

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 ($15 \pm 0.5\%$ O_2 , $\sim 2,400$ m elevation) would improve insulin sensitivity.

Eight healthy obese men (4 Caucasians, 3 African Americans, and 1 Hispanic of mean \pm SEM age 28 ± 1 years, weight 96.5 ± 5.3 kg, and BMI 32.7 ± 1.3 kg/m^2) 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 (Hypoxico Inc., New York, NY) maintained at $\sim 15\%$ O_2 (range 14.5 – 15.5% O_2 , $\sim 2,400$ 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 $mU/m^2/min$ for 180 min; high insulin, 80 $mU/m^2/min$ 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 $37^\circ C$ under normoxic or hypoxic conditions (15% O_2) 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 pO_2 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 $20 \pm 8\%$ 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 ($23 \pm 17\%$) 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 $62 \pm 5\%$ increase ($P = 0.0006$) in insulin-independent glucose uptake in primary myotubes exposed to hypoxia (15% O_2) 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 $56 \pm 7\%$ ($P = 0.02$) and $48 \pm 16\%$ ($P = 0.05$), respectively.

In line with studies performed in rodents and isolated human skeletal muscle,

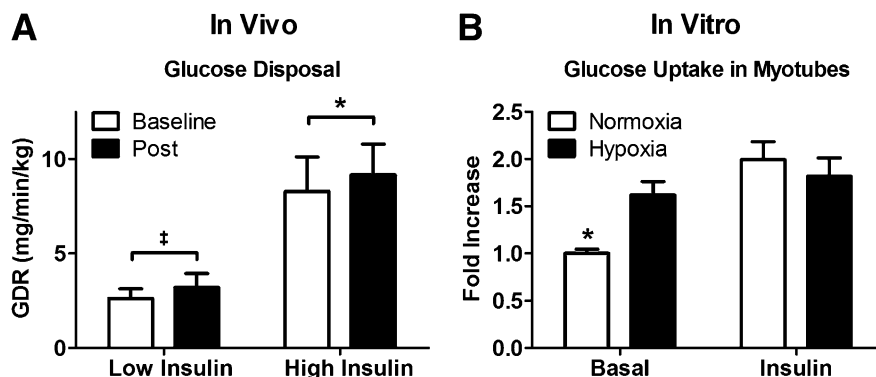


Figure 1—A: 10 nights under moderate hypoxia improves insulin sensitivity, as measured by GDR during a hyperinsulinemic-euglycemic clamp at low (20 $mU/m^2/min$) and high (80 $mU/m^2/min$) insulin infusion. B: 4 h of hypoxia improves basal but not insulin-stimulated glucose uptake in cultured human myotubes. * $P \leq 0.05$, # $P \leq 0.10$.

