

Effect of Prolonged Intermittent Hypoxia and Exercise Training on Glucose Tolerance and Muscle GLUT4 Protein Expression in Rats

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Key Words

Body mass · Diabetes · Glycogen · Insulin resistance · Skeletal muscle

Abstract

We compared the chronic effect of intermittent hypoxia and endurance training on the glucose tolerance and GLUT4 protein expression in rat skeletal muscle. Thirty-two Sprague-Dawley rats were matched for weight and assigned to one of the following four groups: control, endurance training, hypoxia, or hypoxia followed by endurance training. Hypoxic treatment consisted of breathing 14% O₂ for 12 h/day under normobaric conditions, and the training protocol consisted of making animals swim 2 times for 3 h/day. At the end of the 3rd week, an oral glucose tolerance test (OGTT) was performed 16 h after treatments. At the end of the 4th week, GLUT4 protein, mRNA, and glycogen storage in skeletal muscle were determined. Endurance training significantly improved OGTT results. Glycogen content and GLUT4 protein expression in the plantaris and red gastrocnemius, but not in the soleus or white gastrocnemius muscles, were also elevated. Chronic intermittent hypoxia also improved OGTT results, but did not alter GLUT4 protein expression. Additionally, hypoxia followed by

exercise training produced significant increases in GLUT4 protein and mRNA in a greater number of muscles compared to endurance training alone. Both exercise training and hypoxia significantly reduced body mass, and an additive effect of both treatments was found. In conclusion, chronic intermittent hypoxia improved glucose tolerance in the absence of increased GLUT4 protein expression. This treatment facilitated the exercise training effect on muscle GLUT4 expression and glycogen storage. These new findings open the possibility of utilizing intermittent hypoxia, with or without exercise training, for the prevention and clinical treatment of type 2 diabetes or insulin resistance.

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Introduction

Since glucose is a hydrophilic molecule, a transmembrane protein is required to facilitate its movement across the plasma membrane. These transmembrane proteins belong to a family of proteins (termed GLUT) that have different tissue distributions and different amino acid sequences [18]. GLUT4 is the main glucose transporter isoform expressed in skeletal muscle. This protein can be rapidly recruited to the plasma membrane upon insulin

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stimulation, where it increases the permeability of the plasma membrane to glucose. Under postprandial conditions, skeletal muscle becomes the main site for glucose disposal, and hence plays a pivotal role in regulating whole-body glucose homeostasis [8]. Several early studies indicated that the amount of GLUT4 protein expressed is strongly correlated with maximal insulin-stimulated glucose transport in skeletal muscle [17, 19, 23]. Therefore, it was suggested that interventions for enhancing muscle GLUT4 protein expression might be a possible method for treating patients with type 2 diabetes [32, 35].

Increasing oxygen demand by muscle contraction and reducing oxygen delivery by acute hypoxia are both known to accelerate the rate of muscle glucose transport, a process mediated by the rapid recruitment of intracellular GLUT4 protein to plasma membranes [26, 28, 31, 35]. In a chronic state, regular exercise training was found to elevate muscle GLUT4 protein expression, which in turn increases muscle glycogen storage and improves glucose tolerance [17, 23]. In a similar manner, Dill et al. [11] showed that prolonged continuous hypoxic treatment can temporarily activate muscle GLUT4 protein expression, suggesting that long-term hypoxia may influence whole-body glucose metabolism. Most studies examining exercise training were performed with an intermittent mode, in which recovery might have been important for the development of an adaptation against the recurrent metabolic stress. We previously found that GLUT4 protein levels increased to a greater extent when following exercise the muscle recovered for 5–16 h compared to the level immediately after exercise [20]. Therefore, we hypothesized that long-term intermittent hypoxia might, to some extent, simulate the exercise training effect on glucose metabolism. In the current study, we determined the effect of prolonged intermittent hypoxia on oral glucose tolerance as well as observed its link to GLUT4 protein expression. In order to compare differences between exercise and hypoxia adaptation on glucose metabolism, we also studied the independent and interactive effects of exercise training and prolonged intermittent hypoxia on glucose tolerance, muscle GLUT4 protein expression, and glycogen storage.

Methods

Animal Care and Housing

Male Sprague-Dawley rats with a mean body weight of 120 ± 3.5 g were obtained from the Animal Research Laboratory of the National Science Council (Taipei, Taiwan, ROC). The rats were transferred to the Animal Care Facility of the Taipei Physical Educa-

tion College and housed for 1 week to allow them to acclimatize to the new environment. All procedures were approved by the Animal Care and Use Committee of the Taipei Physical Education College, and conformed to the *Guidelines for the Use of Research Animals* published by the Council of Agriculture, Executive Yuan, Taiwan. Rats were maintained on normal rat chow (PMI Nutrition International, Brentwood, Mo., USA) and provided water ad libitum throughout the entire experimental period. The temperature of the animal room was maintained at a constant $21 \pm 1^\circ\text{C}$ with a 12-hour light:12-hour dark cycle.

Experimental Procedures

After allowing acclimatization to their housing, rats were matched for weight and assigned to one of the following four treatment groups: control (C, $n = 8$), endurance training (T, $n = 8$), hypoxia (H, $n = 8$), and hypoxia followed by endurance training (H+T, $n = 8$). Both exercise training and hypoxia were performed 7 days/week for 4 weeks. The hypoxic treatment lasted 12 h/day with food and water remaining accessible. Hypoxia was generated by placing rats in a 4-room isobaric chamber (L = 56 cm; W = 43 cm; H = 39 cm) with a constant fraction of inspired oxygen of 14%. In detail, pure nitrogen gas was used to dilute ambient air in a small gas-mixing chamber. This gas-mixing chamber was connected to the isobaric chamber. An equilibrium state of the fraction of inspired oxygen of 14% was generated inside the chamber throughout the 12-hour period, and the oxygen concentration was monitored with an alarm oxygen sensor (Hypoxico, Cardiff, Calif., USA). In the H+T group, exercise was always elicited 4 h after hypoxia. The exercise protocol of a previous procedure was used [20]. In the 1st week, the exercise protocol consisted of two intervals of 45 min of swimming with 45 min of rest. The swimming duration was gradually extended to two intervals of 3 h of swimming each with a 45-min rest in the 3rd and 4th weeks. At the end of the last bout of swimming, rats with or without hypoxia were allowed to recover for 16 h before tissue collection. All rats were provided glucose (1 g/kg body weight) orally with a stomach tube during the 16-hour recovery after the T and H+T groups had completed their last training bout and the H group had completed its last hypoxic treatment. Food and water were accessible during this recovery period. Hind limb muscles from these four groups were then excised for tissue analysis.

Oral Glucose Tolerance Test and Insulin Response

At the end of the 3rd week of treatment, an oral glucose tolerance test (OGTT) was performed on all animals 16 h after the T and T+H groups had completed their training bout and the H group had completed its last hypoxic treatment. All animals were fasted overnight (12 h) prior to glucose intubation. The 50% glucose (w/v) solution was orally delivered with a stomach tube to rats during the OGTT. Differences in body mass will result in differences in gut mass and muscle mass for glucose absorption as well as differences in metabolic rate, which will affect glucose clearance. Therefore, we provided ingested glucose based on individual body weight (1 g/kg body weight) to remove possible variations caused by different body masses. Blood samples were taken from the tail 0 (fasted sample), 15, and 45 min after the oral glucose load for blood glucose and insulin measurements. A glucose analyzer (LifeScan, Milpitas, Calif., USA) was used for glucose concentration determination. The insulin level was measured by an enzyme-linked immunosorbent assay (ELISA) using an anti-insulin monoclonal antibody. The serum sample was quantified on an ELISA analyzer (Tecan Genios, Salzburg, Austria)

with the use of commercially available ELISA kits (Diagnostic Systems Laboratories, Webster, Tex, USA) according to the manufacturer's procedures.

Muscle Glycogen

In the 4th week, muscle glycogen contents of the soleus, red quadriceps, plantaris, red gastrocnemius, and white gastrocnemius muscles were determined. Muscle glycogen was broken down through enzymatic degradation by amyloglucosidase. A piece of muscle tissue (50–100 mg) was dissolved in 1 ml 1 N KOH at 70 °C for 20 min, mixed, and then incubated for an additional 10 min at 70 °C. An equal volume of 1 N HCl was added to the digested samples and mixed, and aliquots of the neutralized digest were transferred and incubated overnight in 0.3 mol/l sodium acetate buffer, pH 4.8, containing 5 mg/ml amyloglucosidase (Roche Applied Science, Indianapolis, Ind., USA). Glycogen was expressed as glucose units determined by the spectrophotometric Trender reaction (315 Sigma, St. Louis, Mo., USA).

Western Blotting Analysis for GLUT4

About 75 mg of skeletal muscle from the soleus, red quadriceps, plantaris, red gastrocnemius, and white gastrocnemius were homogenized (1:20) in 20 mM ice-cold HEPES, 1 mM EDTA, and 250 mM sucrose buffer (HES buffer, pH 7.4) with a Polytron (Brinkmann Instrument, Littau, Switzerland). The protein concentration of the homogenate was determined using a BioRad protein assay reagent (Richmond, Calif., USA), according to the manufacturer's instructions. Sample homogenates and standards were diluted 1:1 with Laemmli sample buffer (125 mM Tris, 20% glycerol, 2% SDS, 0.008% bromophenol blue, pH 6.8). Seventy-five micrograms of protein from sample homogenates was transferred to a polyvinylidene fluoride membrane as previously described [20]. GLUT4 antiserum directed against the carboxyl terminus was used for immunoblotting in a dilution of 1:5,000 (Chemicon, Temecula, Calif., USA). GLUT4 protein was visualized using an ECL Western Blot Detection Kit containing a secondary antibody against rabbit antibody (Amersham, Arlington Heights, Ill., USA) on Kodak film according to the manufacturer's instructions.

Northern Blotting Analysis for GLUT4 mRNA

The Northern blotting procedure was used to determine GLUT4 mRNA levels according to a method previously described [20]. For RNA extraction, red quadriceps, plantaris, and red gastrocnemius muscles were homogenized in guanidium isothiocyanate- β -mercaptoethanol buffer with a Polytron. Total RNA was purified by a phenol-chloroform extraction method, and the RNA in the aqueous layer was then precipitated in two steps using isopropanol and ethanol. Total RNA (30 μ g) isolated from each tissue sample was denatured by heating at 60 °C for 10 min, then separated on a 1% agarose-formaldehyde gel, and subjected to vacuum transfer onto a nylon membrane. Hybridization with GLUT4 cRNA was performed according to the manufacturer's instructions. A GLUT4 cDNA template was used to generate a fluorescent antisense GLUT4 probe using the Northern Start Kit (Roche Applied Science) for the hybridization procedure. 28 S rRNA on the striped blot of the ethidium-bromide-stained gel was used for determining both the integrity and equal loading of total RNA on the gel. β -Actin mRNA was also determined by hybridization with the striped blots using pre-labeled β -actin cRNA provided with the Northern start kits. β -Actin mRNA was not affected by the experimental treatments (data not shown).

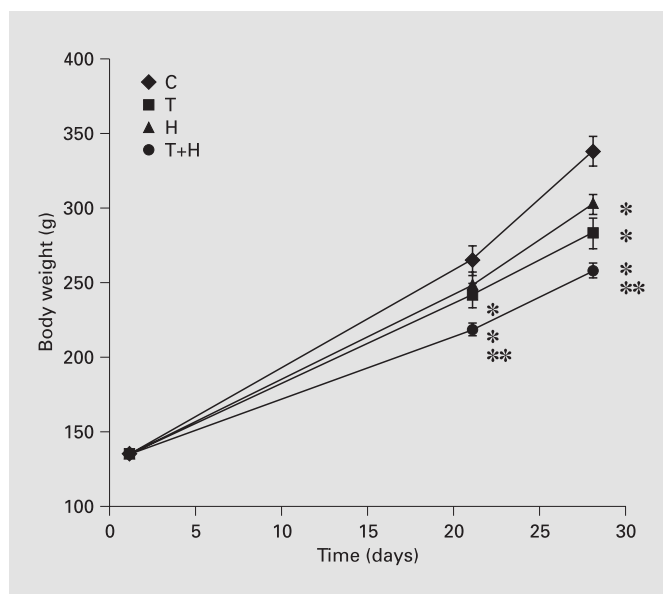


Fig. 1. Body weight: rats with either exercise training or prolonged intermittent hypoxic treatments exhibited lower body weights than the C group. An additive effect of exercise training and hypoxia on body weight was found. * $p < 0.05$ vs. the C group; ** $p < 0.05$ vs. the T group.

Autoradiographs of GLUT4 protein and GLUT4 mRNA were quantitated by a densitometric method with NIH imaging software (National Institutes of Health, Bethesda, Md., USA), according to the software instructions.

Statistical Analysis

A two-way ANOVA among the experimental groups was performed on all variables tested. Fisher's protected least significance test, which holds the value of a type I error constant for each test, was then utilized to distinguish significant differences between pairs of groups. A level of $p < 0.05$ was set for significance for all tests. Data are presented as means \pm SE.

Results

Body Weight

The initial mean body weights of the rats in the four groups before treatment did not differ (fig. 1). At the end of the 4-week treatment, the body weight of the H group was significantly lower than that of the C group ($p < 0.05$). Rats in the T group also exhibited lower body weight than that in the C group ($p < 0.05$). Endurance training and hypoxia had an additive effect on the mean body weight. The mean body weight of the H+T group was significantly lower than those of the H and T groups (both $p < 0.05$).

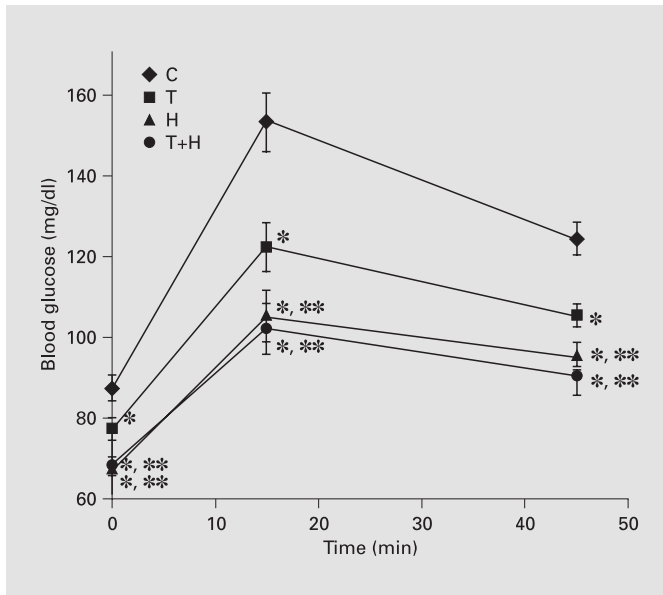


Fig. 2. Blood glucose concentrations during the OGTT. Both exercise training and prolonged intermittent hypoxic treatments improved glucose tolerance. The improvement by hypoxia treatment (with or without exercise training) was significantly greater than that with exercise training alone. * $p < 0.05$ vs. the C group; ** $p < 0.05$ vs. the T group.

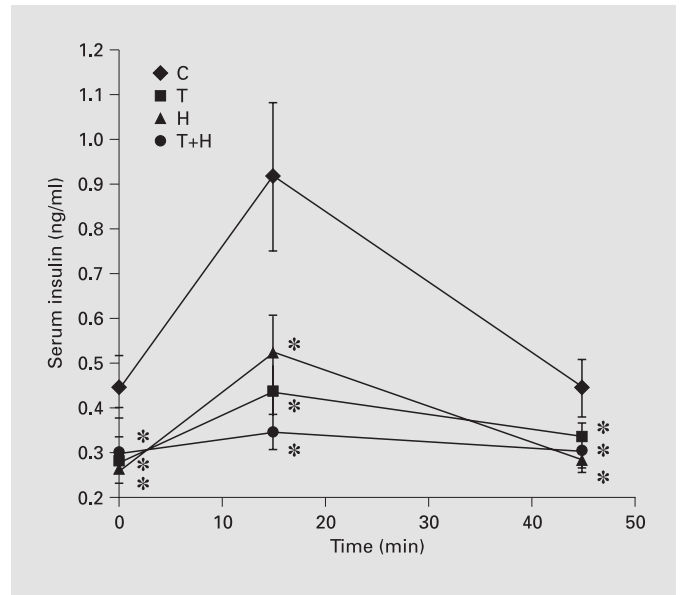


Fig. 3. Serum insulin concentration during the OGTT. Rats in the T, H and T+H groups exhibited lower insulin levels than the C rats, indicating better insulin sensitivity with these treatments. No significant differences in insulin levels were found among the T, H and H+T groups. * $p < 0.05$ vs. the C group.

OGTT and Insulin Response

Concentrations of glucose and insulin during the OGTT are shown in figures 2 and 3. Hypoxia and endurance training significantly reduced the fasting glucose level. Fasting glucose levels in the T, H, and T+H groups were significantly lower than that in the C group ($p < 0.05$). The fasting glucose level in the H group was significantly lower than that in the T group ($p < 0.05$). Glucose levels at 15 min in the T, H, and T+H groups were significantly lower than that in the C group ($p < 0.05$). In the H and T+H groups, the 15-min glucose values were also significantly lower than that in the T group ($p < 0.05$). The 45-min glucose levels in the T, H, and T+H groups were significantly lower than that in the C group ($p < 0.05$). Additionally, the 45-min glucose levels in the H and T+H groups were significantly lower than that in the T group ($p < 0.05$), which indicates that prolonged intermittent hypoxia had a greater effect on glucose tolerance than did endurance training.

The fasting insulin levels (at time 0) in the T, H, and T+H groups were significantly lower than that in the C group ($p < 0.05$; fig. 3). No significant differences in insulin were observed among the T, H, and H+T groups at 15 or 45 min during the OGTT.

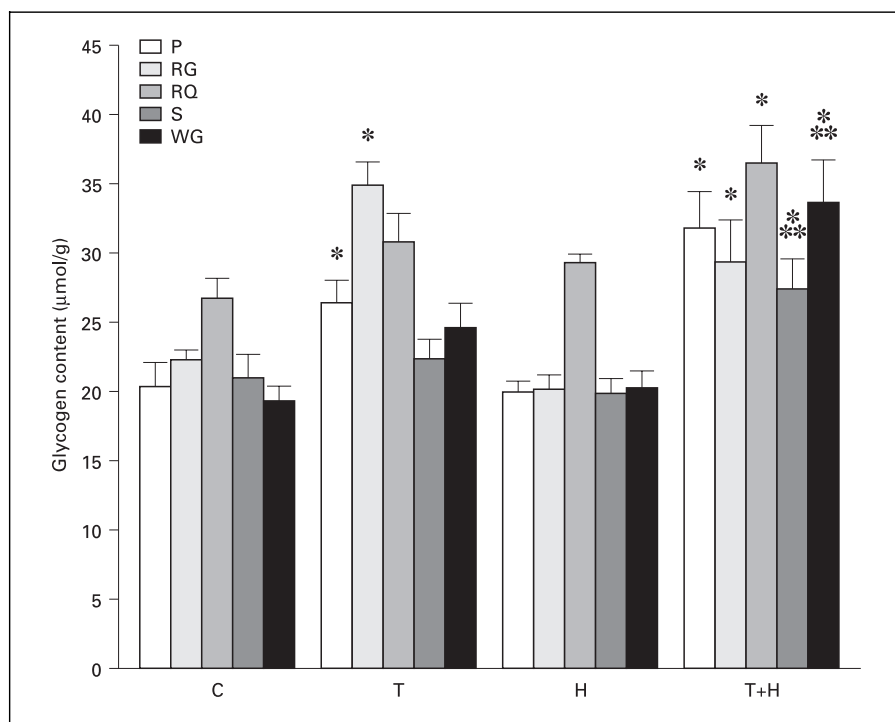
Glycogen Storage

Muscle glycogen storage was measured 16 h after glucose intubation (which occurred immediately after treatments), and the results are shown in figure 4. Hypoxia did not affect glycogen storage in the soleus, red quadriceps, plantaris, red gastrocnemius, or white gastrocnemius muscles. Glycogen storage in the plantaris and red gastrocnemius muscles of the T group was significantly greater than that of the C group. Hypoxia followed by endurance training increased muscle glycogen levels in all five muscles measured. In addition, the glycogen levels of the soleus and white gastrocnemius of the T+H group were significantly greater than that of the T group ($p < 0.05$), suggesting that hypoxia had an additive effect on endurance-induced glycogen supercompensation.

GLUT4 Protein

The muscle GLUT4 protein level was measured 16 h after glucose intubation (fig. 5). The GLUT4 protein level is presented as the percentage of the GLUT4 protein in the same muscle from control rats. Hypoxia did not significantly affect the GLUT4 protein expression in any of the muscles studied. Endurance training significantly increased GLUT4 protein expression in the red quadriceps,

Fig. 4. Glycogen concentrations in hind limb skeletal muscles. Exercise training increased glycogen storage (glycogen supercompensation) in the plantaris (P) and red gastrocnemius muscles (RG). Prolonged intermittent hypoxia enhanced an exercise training effect, as shown by the greater portion of muscles exhibiting glycogen supercompensation. * $p < 0.05$ vs. the C group; ** $p < 0.05$ vs. the T group. RQ = Red quadriceps; S = soleus; WG = white gastrocnemius.



plantaris, and red gastrocnemius when compared to the C group ($p < 0.05$). Hypoxia enhanced the endurance training effect on GLUT4 protein expression. In all muscles except the soleus, GLUT4 protein expression in the T+H group was greater than that of the C group. In addition, the plantaris, red quadriceps, and white gastrocnemius muscles of the T+H group had GLUT4 protein levels greater than those of the T group ($p < 0.05$), indicating that exercise-induced GLUT4 expression was enhanced by hypoxia.

GLUT4 mRNA

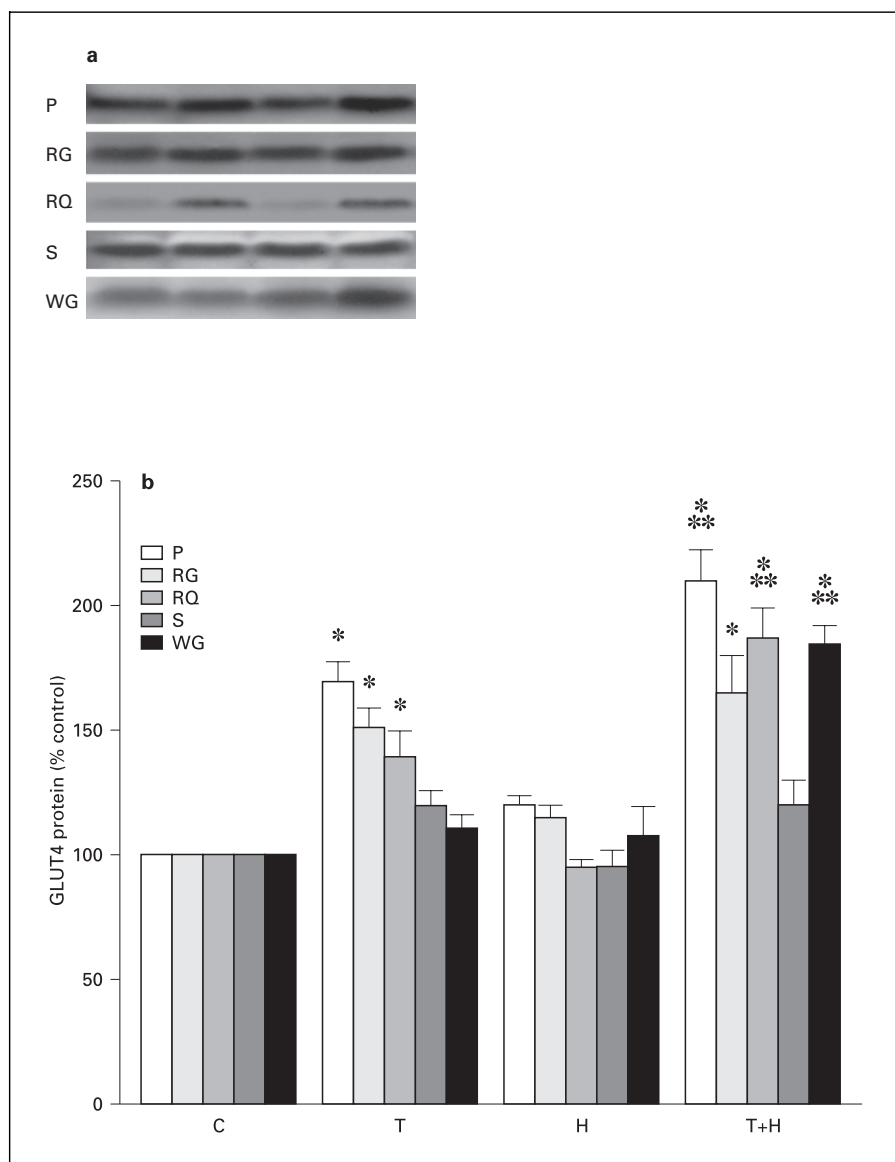
The muscle GLUT4 mRNA level, expressed as a percent of the control, was measured 16 h after glucose intubation (fig. 6). In response to endurance training and hypoxia, the results for GLUT4 mRNA were similar to those of GLUT4 protein expression. Endurance training significantly elevated GLUT4 mRNA in red gastrocnemius and red quadriceps muscles above control levels ($p < 0.05$). Hypoxia treatment did not significantly change GLUT4 mRNA in any muscle measured. Hypoxia treatment further enhanced the exercise training effect on GLUT4 mRNA. The GLUT4 mRNA levels of the red quadriceps, plantaris, and red gastrocnemius muscles of the T+H group were significantly greater than respective values of the T group ($p < 0.05$).

Discussion

To sustain life under lower arterial PO_2 , the reliance on carbohydrate substrates must be increased to compensate for energy deficits due to insufficient fatty acid oxidation. Lowered pO_2 in skeletal muscle may be elicited during intense muscular contraction or acute hypoxia. Similar to the effect of exercise training, Dill et al. [11] found that prolonged continuous hypoxia can regulate the level of GLUT4 protein expression in skeletal muscle. Since muscle tissue is the main site in the entire body for glucose disposal, their finding implies that long-term hypoxic treatment might be able to influence the whole-body glucose tolerance. In the current study, we subjected rats to prolonged but intermittent hypoxia and found that this protocol significantly lowered the fasting glucose level and improved glucose tolerance, supporting our hypothesis. In addition, insulin levels during the OGTT were also lowered in both the H and H+T rats, which indicates that the observed improvement in glucose tolerance by intermittent hypoxia is due to increased insulin sensitivity. However, unlike exercise training, the hypoxia-induced improvement was apparently independent of muscle GLUT4 protein levels.

The observed improvement in glucose tolerance by hypoxia appears to be partly related to a reduction in body

Fig. 5. GLUT4 protein levels in hind limb skeletal muscles (**b**). Representative autoradiographs of the Western blots for each muscle tissue are illustrated in **a**. Exercise training significantly elevated GLUT4 protein levels in the plantaris (P), red gastrocnemius (RG) and red quadriceps muscles (RQ). Prolonged intermittent hypoxia enhanced the exercise training effects. * $p < 0.05$ vs. the C group; ** $p < 0.05$ vs. the T group. S = Soleus muscle; WG = white quadriceps muscle.



weight (fig. 1). Similar to the observation by Xia et al. [36] with continuous hypoxia (9% O₂ for 1 month), we found that the prolonged intermittent hypoxia protocol significantly lowered the body weight of animals. Numerous data in the past have shown that body weight is highly correlated with insulin sensitivity in humans and animals [5, 6, 13, 17]. Evidence from Fushiki et al. [15] indicated that adipose tissue is particularly responsive to prolonged hypoxia compared with muscle tissue. A recent study on developing adipocytes further demonstrated that prolonged hypoxia significantly inhibited adipogenesis [38]. Therefore, the anti-adipogenic effect of hypoxia can be a possible contribution to the improved glucose tolerance

and insulin sensitivity without producing an increase in muscle GLUT4 protein. As we have frequently seen in obese animals, expression levels do not differ from those of lean rats, but less GLUT4 is translocated to plasma membranes under insulin-stimulated conditions [31].

Although the causal link between body weight (or fatness) and insulin sensitivity has been established [5], the study by Fushiki et al. [15] was unable to show an improvement in insulin sensitivity. One major difference between their study and the current one is that we performed the OGTT 16 h after hypoxic treatment. Hypoxia is known to be a metabolic stress which can induce many catabolic endocrine responses, that may in turn cause

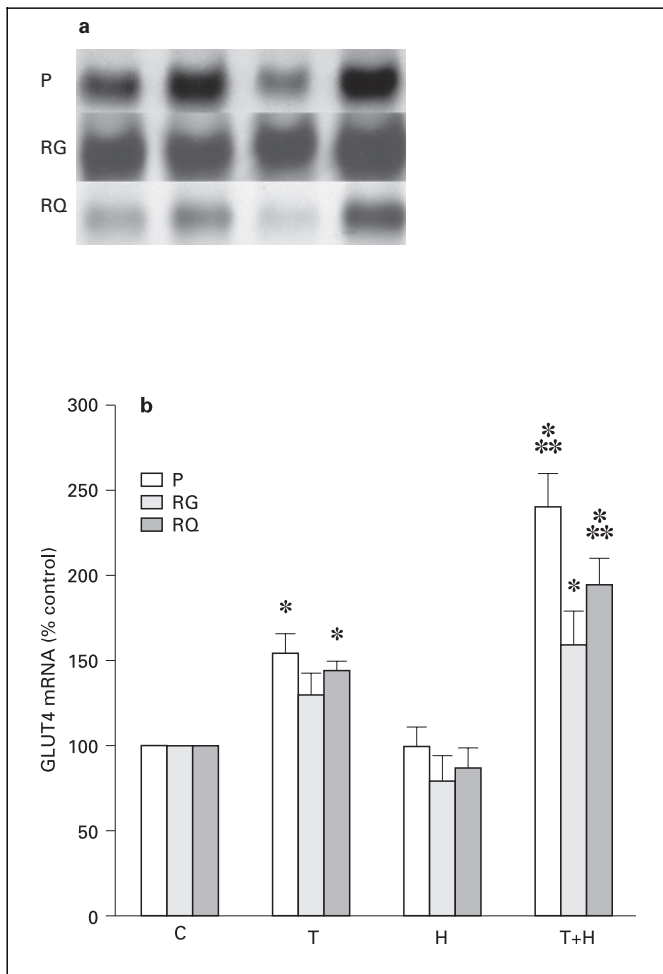


Fig. 6. GLUT4 mRNA levels in hind limb skeletal muscles (**b**). Representative autoradiographs of Northern blots for each muscle tissue are illustrated in **a**. Exercise training significantly elevated GLUT4 mRNA levels in the plantaris (P), red gastrocnemius (RG) and red quadriceps muscles (RQ). Prolonged intermittent hypoxia enhanced the exercise training effects. * $p < 0.05$ vs. C group; ** $p < 0.05$ vs. the T group.

acute elevations in fasting glucose levels and attenuation in insulin sensitivity [22]. Although the expected response was likely attenuated as acclimatization gradually occurred, removal of the hypoxic stimulation during recovery may thus have confounded their observations on insulin sensitivity. Additionally, intermittent hypoxia differs from continuous hypoxia in the sense that the recovery period for adaptation to intermittent hypoxia is probably more sufficient than that to continuous hypoxia. Thus, we speculated that both the recovery time after hypoxic treatment and the mode of the hypoxic regimen may confound observations of insulin sensitivity.

Previous studies have shown that prolonged continuous hypoxia can elevate muscle GLUT4 protein levels, but the present intermittent hypoxia protocol failed to demonstrate the same result [11, 36]. This result is no surprise as it has previously been reported that the hypoxia-induced increase in GLUT4 protein gradually diminishes as the hypoxic exposure is prolonged [11]. Presumably, this fall-back phenomenon is due to some other newly established adaptation relevant to glucose uptake. It has been reported that chronic hypoxia can result in multiple physiological adaptations that are relevant to the regulation of insulin sensitivity independent of the overall level of GLUT4 protein. These potential adaptations include enhancement of GLUT1 expression and basal glucose disposal [7, 33, 36], an increase in angiogenesis [2–4, 10, 21, 29, 30, 34], a redistribution of the subcellular GLUT4 protein [6, 31] and modulation of insulin receptor density [11–13]. In particular, the well-recognized angiogenesis response with hypoxia can presumably enhance deliveries of substrate, insulin, and other relevant signals for training adaptation of muscle tissues. In the present study, intermittent hypoxic treatment was found to enhance exercise-training-induced increases in glycogen storage and GLUT4 protein expression in skeletal muscle (figs. 4–6). The exercise training response was capable of amplification due to the greater signal delivery to better-vascularized tissue, and thus training adaptation was enhanced.

The fact that only exercise-trained muscle exhibited higher glycogen storage above the control level (fig. 3), but not hypoxic muscles, implies the importance of muscle fiber recruitment for stimulating muscle GLUT4 protein expression. The rate of glycogen storage of muscles is usually related to the level of GLUT4 protein expression [32]. In the present study, hypoxic treatment alone did not elevate muscle GLUT4 protein levels, as did exercise training. It is generally known that neuromuscular activity during exercise not only recruits muscle fibers, but also produces neurotrophic action for regulating protein expression in the recruited muscles [9, 16]. Apparently, such neuromuscular activity is minimal during hypoxia. A previous study suggested that neurogenic factors are involved in the regulation of muscle GLUT4 expression at the pre-translational level [24]. Denervation can cause a decline in muscle GLUT4 protein expression [27], whereas chronic electrical stimulation of skeletal muscle through the sciatic nerve can increase GLUT4 protein expression [37]. Thus, a lack of neuromuscular recruitment during hypoxia can, in part, explain the higher GLUT4 expression and glycogen storage induced by exercise training but not by intermittent hypoxia.

In the present study, the lowering effect of hypoxic treatment on the basal glucose level could have also partly contributed to the observed improvement in glucose tolerance. According to the OGTT curve, the mean difference in blood glucose levels after the oral glucose load approximated the difference in the fasting glucose level between the hypoxic and non-hypoxic groups. A previous study showed that chronic continuous hypoxia for more than 1 week does not seem to affect hepatic glucose output [22]. Therefore, the uptake of glucose by other peripheral tissues should largely dictate glucose homeostasis. Numerous studies have documented how prolonged hypoxia can significantly enhance basal glucose transport and GLUT1 protein expression in several tissues of the body [7, 33, 36]. Such an adaptation should help lower fasting glucose levels by enhancing the basal glucose uptake of tissues, and thus should account for part of the improvement in the whole-body glucose tolerance.

The current results on improved glucose tolerance and insulin sensitivity imply the possibility of utilizing non-pharmacological interventions, with either chronic intermittent hypoxia or a combination of endurance training and hypoxia for the prevention and treatment of type 2 diabetes or insulin resistance. These novel findings are also important in the sense that insulin sensitivity to glucose disposal in adults has been considered to be a common origin for several age-associated diseases [14]. This is supported by a longitudinal study which revealed that middle-aged individuals with the top 1/3 of insulin sensitivity had the lowest probability of developing age-associated diseases, including type 2 diabetes, hypertension, coronary heart disease, stroke, and cancer [14]. It is generally known that these age-associated metabolic disorders account for great proportions of the mortality and mor-

bidity in most industrialized societies. Coincidentally, an early study reported that people living in higher-altitude areas exhibit lower mortality rates from insulin-resistance-related diseases such as coronary heart disease [25] and cancer [1] compared to residents living in lower-altitude areas. Thus, the current results suggest that prolonged hypoxic exposure can be beneficial for preventing chronic metabolic disorders mediated by improvements in insulin sensitivity. In the future, identification of hypoxia-induced factors relevant to the hypoxia-induced adaptation on insulin sensitivity may be helpful for developing new pharmaceutical modalities for the prevention and treatment of type 2 diabetes, as well as those of age-associated metabolic disorders.

In conclusion, results of the current study demonstrate that prolonged intermittent hypoxia significantly improved glucose tolerance and insulin sensitivity in the absence of increased muscle GLUT4 protein expression. This is also the first study which shows that intermittent hypoxic treatment can enhance exercise-training-induced increases in GLUT4 protein expression and glycogen storage in skeletal muscle. These results suggest the possible value of using chronic intermittent hypoxia or a combination of exercise and hypoxia for prevention or correction of metabolic defects associated with insulin resistance and type 2 diabetes.

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