**OBSERVATIONS**

Ten Nights of Moderate Hypoxia Improves Insulin Sensitivity in Obese Humans

Hypoxia in obese adipose tissue (AT) plays an important role in the development of whole-body insulin resistance by inducing local inflammation and the release of proinflammatory cytokines (1). Yet, living at high altitude is associated with a lower prevalence of impaired fasting glucose and type 2 diabetes compared with living at low altitude (2). Furthermore, exposure to hypoxic environments increases whole-body glucose fluxes in healthy males and glucose uptake in human and murine skeletal muscle (3). In addition, exercising under hypoxic conditions improves glucose tolerance more than exercising under normoxia (4), strongly suggesting an insulin-sensitizing effect of hypoxia. Therefore, we hypothesized that exposing obese men to 10 consecutive nights of moderate hypoxia (15 ± 0.5% O2, ~2,400 m elevation) would improve insulin sensitivity.

Eight healthy obese men (4 Caucasians, 3 African Americans, and 1 Hispanic of mean ± SE age 28 ± 1 years, weight 96.5 ± 5.3 kg, and BMI 32.7 ± 1.3 kg/m²) without evidence of chronic disease or sleep apnea and taking no medication participated in this study. The protocol was approved by the institutional review board at Pennington Biomedical Research Center (Baton Rouge, LA). Subjects slept for 10 consecutive nights (~10 h/night, ≥100 h in total) in a hypoxic tent (Hypoxicco Inc., New York, NY) maintained at ~15% O2 (range 14.5–15.5% O2, ~2,400 m above sea level) using nitrogen dilution. Biopsies of abdominal subcutaneous AT and skeletal muscle were obtained at baseline and on day 11 under normoxic and hypoxic (AT only) conditions. The oxygen tension in subcutaneous AT was also measured in normoxia at baseline and under hypoxia and normoxia on day 11 using dual temperature-oxygen tension probes (Licox, Integra LifeSciences, Plainsboro, NJ) as previously described (5).

In vivo insulin sensitivity was measured by a two-step hyperinsulinemic-euglycemic clamp (low insulin, 20 mU/m²/min for 180 min; high insulin, 80 mU/m²/min for 120 min), and the glucose disposal rate (GDR) was calculated. Substrate oxidation rates and energy expenditure were assessed by indirect calorimetry (Deltatrac II; Datex-Ohmeda) at the end (30 min) of each stage of the clamp. In vitro, myotubes obtained from biopsied muscle were cultured and differentiated for 5 days and then incubated at 37°C under normoxic or hypoxic conditions (15% O2) for 4 h with measures of glucose uptake (6). Protein and gene expression were measured in skeletal muscle using Western immunoblotting and real-time PCR and adjusted to glyceraldehyde-3-phosphate dehydrogenase or Ponceau S stain and to cyclophilin A expression, respectively.

In response to the 10-night hypoxia treatment, subjects lost an average of 1.2 ± 0.3 kg (P = 0.003), and AT pO2 tended to decrease from 51.1 ± 5.7 to 40.9 ± 2.1 mmHg (P = 0.07). This was accompanied by a decrease in fasting glucose from 94.8 ± 3.3 mg/dL at baseline to 91.8 ± 2.7 mg/dL on day 12 (P = 0.04) but unchanged fasting insulin (11.1 ± 2.9 vs. 10.3 ± 2.2 mU/L; P = 0.28). At high insulin infusion, GDR increased from 8.3 ± 1.8 to 9.2 ± 1.6 mg/kg/min (P = 0.02), indicating improved whole-body insulin sensitivity (Fig. 1A). The relative change in GDR at high insulin was 20 ± 8% and was inversely correlated with baseline GDR (r = −0.71, P = 0.05) but did not correlate with weight loss (P = 0.22). GDR was somewhat increased (23 ± 17%) at low insulin infusion from 2.6 ± 0.5 to 3.2 ± 0.7 mg/kg/min (P = 0.09). Impressively, half of the subjects experienced at least a 38% improvement in GDR at either low or high insulin.

These in vivo improvements in insulin sensitivity were corroborated by in vitro experiments showing a 62 ± 5% increase (P = 0.0006) in insulin-independent glucose uptake in primary myotubes exposed to hypoxia (15% O2) for 4 h and no change in insulin-dependent uptake (Fig. 1B). In AT, hypoxia-inducible factor 1α expression tended to be higher under hypoxia than normoxia either at baseline or at postintervention (P = 0.09). Interestingly, muscle expression of the insulin signaling proteins Akt and IRS1 and of the mitochondrial complexes I-V and peroxisome proliferator-activated receptor γ coactivator 1α were unchanged after the hypoxia treatment. However, gene expression of peroxisome proliferator-activated receptor γ coactivator 1α and COL6A3 decreased by 56 ± 7% (P = 0.02) and 48 ± 16% (P = 0.05), respectively.

In line with studies performed in rodents and isolated human skeletal muscle,
this study reports for the first time a reduced fasting glucose level and improved whole-body (skeletal muscle) and hepatic insulin sensitivity after nightly exposure to moderate hypoxia. Indeed, controlled studies at altitude and after acute exposure to hypoxia during exercise report enhancements in carbohydrate metabolism, including increased glucose oxidation, reduced fasting insulin and glucose, and higher fluxes of glucose (4).

The improvements in GDR at low and high fluxes of glucose (4).

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V.L. designed the study, performed the tests and laboratory analysis, drafted the manuscript, recruited participants, and revised the manuscript. C.M.P. contributed equally to this study. E.R. designed the study, performed the tests and laboratory analysis, drafted the manuscript and revised the manuscript. J.D.C. recruited participants, and revised the manuscript. J.M.S. designed the study, performed the tests and laboratory analysis, and revised the manuscript. E.R. is the guarantor of this work and, as such, had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

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References
1. Trayburn P. Hypoxia and adipose tissue function and dysfunction in obesity. Physiol Rev 2013;93:1–21
2. Santos JL, Pérez-Bravo F, Carrasco E, Calvillán M, Albá C. Low prevalence of type 2 diabetes despite a high average body mass index in the Aymara natives from Chile. Nutrition 2001;17:305–309
3. Azevedo JL Jr, Carey JO, Pories WJ, Morris PG, Dohm GL. Hypoxia stimulates glucose transport in insulin-resistant human skeletal muscle. Diabetes 1995;44:693–698
4. Mackenzie R, Maxwell N, Castle P, Elliott B, Brickley G, Watt P. Intermittent exercise with and without hypoxia improves insulin sensitivity in individuals with type 2 diabetes. J Clin Endocrinol Metab 2012;97:E546–E555
5. Pasarica M, Sereda OR, Redman LM, et al. Reduced adipose tissue oxygenation in human obesity: evidence for rarefaction, macrophage chemotaxis, and inflammation without an angiogenic response. Diabetes 2009;58:718–725
6. Henry RR, Abrams L, Nikouliha S, Ciaraldi TP. Insulin action and glucose metabolism in nondiabetic control and NIDDM subjects. Comparison using human skeletal muscle cell cultures. Diabetes 1995;44:936–946
7. Cartee GD, Douen AG, Ramjal T, Klip A, Holloszy JO. Stimulation of glucose transport in skeletal muscle by hypoxia. J Appl Physiol 1991;70:1593–1600