0.04) mg/kg · min⁻¹). No difference was found when the same calculations were carried out for both intermittent exercise trials. These data suggest that the trend for higher rates of Rd were matched with a subsequent increase in endogenous R₄ during intermittent exercise, resulting in smaller changes in circulating blood glucose when compared with HyEx60.

It is recognized that glucose uptake and production increase with exercise intensity as a result of greater reliance of skeletal muscle on glucose oxidation (40). It is also known that Ra increases at a greater pace than Rd during sustained high-intensity exercise (>80% VO₂max). Some reports (16, 41, 42) found glucose utilization to be 1.2 times higher during intermittent compared to continuous exercise in healthy controls. The current study does not support this notion, which may reflect differences in the measurement methods used. Christmass et al. (16) measured substrate oxidation using indirect calorimetry, which overestimates carbohydrate but underestimates fat oxidation, during high-intensity exercise via the depletion of the HCO₃⁻ pool and nonmetabolic production of CO₂ (43).

To the best of the authors’ knowledge, this is the first study to assess the effects of continuous and intermittent hypoxic exercise in T2D. The novel data presented therefore makes literature-based conclusions difficult. Both Nor5:5 and Hy5:5 have the ability to reduce circulating blood glucose levels in T2D to a similar degree. This finding was surprising, given that hypoxic exposure has been
shown to stimulate glucose transport independent of exercise (22, 25). It was proposed that intermittent exercise in hypoxia, separated with passive (hypoxic) recovery, would stimulate glucose transport to a greater degree than the same exercise in normoxia. A mechanistic standpoint would support such a proposal in that as the exercise-induced stimulus for glucose transporter (GLUT)-4 activation/translocation (37) wears off; it would be replaced by a hypoxic-induced stimulus known to cause the same glucose transport effect (22, 24, 44). Because glucose Ra, Rd, and arterialized blood glucose concentrations changed to a similar extent for both Nor5:5 and Hy5:5, it was concluded that intermittent exercise in hypoxia had no additive effect on short-term glucose metabolism in T2D.

Exercise increases insulin-dependent glucose transport (45–47) and insulin sensitivity (48–51) in T2D subjects. The mechanisms surrounding this are thought to include increased GLUT-4 translocation via an insulin-dependent pathway that is reliant on a well-defined signaling nexus including, but not exclusive of, insulin receptor substrate-1/phosphatidylinositol 3-kinase/Akt-AS160 (52–55). The main finding from the current study was that HyEx60 had the most prominent effect on postexercise measures of insulin sensitivity/resistance, demonstrating continuous exercise at 90% LT for 60 min as an effective intervention for improving glucose control.

Although blood glucose concentrations decreased and glucose Rd increased to a similar degree during both Nor5:5 and Hy5:5, only the latter trial improved insulin resistance (HOMAIR and FRI) in the 24 h after exercise. This may be explained by the ability of hypoxia to increase insulin sensitivity independent of contractile activity (24, 25). The postexercise improvements in insulin sensitivity may be attributed to the degree of glycogen depletion during exercise (56) and hypoxia (57), improvements in postexercise vasodilatory function (58), and increased muscle GLUT-4 membrane content (59).

Hy5:5 also increased β-cell function (HOMAβ-cell) in the 48 h after exercise. The reason for this improvement is not clear and was not maintained during HyEx60. This is hard to interpret, given that HOMAβ-cell is merely an estimate of insulin secretion capacity. However, β-cell function has been reported to improve after exercise training in diabetic rodents (60) and humans (61). In a companion publication, hypoxia was shown to improve β-cell function, as measured using a labeled iv glucose tolerance test, in the same population (25). It is possible that an acute bout of intermittent exercise in hypoxia may increase.
GLUT-2 membrane content within the β-cells, thereby improving the glucose sensory and insulin secretory capacity of the pancreas (62–64). In addition, and more probable, given the acute nature of the current study, the improvements in β-cell function may be the consequence of the homeostatic feedback relationship that exists between insulin sensitivity and insulin secretion, suggesting that the improvement seen in HOMA\textsubscript{IR} would potentially result in a decrease in insulin requirements and insulin release due to elevated insulin-stimulated glucose clearance.

Exercise in hypoxia reduced TNF-α concentrations in the days after exercise. The finding that exercise with hypoxia can reduce circulating TNF-α levels is novel. The reason for this decrease may be associated with improvements in insulin sensitivity witnessed in the same trials. TNF-α is widely implicated in insulin resistance, via its involvement in serine phosphorylation of insulin receptor substrate-1. Due to its role in insulin resistance, TNF-α concentrations were correlated with changes in indices of glucose control. The results obtained demonstrated no relationship between TNF-α and insulin resistance. This is in direct contrast with Plomgaard et al. (65), who found an association ($P < 0.01$) between TNF-α and HOMA\textsubscript{IR} in T2D. Attempts to reduce insulin resistance in individuals with T2D by suppressing TNF-α activity with TNF-α inhibitors is ineffective (28, 65). Taken together, these data provide indirect evidence that the short-term reversal of insulin resistance seen with exercise cannot be completely attributed to reductions in circulating TNF-α and that improvement in postexercise insulin sensitivity in T2D may be the result of increased peripheral blood flow and endothelium function (58).

**FIG. 6.** Values are presented as means (SEM) for Hy5:5. Fasting blood glucose (A), HOMA\textsubscript{IR} (B), FIRI (C), plasma insulin (D), QUICKI (E), and HOMA\textsubscript{β-cells} (F) ($n = 8$). *, Significant difference from d 1 at $P < 0.05$; †, significant difference from d 1 at $P < 0.01$.

**FIG. 7.** TNF-α concentrations at baseline (d 1) and 24 h (d 2) and 48 h (d 3) after exercise ($n = 8$). *, Significant difference from d 1 within the trial ($P < 0.05$).
The conclusions from the current work are that continuous moderate-intensity exercise in hypoxia provides the greatest improvements in acute and moderate-term glucose control in T2D. Intermittent exercise also stimulated glucose disposal and improved insulin sensitivity, which was further enhanced when exercise was combined with Hy5:5. The data presented with the current and previous work (25) propose that 60 min of continuous exercise in hypoxia provides the greatest improvement in glycemic control and suggest the possible use of hypoxic exercise in T2D treatment.

Acknowledgments
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