Effects of Hypoxic Living and Training on Gene Expression in an Obese Rat Model

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ABSTRACT

HE, Z., L. FENG, L. ZHANG, Y. LU, J. XU, and A. LUCIA. Effects of Hypoxic Living and Training on Gene Expression in an Obese Rat Model. *Med. Sci. Sports Exerc.*, Vol. 44, No. 6, pp. 1013–1020, 2012. **Purpose**: The study's purpose was to determine in a rat obesity model the effects of normoxic training, sedentary hypoxic living, or hypoxic living plus training on the skeletal muscle messenger RNA (mRNA) levels of 14 genes involved in oxygen sensing (hypoxia-inducible factor 1α, vascular endothelial growth factor, myoglobin), glucose metabolism (glucose transporter 4, muscle phosphofructokinase), mitochondrial biogenesis (peroxisome proliferator–activated receptor γ coactivator 1-α, nuclear respiratory factor 1) and function (citrate synthase, mitochondrial-encoded cytochrome oxidase subunit 1), pH regulation (monocarboxylate transporter 1, carbonic anhydrase 3), and antioxidant defense (manganese superoxide dismutase, copper/zinc superoxide dismutase, glutathione S-transferase pi). **Methods**: One hundred thirty male 3-wk-old Sprague–Dawley rats were fed a high-fat diet (4100 kcal·kg^{-1}) for 3 months (all reaching a final weight >415 g) and then randomly assigned to the following groups (n = 10 per group): C (control, 2 d of sedentary living in normoxic conditions), TN1–TN4 (1–4 wk of normoxic treadmill training), SH1–SH4 (1–4 wk of sedentary hypoxic living (13.6% O_2)), or TH1–TH4 (1–4 wk of hypoxic living (13.6% O_2) + hypoxic treadmill training). Individual mRNA levels recorded for TN1–TN4, SH1–SH4, and TH1–TH4 were expressed relative to the mean obtained in C for each gene. **Results**: Through a two-way ANOVA, a significant interaction (treatment × treatment duration) effect was detected on expression levels of mRNAs for hypoxia-inducible factor 1α, vascular endothelial growth factor, myoglobin, nuclear respiratory factor 1, citrate synthase, carbonic anhydrase 3, monocarboxylate transporter 1, copper/zinc superoxide dismutase, glutathione S-transferase pi, and manganese superoxide dismutase. Expression levels were overall highest when training and living under hypoxia, usually after 3 wk (TH3), i.e., 79%–99% higher than the lowest values (usually corresponding to TN2) and 15.5%–53.9% higher than the second highest values (usually TH4). Normoxic training elicited no greater response than hypoxic sedentary living. **Conclusions**: In our obese rat model, hypoxic living conditions, especially if accompanied by hypoxic exercise training, can lead to health-related molecular adaptations at the skeletal muscle level. **Key Words:** OXYGEN SENSING, FAT LOSS, MITOCHONDRIA, HIGH-FAT DIET

Regular exercise is strongly advocated as a first-line intervention for the prevention and treatment of obesity (22). Although it is clear that any exercise-induced benefits will depend on the type, frequency, intensity, and duration of exercise, data from human and animal studies suggest that environmental conditions, notably hypoxia, may also play an important role. At altitudes above 5000 m, loss of appetite and weight is frequently observed in humans (25). In obese subjects, low-intensity physical exercise in conditions of normobaric hypoxia leads to more weight loss than the same intervention under normal conditions (18). Also, lower workloads are needed for hypoxic training to elicit similar improvements in terms of physical fitness, metabolic risk markers, and body composition as normoxic exercise training (2,28). In animal models, it has been observed that rats exposed to hypoxia since birth experience significant fat loss (17) and that subjecting obese mice to moderate intermittent hypoxia for 40 d reduces body weight by increasing leptin concentration and enhancing liver leptin expression while decreasing blood levels of glucose and cholesterol (12).

Regular normoxic endurance exercise induces a variety of adaptive responses that could explain its benefits in obese people, including enhanced skeletal muscle oxidative capacity and metabolic efficiency (13,16). However, the molecular mechanisms that mediate the potential health benefits of hypoxic exposure remain unclear. Some responses to exercise training can be attributed, at least partly, to gene expression changes at the skeletal muscle level (15). Thus, preexercise RNA levels of metabolic factors in skeletal muscles have been reported to increase after repeated endurance exercise stimuli (19,20). Steady-state transcript-level adaptations in skeletal muscles are therefore sensitive

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indicators of the specific muscular adaptations induced by endurance exercise training (31).

The main purpose of our study was to determine, in a diet-induced obesity animal model, the effects of normoxic training, hypoxic sedentary living, or both hypoxic living and training on skeletal muscle messenger RNA (mRNA) levels of several genes associated with endurance exercise–related phenotypes, i.e., those involved in oxygen sensing (myoglobin (*Mb*), vascular endothelial growth factor (*VEGF*), hypoxia-inducible factor 1α (*HIF-1*α)), glucose metabolism (glucose transporter 4 (*GLUT-4*), muscle phosphofructokinase (*PFKm*)), mitochondrial biogenesis (peroxisome proliferator–activated receptor γ coactivator 1-α (*PPARGC1*α), nuclear respiratory factor 1 (*NRF1*)) and function (citrate synthase (*CS*), mitochondrial-encoded cytochrome oxidase subunit 1 (*COX-1*)), pH regulation (monocarboxylate transporter 1 (*MCT1*) and carbonic anhydrase 3 (*CA3*)), and antioxidant defense (manganese superoxide dismutase (*MnSOD*), copper/zinc superoxide dismutase (*Cu/ZnSOD*), and glutathione S-transferase pi (*GSTPi*)). These gene transcripts were determined on the basis of the findings of recent studies analyzing hypoxia adaptations (27,31). Our working hypothesis was that, in conditions of obesity, the combination of hypoxic living and hypoxic training might exert a synergistic effect on skeletal muscle gene expression profiles compared with normoxic training or sedentary living under hypoxia.

MATERIALS AND METHODS

Animal Care

All experimental procedures were conducted in accordance with the *Guide for the Care and Use of Laboratory Animals* of the China Institute of Sport Science. This guide conforms with the policy statement of the American College of Sports Medicine on research using experimental animals as published in *Medicine & Science in Sports & Exercise*®.

Feeding Methods

Three-week-old male Sprague–Dawley rats (n = 260, initial body weight (mean ± SD) = 42.7 ± 4.7 g) purchased from Vital River (Beijing, China) were used in this study. The rats were housed five per cage with natural light and free access to water during the entire study period. Room temperature and humidity were kept at 21°C–23°C and 40%–60%, respectively. The rats were fed a high-fat diet for 3 months, i.e., 4100 kcal·kg^{-1} (Keaoxieli Limited Corporation, Beijing, China; license number: Beijing feed No. 238, SCXK (Beijing) 2005-0007; implementation of the standard GB14924 • 3-2001). This diet consists of 61.5% normal chow, 12% lard, 10% egg, 5% sucrose, 5% milk powder, 5% peanut, 1% sesame oil, and 0.5% salt.

Only rats with a body weight >415 g (n = 160, mean ± SD body weight = 460.2 ± 35.8 g) after the 3-month diet were included in the study. This cutoff was based on a preliminary validation of our obesity model (including 10 obese rats and 20 control rats). This study revealed that only rats reaching a body weight >415 g at the end of the diet had higher mean body fat–to–body weight ratios and serum cholesterol, triglyceride, and LDL levels than control rats fed a normal diet during the same period.

Adaptation to Treadmill Running (Normoxia)

The 160 obese rats enrolled were first familiarized with the treadmill (PT200, Hangzhou LITAI Electrical Mechanical Equipment Co, LTD, Hangzhou, China) running protocol (three daily sessions, 20 min per session, speed = 12–16 m·min^{-1}) under normal (normoxic) conditions. After this protocol, they underwent a 2-wk adaptation period (six sessions per week, with gradual increases in session duration (20–60 min) and running speed (16 to 25–26 m·min^{-1})).

To prescribe comparable relative training intensities in normoxic and hypoxic conditions (see below), we determined mean blood lactate levels (blood collected from the tail vein) using an automated analyzer (YSI 1500; Yellow Springs Instruments, Yellow Springs, OH) in eight rats made to run at 26 m·min^{-1} under normal (normoxic) conditions. This gave a mean ± SD value of 2.96 ± 1.36 mmol·L^{-1}. Thereafter, we determined which treadmill speed elicited a blood lactate concentration closest to 2.96 mmol·L^{-1} under hypoxia (13.6% O_2 in breathing air). This speed was 21 m·min^{-1}, corresponding to a blood lactate concentration of 3.08 ± 1.45 mmol·L^{-1}.

Experimental Groups

Thirty rats were eliminated from the study for one of the following reasons: body weight >550 g, injury, or inability to adapt to treadmill running. The remaining 130 rats were randomly assigned to one of 13 groups (n = 10 per group) according to the living/training conditions and duration of treatment:

- C (control): animals subjected to normal (normoxic) living conditions for 2 d before their sacrifice (initial body weight (mean ± SD) = 464.9 ± 29.1 g).
- TN1, TN2, TN3, and TN4: animals living and training in conditions of normoxia for 1 wk (466.8 ± 24.9 g), 2 wk (464.8 ± 26.3 g), 3 wk (465.6 ± 34.6 g), or 4 wk (465.0 ± 33.3 g), respectively.
- SH1, SH2, SH3, and SH4: animals living in conditions of sedentarism and normobaric hypoxia for 1 wk (464.8 ± 28.8 g), 2 wk (465.2 ± 27.6 g), 3 wk (466.1 ± 37.2 g), or 4 wk (464.6 ± 29.6 g), respectively.
- TH1, TH2, TH3, and TH4: animals living and training in conditions of normobaric hypoxia for 1 wk (467.3 ± 28.7 g), 2 wk (464.0 ± 33.3 g), 3 wk (468.6 ± 37.2 g), or 4 wk (467.0 ± 38.6 g), respectively.

Mean initial body weight (values shown above) did not differ among the groups (P > 0.05). All the groups followed the same type of diet, i.e., *ad libitum* diet of high-fat chow and water. In SH1–SH4 and TH1–TH4, animals were exposed for 24 h·d^{-1} to hypoxic conditions achieved using air

compressors (GA15FF-13; Atlas Copco Stockholm, Sweden) and a nitrogen generator (CA-200AT; CNRO, Tianjin, China) to generate a constant O_2 concentration of 13.6% O_2 in breathing air (corresponding to an altitude of 3500 m). Animals in groups TN1–TN4 and TH1–TH4 were exercised 1 h per session (6:00–7:00 p.m.) during 6 $d \cdot wk^{-1}$ (Sunday to Friday). Treadmill speed was 26 $m \cdot min^{-1}$ for TN1–TN4 and 21 $m \cdot min^{-1}$ for TH1–TH4.

Body Weight and Fat

At the end of the study (immediately before gene expression analysis—see below), all rats were anesthetized by intraperitoneal injection of a 10% chloral hydrate solution (0.3 mL per 100 g of body weight). After fixation and laparotomy, the left perineal and epididymal fat pads were quickly separated; the latter were weighted with an accuracy of 0.001 after rinsing blood in the precooling saline and dried with filter paper. We determined the ratio of body fat to body weight ([weight [g] of the left perineal fat + weight [g] of the epididymal fat] / body weight [g]).

Gene Expression in Skeletal Muscles

Muscle preparation. All rats were anesthetized at the same time of the week (Sunday, 9:00 a.m.), i.e., 38 h after the last bout of exercise training (Friday, 7:00 p.m.), by intraperitoneal injection of 10% chloral hydrate solution (0.3 mL per 100 g of body weight). The gastrocnemius muscle was dissected out, rapidly frozen with tongs, cooled to the temperature of liquid N_2, and stored at −80°C until analyzed for gene mRNA content. The order of anesthetization and excision was randomized among the groups.

RNA isolation and real-time polymerase chain reaction. Total RNA was extracted from muscle tissue using the RNeasy Mini Kit (Qiagen, Basel, Switzerland) according to the manufacturer's instructions. The integrity of RNA was electrophoretically verified by ethidium bromide staining and confirmation of an OD260/OD280-nm absorption ratio >1.95. Next, 5 μg of the total RNA was reverse transcribed using 2.5-μM random primers (Invitrogen, Life Technologies, Carlsbad, CA) and 100 U of SuperScript II reverse transcriptase (Invitrogen, Life Technologies, Carlsbad, CA). Real-time polymerase chain reaction (PCR) amplification reactions were carried out in triplicate in 8-μL aliquots in a 96-well plate using an ABI Prism 7500 Sequence Detection System (Applied Biosystems, Foster City, CA) with complementary DNA (cDNA) signal detection via SYBR Green (PE Biosystems, Rotkreuz, Switzerland). Primers were designed using the Primer Express software (PE Biosystems, Rotkreuz, Switzerland). Primer sequences are provided in Table 1. Individual PCR efficiency was calculated from the mean slope of the SYBR Green intensity monitored within the linear phase of cDNA amplification. The specificity of amplified cDNA was verified from the dissociation curve, as determined on the ABI Prism 7500 and by checking the amplified fragment for correct size after separation of the PCR reaction products on a 1% agarose gel. Cycle threshold coefficients of variation were <0.1% in duplicate samples, confirming negligible differences in efficiency and RNA loading.

Statistical Analysis

Individual mRNA data for each of the 14 genes in the different treatment groups (TN1–TN4, SH1–SH4, and TH1–TH4) were "normalized" relative to the mean value obtained in the C or "baseline" group for each particular gene, e.g., the individual *HIF-1α* transcript level recorded for an animal in TN1 was expressed as the actual individual level divided by the mean *HIF-1α* mRNA level obtained in C. Thereafter, we performed a two-way (treatment × treatment duration) ANOVA using a 3 × 4 factorial design (i.e., three treatment levels—normoxic living and training, hypoxic sedentary living, hypoxic living and training—and four treatment duration levels—1, 2, 3, and 4 wk) for all anthropometric and gene expression data, using animal age as a covariate. For *post hoc* analysis, we used the Tukey test. To minimize the risk of statistical type I error arising from numerous between-group comparisons, all statistical analyses were corrected for mass significance (7) as follows: the *P*

TABLE 1. Gene bank accession code, product length, primer, and oligodeoxynucleotide location.

	Sequence	Gene Bank Access Code	Primer Location		Product Length (bp)
			5 End	3 End	
1	*HIF-1α*	NM_024359.1	F:CTCAGTCGACACAGCCTCGATA(730–751)	R:TAAATTGAACGGCCCAAAAGTT(809–830)	101
2	*VEGF*	NM_001110333.1	F:GGTTTCGGGAACTAGACCTCTCA(1617–1639)	R:AGGCTTTCTGGATTAAGGACTGTTCT(1693–1718)	102
3	*Mb*	NM_021588.2	F:CCACGCCACCAAGCACAAG(279–297)	R:CAATGTCATTCCGGAACAGCTC(409–430)	152
4	*GLUT-4*	D28561.1	F:TGCTGGCCAACAATGTCTTG(475–494)	R:AATGAGGAACCGTCCGAGAA(556–575)	101
5	*PFKm*	BC094212.1	F:CGTTTTCCTGGTTGGCTCAA(496–515)	R:TGGCGTCCACGATCTCTACA(577–596)	101
6	*PPARGC1α*	AY237127.1	F:TTCGATGTGTCGCCTTCTTG(1594–1613)	R:TCGGGAACACGACCTGTGT(1716–1734)	141
7	*NRF1*	BC161956.1	F:CTCAGCTTCGGGCATTTATCC(853–870)	R:TTCTGGCCACCAGATAGGCT(944–963)	101
8	*COX-1*	S79304.1	F:GCAGTATTCGCCATCATAGC(508–527)	R:TCAGAGTAACGACGAGGTATCC(659–680)	173
9	*CS*	DQ403126.1	F:CAGGGCCTCTGCATGGACTA(224–243)	R:CCGTCCCGAGTTGAGTGTGT(335–354)	131
10	*CA3*	BC061980.1	F:CCTGCTTGCCGGGACTATT(598–616)	R:CTCACTGTCATGGGCTCTTTCA(677–698)	101
11	*MCT1*	BC078877.1	F:TGGCAGGCAGCCCAGTGTTC(643–662)	R:ACCTTGCCTTGCTGAGGCCC(786–805)	163
12	*Cu/ZnSOD*	BC082800.1	F:GCGGTCCAGCGGATGA(251–306)	R:GTCCTTTCCAGCAGCCACAT(333–352)	62
13	*GSTPi*	FJ179407.1	F:GGTTACCATAGATGTCTGGCTTCA(99–122)	R:AGGTCCTCCACCCCATCATT(280–299)	201
14	*MnSOD*	Y00497.1	F:ACCACGCGACCTACGTGAA(172–190)	R:TGCAGGCTGAAGAGCAACCT(253–272)	101

Abbreviations: *PFKm*, muscle phosphofructokinase gene; bp, base pairs.

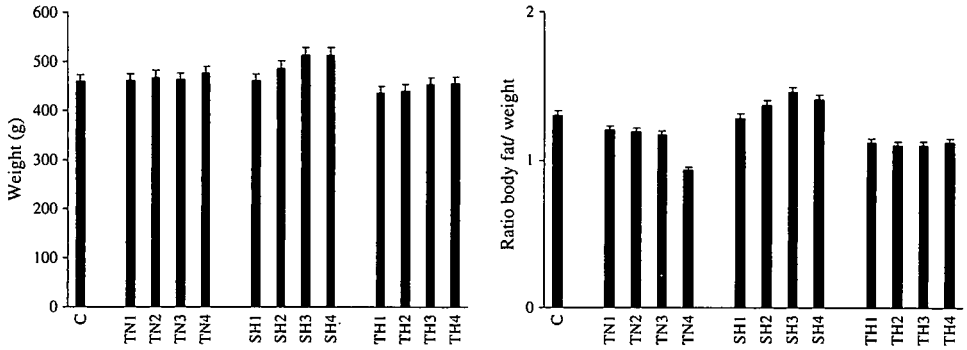

FIGURE 1—Between-group comparisons of body weight and fat (see text for group abbreviations). Data are expressed as mean ± SEM. After adjusting for multiple comparisons, no significant differences between groups were detected in *post hoc* comparisons (all $P > 0.001$).

values of the k tests are ranked in increasing order: $P_1, P_2, \ldots, P_i, \ldots, P_k$. Then, if $P_1 > \alpha/k$, then none of the k tests are significant, and the test procedure is finished. If $P_1 \leq \alpha/k$, then test 1 is significant, and P_2 is now examined. If $P_2 > \alpha/(k-1)$, then none of the $(k-1)$ remaining tests are significant, but if $P_2 \leq \alpha/(k-1)$, then test 2 is significant, and P_3 is examined. This procedure goes on until $P_i > \alpha/(k-i+1)$, and the procedure is interrupted. This method keeps the familywise error rate less than α. The familywise error rate is defined as the probability that one or more false results of the k tests will be less than or equal to α. All statistical analyses were performed using the SPSS software (version 13.0; SPSS, Inc., Chicago, IL). Values are expressed as means ± SEM.

RESULTS

Treatment Effects on Body Weight and Fat

Body weights and body fat–to–body weight ratios obtained at the end of the study in the different groups are provided in Figure 1. After adjusting for multiple comparisons (7), a significant treatment effect on body weight and body fat–to–body weight ratio (both $P < 0.001$) was observed, but no significant effect of treatment duration ($P = 0.829$, body weight; $P = 0.510$, body fat–body weight ratio) or of the interaction (treatment × treatment duration) ($P = 0.131$, body weight; $P = 0.496$, body fat–body weight ratio) emerged.

Treatment Effects on Gene Expression Levels in Skeletal Muscles

Oxygen-sensing system. Significant treatment and interaction (treatment × treatment duration) effects were observed on expression levels of mRNA for *HIF-1α*, *VEGF*, and *Mb* (all $P < 0.001$), as well as a significant treatment duration effect for *HIF-1α* ($P < 0.001$). Thus, expression levels of *HIF-1α* mRNA were significantly higher after ≥2 wk of hypoxic living + hypoxic training (TH2–TH4) compared with most groups not subjected to this intervention

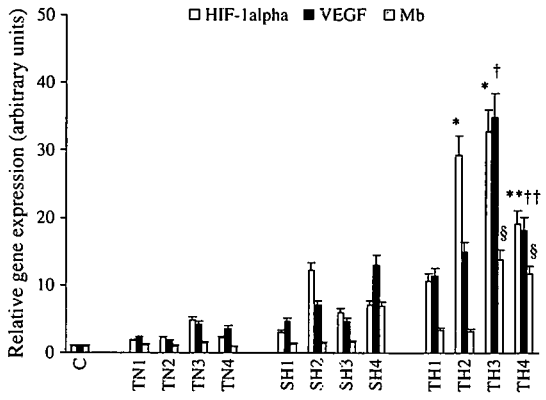

FIGURE 2—Between-group comparisons of skeletal muscle expression levels of genes involved in the oxygen-sensing system (see text for group and gene abbreviations). Data are expressed as mean ± SEM. The individual mRNA data corresponding to each gene in the different groups were expressed relative to the mean value obtained in the C group (see text) for each particular gene. Symbols: *$P < 0.001$ for *HIF-1α* in both TH2 and TH3 versus the remaining groups; **$P < 0.001$ for *HIF-1α* in TH4 versus TN1–TN4 and SH1; †$P < 0.001$ for *VEGF* in TH3 versus the remaining groups; ††$P < 0.001$ for *VEGF* in TH4 versus TN1–TN4; §$P < 0.001$ for *Mb* in both TH3 and TH4 versus remaining groups.

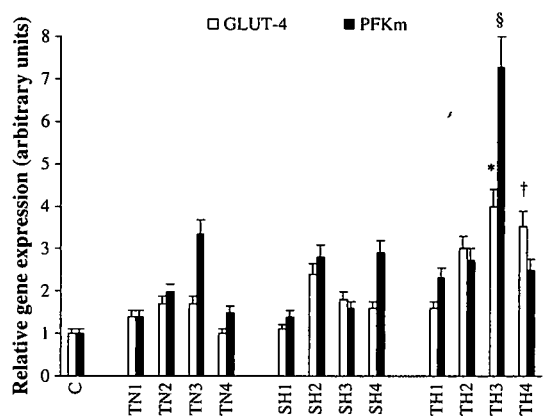

FIGURE 3—Between-group comparisons of skeletal muscle expression levels of genes involved in CHO metabolism (see text for group and gene abbreviations). Data are expressed as mean ± SEM. The individual mRNA data corresponding to each gene in the different groups were expressed relative to the mean value obtained in the C group (see text) for each particular gene. Symbols: *$P < 0.001$ for *GLUT-4* in TH3 versus TN1–TN4, SH1, SH3, SH4, and TH1; †$P < 0.001$ for *GLUT-4* in TH4 versus TN1, TN4, and SH1; §$P < 0.001$ for *PFKm* in TH3 versus TN1, TN2, TN4, SH1, and SH3.

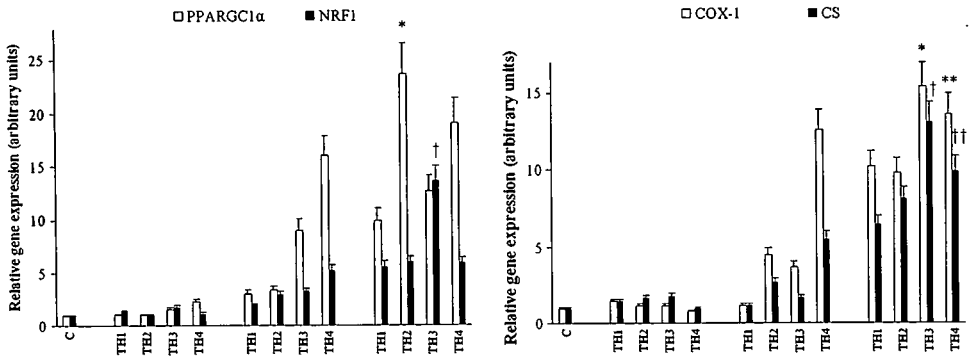

FIGURE 4—Between-group comparisons of skeletal muscle expression levels of genes involved in mitochondrial biogenesis (*upper panel*) and function (*lower panel*) (see text for group and gene abbreviations). Data are expressed as mean ± SEM. The individual mRNA data corresponding to each gene in the different groups were expressed relative to the mean value obtained in the C group (see text) for each particular gene. *Upper panel* symbols: *$P < 0.001$ for *PPARGC1α* in TH2 versus TN1–TN4, SH1, and SH2; †$P < 0.001$ for *NRF1* in TH3 versus TN1–TN4, SH1–SH4, and TH1. *Lower panel* symbols: *$P < 0.001$ for *COX-1* in TH3 versus TN1–TN4 and SH1–SH4; **$P < 0.001$ for *COX-1* in TH4 versus TN1–TN4 and SH1; †$P < 0.001$ for CS in TH3 versus TN1–TN4, SH1, and SH2; ††$P < 0.001$ for CS in TH4 versus TN1–TN4 and SH1.

(Fig. 2). Similar findings were obtained for *VEGF* and *Mb* mRNA levels after ≥3 wk of hypoxic living + hypoxic training (TH3–TH4).

CHO metabolism. We observed a significant treatment effect on expression levels of mRNAs for *GLUT-4* ($P < 0.001$) and a significant treatment duration effect for *PFKm* ($P = 0.001$). Thus, expression levels of *GLUT-4* and *PFKm* mRNAs were significantly higher after ≥3 wk of hypoxic living + hypoxic training compared with most other groups (Fig. 3).

Mitochondrial biogenesis and function. A significant treatment effect on *PPARGC1α* mRNA ($P < 0.001$) and *NRF1* mRNA ($P < 0.001$) and a significant interaction (treatment × treatment duration) effect on *NRF1* mRNA ($P = 0.001$) were detected. Expression levels of *PPARGC1α* and *NRF1* mRNAs were highest after 2 (TH2) and 3 wk of hypoxic training + hypoxic living (TH3), with significant differences emerging with respect to most other groups in which animals were not subjected to living *and* training in hypoxia, especially the groups undergoing training and living in normoxia (TN1–TN4) (Fig. 4, upper panel). We found a significant treatment effect for *COX-1* ($P < 0.001$) and *CS* ($P < 0.001$) and a significant interaction (treatment × treatment duration) effect for *CS* ($P < 0.001$). Accordingly, expression levels of *COX-1* and *CS* mRNAs were higher in TH3 and TH4 compared with most other groups, especially TN1–TN4 (training and living in conditions of normoxia) (Fig. 4, lower panel).

pH regulation. Significant treatment and interaction (treatment × treatment duration) effects were observed on mRNA expression levels for *CA3* ($P < 0.001$) and *MCT1* ($P < 0.001$). Expression levels of mRNAs for both genes were significantly higher in TH3 compared with most groups not exposed to hypoxic living + hypoxic training (Fig. 5, upper panel).

Defense against oxidative stress. Significant treatment, treatment duration, and interaction (treatment × treatment duration) effects were detected on expression levels of

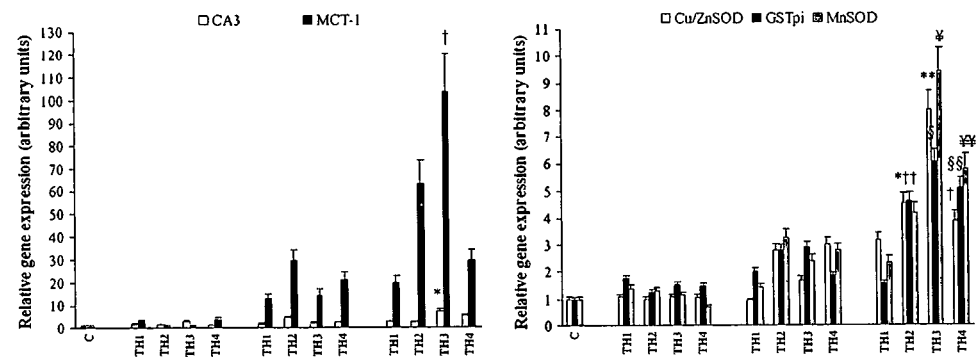

FIGURE 5—Between-group comparisons of skeletal muscle expression levels in genes involved in pH regulation (*upper panel*) and antioxidant defense (*lower panel*) (see text for group and gene abbreviations). Data are expressed as mean ± SEM. The individual mRNA data corresponding to each gene in the different groups were expressed relative to the mean value obtained in the C group (see text) for each particular gene. *Upper panel* symbols: *$P < 0.001$ for *CA3* in TH3 versus TN1–TN4; SH1, SH3, and SH4; and TH1. †$P < 0.001$ for *MCT1* in TH3 versus TN1–TN4, SH1–SH4, and TH1. *Lower panel* symbols: *$P < 0.001$ for *Cu/ZnSOD* in TH2 versus TN1–TN4 and SH1; **$P < 0.001$ for *Cu/ZnSOD* in TH3 versus TN1–TN4, SH1–SH4, and TH1; †$P < 0.001$ for *Cu/ZnSOD* in TH4 versus TN1–TN4, SH1, and SH3; ††$P < 0.001$ for *GSTPi* in TH2 versus TN1–TN4 and SH1; §$P < 0.001$ for *GSTPi* in TH3 versus TN1–TN4, SH2, SH3, and SH4; §§$P < 0.001$ for *GSTPi* in TH4 versus TN1–TN4, SH1, and SH4; ¥$P < 0.001$ for *MnSOD* in TH3 versus TN1–TN4, SH1–SH4, and TH1; ¥¥$P < 0.001$ for *MnSOD* in TH4 versus TN1–TN4 and SH1.

Cu/ZnSOD, *GSTPi*, and *MnSOD* mRNAs (all $P < 0.001$). Thus, expression levels of mRNAs for *Cu/ZnSOD* and *GSTPi* were significantly higher in TH2–TH4, especially TH3, compared with most other groups (Fig. 5, lower panel). Similar findings (i.e., higher expression levels compared with most other groups not exposed to hypoxic living + hypoxic training) were obtained for *MnSOD* mRNA in TH3 and TH4, especially in TH3.

DISCUSSION

The main finding of our study was the detection of changes in the transcripts of 14 genes expressed in the skeletal muscle of obese rats in response to exercise habits (training vs sedentary) and the environment (hypoxic vs normoxic). Compared with sedentary living and especially with living and training in normal (normoxic) conditions, the combination of hypoxic living and hypoxic training seemed to exert a synergistic effect on the expression levels of several genes associated with exercise-related phenotypes. Hence, a significant interaction effect of treatment and treatment duration was observed on transcript levels of genes with a known role in oxygen sensing (*HIF-1α*, *VEGF*, *Mb*), mitochondrial biogenesis (*NRF1*) and function (*CS*), pH regulation (*CA3*, *MCT1*), and antioxidant defense (*Cu/ZnSOD*, *GSTPi*, and *MnSOD*). Gene expression was overall highest when the animals trained and lived in conditions of hypoxia (usually after 3 wk). Normoxic training did not elicit a greater response compared with sedentary living under hypoxia. In effect, we noted a trend toward higher expression levels of most genes (except those involved in glucose metabolism) in conditions of hypoxic sedentary living compared with normoxic training. The lack of statistical power could be attributed to the low number of animals per group masking a potential significant difference between the two treatments. Interestingly, gene expression was usually highest after 3 wk of hypoxic living combined with hypoxic training (i.e., TH3 group), which suggests certain downregulation of gene expression after longer periods of hypoxia.

HIF-1α is a transcription factor that plays a key role in mediating several muscle adaptations to hypoxia and exercise (13). Thus, its higher expression detected in our study in response to hypoxic living plus hypoxic training is consistent with previous data (27,31) and possibly reflects downstream activation of HIF-1α–dependent pathways, leading to several possible muscle adaptations such as increased angiogenesis, increased glycolytic flux, and improved potential for pH regulation (6,14,31). Indeed, a similar behavior to *HIF-1α* mRNA expression levels, that is, the highest levels for hypoxic living (SH3) or hypoxic living and training for 3 wk (TH3), was detected here for mRNA levels corresponding to several genes involved in angiogenesis (*VEGF*), glucose metabolism (*GLUT-4* and *PFKm*), and pH regulation (*CA3*, *MCT1*). As observed for *HIF-1α* and *VEGF*, the highest *Mb* transcript levels were recorded for the treatment 3 wk of hypoxic living and hypoxic training (TH3). This finding is in line with the results of studies indicating that increased *Mb* expression is an adaptive response to hypoxia (9,24), although this response might not be related to HIF-1α–dependent pathways (9).

Overall, the combined effect of hypoxic living and hypoxic training led to the highest expression levels of the genes involved in mitochondrial biogenesis (*PPARGC1a*, *NRF1*) and function (*COX-1* and CS). This is in agreement with the findings of a recent study by Schmutz et al. (21), who reported that in previously untrained men, endurance training (cycling) in conditions of hypoxia (12% O_2) gave rise to a greater increase in subsarcolemmal mitochondrial density in the vastus lateralis muscle compared with the same intervention in normoxic conditions. In their study, such ultrastructural adaptations to hypoxia were accompanied by specific adjustments in the muscle transcriptome, i.e., in genes involved in energy provision in mitochondria (21). In addition, many postexercise transcript level alterations observed by Schmutz et al. were tightly correlated with the level of muscle oxygenation, and the majority of the observed gene messages were targets of *HIF-1α*, consistent with the significant interaction effect on this gene observed here. The *NRF1* gene plays a role in the transcriptional control of many genes involved in mitochondrial function and biogenesis (10), whereas the *PPARGC1A* gene is one of the main regulators of mitochondrial biogenesis, i.e., it is a coactivator of the subset of oxidative phosphorylation genes that control lipid transportation and oxidation (23,26). Recent research has also identified a role of PPARGC1A as a key component of the oxygen response system (22). The expression of *PPARGC1A* is induced by a lack of nutrients and oxygen. Besides its function in mitochondrial biogenesis, *PPARGC1A* regulates *VEGF* expression and angiogenesis in cultured muscle cells and skeletal muscle *in vivo*, enhancing oxygen and nutrient delivery (1). The COX-1 protein is encoded by mitochondrial DNA, and its transcript levels have been shown to increase after hypoxic training in humans (31). Transcript levels of CS, a key regulatory enzyme of the Krebs cycle, also increase with hypoxic training (31). Thus, overall, our findings point to a major role of hypoxic exposure (especially when combined with hypoxic training) in upregulating genes involved in mitochondrial function. This observation has important implications because given their energy production and substrate metabolism regulation functions, mitochondria play a key role in the pathophysiology of obesity and insulin resistance, as well as in mechanisms that affect adipogenesis and mature adipocyte function (3). Nevertheless, in our obese rat model, it is likely that higher hypoxia levels (i.e., above 13.6% O_2 or an altitude of 3500 m) are needed to induce the metabolic adaptations that lead to significant fat loss.

In our diet-induced obese rat model, the combination hypoxic living/hypoxic training resulted in higher mRNA levels for MCT1, a protein needed for effective pH regulation

during exercise. This finding is in agreement with the findings of a human study suggesting that transient (31) or permanent hypoxia exposure modifies skeletal muscle acid–base control through an increased capacity for lactate, HCO_3^-, and H^+ fluxes from muscle to blood (31). On the other hand, our detection of an effect of hypoxic living and training on *CA3* mRNA is inconsistent with the results of a previous human study (8).

Our observation that hypoxic living + training for ≥2 wk led to the highest expression levels of genes encoding antioxidant enzymes (*Cu/ZnSOD*, *GSTPi*, and *MnSOD*) is consistent with reports of human studies (30). Indeed, compared with normoxia, hypoxia seems to induce an additional increase in the reactive oxygen species generated by mitochondria (4,30). Thus, a compensating increase in antioxidant defense would be expected, as confirmed in humans training in hypoxic conditions (31). For instance, Juel et al. (8) noted a trend toward elevated muscle Cu/ZnSOD levels in response to 6 wk of hypoxic training (14.5% O_2).

Our study is not without limitations. Thus, ideally, we should have included a group of sedentary rats living under normoxic conditions for 1 to 4 wk. This would have given a more complete picture of how hypoxic living or training and these two factors combined drive muscle gene adaptations that could be associated with a healthier metabolic and body composition phenotype, compared with normoxic conditions. In addition, no endogenous gene was used to generate control data because the housekeeping genes traditionally used in muscle gene expression studies (notably, glyceraldehyde 3-phosphate dehydrogenase) are not hypoxia stable (5). In effect, glyceraldehyde 3-phosphate dehydrogenase mRNA levels were found to similarly increase in response to hypoxia exposure ($P < 0.001$ for treatment effect) to mRNA levels of the genes examined here (data not shown). Our study also failed to examine the effects of treatment on other important genes such as those involved in lipid metabolism. Further, despite the significant molecular adaptations induced by hypoxic living and particularly by the combination hypoxic living/hypoxic training, none of these interventions was able to induce significant benefits in terms of body composition. Keeping in mind the aforementioned limitations, our data do nevertheless reveal that hypoxic living, especially if combined with hypoxic exercise training, can lead to health-related adaptations at the skeletal muscle level, notably in mitochondrial function and biogenesis, and thus produce a shift toward a "healthier" muscle transcriptome profile. In obese subjects, such adaptations could be of potential interest, for example, to reduce the risk of other diseases (notably cardiovascular disorders) associated with obesity. Effectively, in the classic studies in rats conducted by the group of Koch and Britton (11), the improvement of oxidative pathways in mitochondria was identified as a common factor linking physical fitness and a reduced risk of cardiovascular and metabolic diseases (29). In future work, it needs to be determined if, besides the beneficial molecular skeletal muscle adaptations observed here, more intense hypoxic stimuli (>13.6% O_2 or >3500-m altitude) will also promote fat loss in obese populations. Finally, apart from an overall large sample size (n = 130 rats) and a large number of treatment groups, an additional strength of our study was that exercise intensity (i.e., treadmill speed) was well controlled through blood lactate levels. Recent research has indeed shown that lactate levels (a marker of glycolytic stress) correlate with hypoxia-modulated patterns of transcript alterations after endurance exercise (21).

In summary, compared with both normoxic training and sedentary living under hypoxia (especially with the former), the combination of hypoxic living plus hypoxic training seems to exert a major effect on the expression in skeletal muscle of several genes involved in oxygen sensing, mitochondrial biogenesis and function, and pH regulation. The results of our study provide insight into the molecular mechanisms underlying the health benefits of hypoxic living and training in obese subjects and provide direction for future studies.

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