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Hyperinsulinemia and overweight in obese Zucker rats effectively suppressed by exercise training with hypoxia recovery

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ORIGINAL ARTICLE

Hyperinsulinemia and overweight in obese Zucker rats effectively suppressed by exercise training with hypoxia recovery

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Abstract
It is currently unknown whether hypoxia training can effectively suppress overweight and hyperinsulinemia in genetically obese animals. In this study, both lean and obese Zucker rats were randomly assigned into the following groups: control (CON, $n=7$), exercise training (EX, $n=7$), hypoxia (HYP, $n=7$) and exercise training with hypoxia recovery (EX + HYP, $n=7$). During a 6-week training period, rats performed swimming exercise progressively from 30 to 180 min·day^{-1}, and recovered under hypoxia (14% oxygen for 8 h·day^{-1}). Obese Zucker rats exhibited substantially greater fasting insulin levels, and exaggerated glucose and insulin responses following an oral glucose challenge compared with lean rats. At the beginning of week 6, body weight, fasting glucose, fasting insulin, area under curve of glucose (GAUC) and insulin (IAUC) in the EX + HYP group were significantly lower than CON group among the obese rats. Meanwhile, only GAUC was significantly lower in the EX group compared to the CON group. At the end of week 6, capillaries to fibre ratio (C/F), capillary density (CD) and type IIa fibre proportion of the plantaris muscle in the EX group were significantly greater than the CON group ($P<0.05$), but no additive effect of hypoxia on exercise training was observed. Our data demonstrate that exercise training with prolonged hypoxia recovery offers better metabolic benefits than exercise training alone for the obese Zucker rats. This advantage was closely associated with effective weight reduction.

Keywords: *Muscle fibre transformation, capillary density, insulin resistance, AMPK, PGC-1α*

Introduction

The obese Zucker rat is a widely used insulin resistance model with similar features of human insulin resistance, such as marked hyperinsulinemia and impaired glucose tolerance (Ivy, 2004; Leonard, Watson, Loomes, Phillips, & Cooper, 2005). A substantial number of studies have demonstrated that endurance training can lower the fasting blood insulin and improve glucose and insulin responses to a glucose challenge in obese Zucker rats (Ivy, 2004; Király et al., 2007). Altitude training is generally considered a more potent method to produce training effect for endurance athletes. However, exercise training under moderate hypoxia (15% O_2, 60 min a day, 3 days a week) failed to produce additional benefits in reversing the hyperinsulinemia compared to exercise training alone (Wiesner et al., 2010). This is probably related to reduced training intensity under

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the hypoxic environment. One way to circumvent this drawback would be to perform exercise training under normoxic condition but recover under moderate hypoxia. It was previously unknown whether exercise training with hypoxia recovery can produce equivalent or even better metabolic benefits than exercise training alone for genetically obese animals, given that obese rats are less responsive to some of the exercise training effects in suppressing insulin resistance (Kibenge & Chan, 2002; Király et al.2008), such as increasing capillary supply (Lash, Sherman, & Hamlin, 1989) and altering intracellular signaling system related to glucose metabolism (Lee-Young et al., in press; Sriwijitkamol et al., 2006).

Skeletal muscle is the major site for insulin-stimulated glucose disposal (DeFronzo et al., 1981; Ivy, Zderic, & Fogt, 1999). GLUT4, as an insulin-responsive glucose transporter protein expressed in skeletal muscle, functions to accelerate glucose transport across plasma membrane after a meal (Ivy & Kuo, 1998; Saengsirisuwan, Kinnick, Schmit, & Henriksen, 2001). Increasing GLUT4 protein expression in skeletal muscle has been shown to improve the whole-body glucose disposal in animals (Leturque, Loizeau, Vaulont, Salminen, & Girard, 1996; Wallberg-Henrikss & Zierath, 2001). Muscle fibre composition is one of the factors that can influence whole-body glucose homeostasis. Type IIb muscle fibres have substantially less amounts of GLUT4 protein and capillary numbers than the rest of muscle fibre types (Ivy et al.1999; Ripoll, Sillau, & Banchero, 1979; Waters, Rotevatn, Li, Annex, & Yan, 2004). It is known that both GLUT4 protein expression (Winder & Hardie, 1999) and angiogenesis (Ouchi, Shibata, & Walsh, 2005) are regulated by AMP-activated protein kinase (AMPK) in contracted skeletal muscle. Chronic activation of AMPK can elevate GLUT4 expression in skeletal muscle, suggesting that the endurance training effect is mediated by AMPK activation (Holmes, Kurth-Kraczek, & Winder, 1999; Ojuka, Nolte, & Holloszy, 2000). Furthermore, repeated AMPK activation by muscle contraction has also been proposed as an underlying mechanism for muscle fibre transformation, which is mediated by peroxisome proliferator-activated receptor-γ coactivator-1α (PGC-1α) protein (Röckl et al., 2007). PGC-1α is a transcriptional coactivator that interacts with several nuclear transcriptional factors governing genes regulating carbohydrate metabolism in skeletal muscle (Lin et al., 2002).

In lean animals, increasing AMPK expression causes muscle fibre transformation from type IIb to IIa (Röckl et al., 2007). However, the AMPK signalling system is less responsive to muscle contraction in obese Zucker rats compared with lean rats (Barnes et al., 2002; Sriwijitkamol et al., 2006). It is unknown whether exercise training with hypoxia recovery can alter the aforementioned muscle properties that are jointly affecting whole-body glucose homeostasis for genetically obese animals. In this study we determined the effectiveness of exercise training with prolonged hypoxia recovery on suppressing hyperinsulinemia and overweight in obese Zucker rats. The involvement of AMPK-PGC-1α signalling pathway and the morphology of skeletal muscle were also examined.

Methods

Animals

Male obese (*fa*/*fa*) Zucker rats and their lean (*fa*/–) littermates (aged 13–14 weeks) were housed in a temperature-controlled room ($21 \pm 2^{\circ}$C) with a 12-hour light/12-hour dark cycle. These rats were allowed free access to water and chow (PMI Nutrition International, Brentwood, MO, USA) except when indicated. All experimental 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 made by the Council of Agriculture, Executive Yuan, Taiwan (ROC).

Experimental design and procedures

After acclimatization to their housing environment, weight-matched obese Zucker rats were evenly assigned into the following groups: control (CON, $n = 7$), exercise training (EX, $n = 7$), hypoxia (HYP, $n = 7$) and exercise training with hypoxia recovery (EX + HYP, $n = 7$). The same number of lean Zucker rats for each group was used as reference for comparison against obese Zucker rats. Both exercise training and hypoxia performed 7 days/week for 6 weeks. The treatment procedure was consistent with our previous study (Chiu et al., 2004).

The exercise protocol consisted of 30 min of swimming with 2% weight load on the first day. Swimming duration was gradually increased from 30 min to 90 min for the first two weeks. From week 5, swimming duration was further extended to 180 min. The hypoxic challenge lasted 8 h a day with water remaining accessible. During hypoxia, rats were placed in a 4-room isobaric chamber (L = 56 cm; W = 43 cm; H = 39 cm) with a constant fraction of inspired oxygen of 14% by using hypoxic air generator (GAO_2 Altitude, Melbourne, Australia). The oxygen concentration in isobaric chambers was monitored with an oxygen sensor (GAO_2 Altitude) throughout the 8-hour period. In the EX + HYP group, the last bout of exercise was performed 3–4 h after the last hypoxia.

At the first day of week 6, oral glucose tolerance test (OGTT) and insulin response were measured 16 h after the last bout of swimming under overnight fasted condition. At the end of week 6, all rats were anaesthetized with an intraperitoneal injection of pentobarbital sodium (65.0 mg·kg^{-1} body wt) 16 h following glucose intubation (0.5 g·k^{-1} body wt). Food and water remained accessible during the 16 h recovery period. The plantaris muscles were freeze-clamped with tongs cooled in liquid N_2 for glycogen assay, citrate synthase activity, western blotting, and histochemistry analysis. Following muscle tissue collection, rats were euthanized by cardiac injection of pentobarbital sodium.

Oral glucose tolerance test (OGTT) and insulin response

The glucose solution (1 g·kg^{-1} body wt) was orally delivered with a stomach tube to rats during an OGTT. Blood samples were collected from the tail 0 (fasting sample), 30, 60, 90, and 120 min after an oral glucose load for glucose and insulin measurements. The trapezoidal rule was used for calculation of area under curves for glucose (GAUC) and insulin (IAUC). Glucose concentration was determined by glucose oxidase method using a glucose analyser (LifeScan, Milpitas, CA, USA). Insulin concentration was measured by an enzyme-linked immunosorbent assay (ELISA) using an anti-insulin monoclonal antibody. Samples were quantified on an ELISA analyser (Tecan Genios, Salzburg, Austria) with the use of commercially available ELISA kits (Diagnostic Systems Laboratories, Webster, Tax, USA) according to the manufacturer's procedures.

Glycogen assay

About 50 mg muscle samples were dissolved in 1N KOH at 70°C for 30 min. Dissolved homogenate was neutralized by glacial acetic acid and incubated overnight in sodium acetate buffer (0.3 M, pH 4.8) containing amyloglucosidase (Boehringer Mannheim, Indianapolis, IN, USA). The reaction mixture was neutralized with 1N NaOH, and then the samples were analysed by measuring glucosyl units by the Trinder reaction (Sigma, St. Louis, MO, USA).

Citrate synthase activity

Citrate synthase (CS) activity was assayed spectrophotometrically by the method of Srere (1969). Muscle samples were homogenized in HES buffer (20 mM ice-cold HEPES, 1 mM EDTA, and 250 mM sucrose buffer, pH 7.4, 1:20 wt·vol). A 300 μl aliquot was further diluted 1:2 with HES buffer and 1:10 in 0.1 mol·l^{-1} Tris and 0.4% Triton X-100, pH 8.1.

Histochemistry

Plantaris muscles from the left hind limbs were excised, weighed, and frozen in pre-cooled isopentane. Transverse 10 μm serial sections from the muscle mid-belly were sliced in a cryostat (Leica CM1850, Germany) at -22°C. These sections were stained for alkaline phosphatase activity to identify capillaries according to the method of Cassin, Gilbert, Bunnell, and Johnson (1971). Sections were also used for myosin ATPase staining and succinate dehydrogenase (SDH) staining, with preincubated at pH 4.55, to distinguish the three main muscle fibre types and mitochondria content (Brooke & Kaiser, 1970; Deveci, Marshall, & Egginton, 2001). In the stains for myosin ATPase activity, dark staining indicates type I (slow oxidative), light staining indicates type IIa (fast oxidative glycolytic), and intermediate staining intensity indicates type IIb (fast glycolytic) fibre types (see Figure 1). The stained muscle sections were observed under a light microscope and a digital image was captured by digital camera (Olympus BX51, Tokyo, Japan). Within each muscle section, all analyses were always performed in the same position relative to the long axis of the section boundaries. Within each circumscribed region, the relative muscle cross-sectional area occupied by each of the three main fibre types was quantified by a stereological point-counting method. Each section containing >300 fibres was counted to estimate the CD (number of capillaries/mm^2), the C/F (number of capillaries/total number of fibres), and numerical fibre type composition of each fibre type (the total number of fibres in a sample/the appropriate number of fibres). For the measurement of the average fibre cross-sectional area (FCSA) of each fibre type (fraction of the sample area occupied by each fibre type in μm^2/the appropriate number of fibres), 20–40 of each fibre type were counted from each muscle (Armstrong & Phelps, 1984; Mattson,
Miller, Poole, & Delp, 2002). All fibres (type I, IIa and IIb) were measured by NIH Image software (developed by Research Services Branch (RSB) of the National Institute of Mental Health).

Western blotting

About 50 mg skeletal muscles were homogenized (1:20 wt·vol^{-1}) in 20 mM ice-cold HEPES buffer, containing 1 mM EDTA, and 250 mM sucrose buffer (HES buffer, pH 7.4) by a Polytron (Brinkmann Instrument, Littau, Switzerland). The protein concentration of the homogenate was determined using a BioRad protein assay reagent (Richmond, CA, 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). Muscle samples from lean (four treatment groups) and obese (four treatment groups) rats were run in parallel on the same gel for comparisons. Fractionated protein from sample homogenates was transferred to a polyvinylidene fluoride (PVDF) membrane. After blocking in Tris-buffered saline with 7.5% nonfat dry milk, the membranes were incubated overnight at 4°C with the primary antibody against AMPKα, phospho-AMPK Thr^{172}, PGC-1α, β-actin and GLUT4 purchased form Cell Signaling Technology, Inc. (Temecula, CA, USA) and Santa Cruz Biotechnology, Inc. (Santa Cruz, CA, USA). Each protein value in Western blotting analysis was quantified on NIH image and expressed as percentage of lean control muscle. β-actin was used as internal standard. All muscle proteins were visualized using an ECL Western Blot Detection Kit containing a secondary antibody against rabbit antibody (Amersham, Arlington Heights, IL, USA) on Kodak film according to the manufacturer's instructions.

Statistical analysis

A two-way analysis of variance (ANOVA) among the experimental groups was performed on all variables to determine interactive effect. One-way ANOVA was used to compare the mean difference among groups. Fisher's LSD test was used for multiple comparisons. The value of a type I error to 0.05 for each test was utilized to distinguish significant differences between pairs of groups. A level of $P < 0.05$ was set for significance for all tests, and all values are expressed as means $\pm$ *s*.

Results

The effect of 6-week exercise training on the obese Zucker rats was verified by higher citrate synthase activity and glycogen supercompensation of plantaris muscle against the CON group (Table 1, $P < 0.05$). Body weight (Table 1) and area under curves of

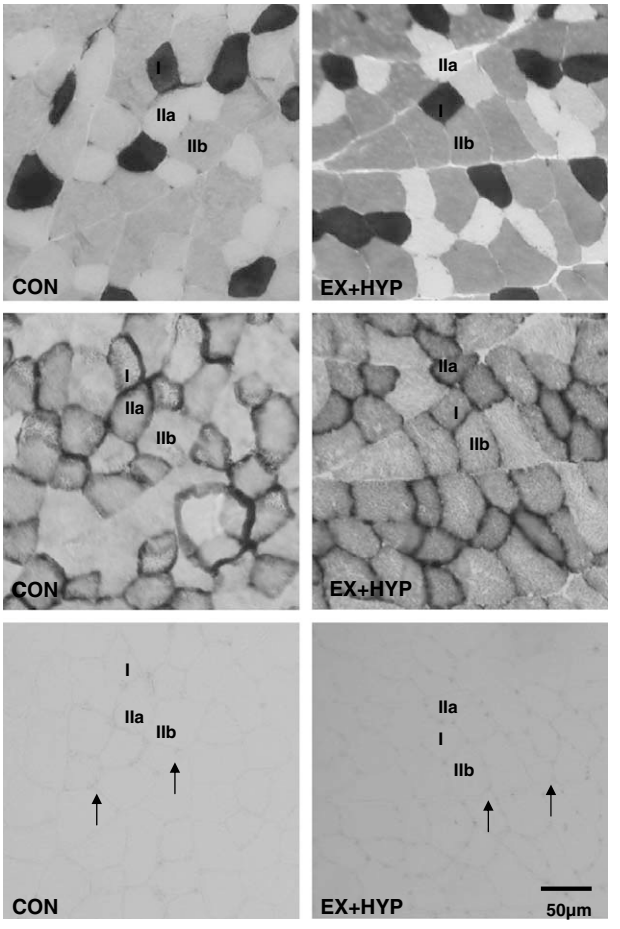

Figure 1. Muscle fibre type and capillary density in obese Zucker rats. Serial sections of plantaris muscle were stained for myofibrillar ATPase and succinate dehydrogenase (SDH) to identify fibre types and mitochondria content, and alkaline phosphatase to localize capillaries (arrows), respectively. Muscle fibres are labelled as type I, IIa and IIb.

Table 1. Body weight, muscle glycogen content, and citrate synthase (CS) activity of lean and obese Zucker rats in the control (CON), exercise training (EX), hypoxia (HYP), and exercise training with hypoxia recovery (EX+HYP) groups.

	CON	EX	HYP	EX+HYP
Body weight (g)				
Lean	318.2±13.5	306.7±19.4	306.3±17.9	274.6±19.0*
Obese	451.6±24.4†	440.1±14.5	462.3±18.4	369.8±17.3*
Muscle glycogen ($\mu mol \cdot g^{-1}$)				
Lean	37.3±1.1	43.3±1.0*	38.1±0.9	38.9±1.5
Obese	40.5±1.3	50.1±3.5*	43.3±1.6	47.8±2.2*
CS activity ($\mu mol \cdot min \cdot g^{-1}$)				
Lean	34.5±1.0	43.2±1.4*	35.7±3.9	37.2±3.8
Obese	37.8±3.2	48.4±1.6*	39.2±1.3	47.3±0.7*

* denotes significance against the CON group, $P < 0.05$; † denotes significance against the lean group, $P < 0.05$.

glucose and insulin responses after an oral glucose challenge (Figure 2A and 2B) in the obese rats were greater than the lean littermates ($P < 0.05$). Body weight, fasting glucose, fasting insulin, GAUC (Figure 2C), and IAUC (Figure 2D) in the EX + HYP group were significantly lower than those the CON group for the obese rats ($P < 0.05$). Without hypoxia exposure, only GAUC was significantly lower in the EX group compared to the CON group of the obese rats. Hypoxia treatment alone did not show any significant difference on all aforementioned variables against the CON group for the obese rats.

We found no significant differences in muscle fibre composition, cross-sectional area and capillary supply in plantaris muscle between the lean and obese rats (Table 2). For both lean and obese rats, significantly greater proportion of type IIa fibres and lower proportion of type IIb fibres were found in the EX group compared to the CON group, following 6-week treatments (Table 2, $P < 0.05$). Meanwhile, no significant muscle fibre transformation was detected in the EX + HYP group compared with the CON group for the obese Zucker rats (Table 2). Furthermore, muscle capillaries to fibre ratio (C/F) and capillary density (CD) in EX and EX + HYP groups were significantly greater than the CON group ($P < 0.05$; Table 2). Despite no significant difference in plantaris muscle weight among all groups in the obese rats, fibre cross-sectional areas of all fibre types in the HYP group was significantly lower than the CON group ($P < 0.05$; Table 3). Representative images of histochemistry stains for comparing the CON and EX + HYP groups are shown in (Figure 1).

(Figure 3) presents data from Western blot analysis for plantaris muscle after 6-week treatments. For the lean rats, GLUT4 (Figure 3A) and PGC-1α (Figure 3B) protein concentrations in the EX group were significantly greater than those in the CON group ($P < 0.05$). However, these differences were not detected in the obese rats. PGC-1α protein in the obese rats was significantly greater than in the lean littermates (Figure 3B, $P < 0.05$). For the lean rats, AMPKα protein (Figure 3C; $P < 0.05$) was significantly greater in the HYP group compared to CON group, while no change in phoshpo-AMPKα Thr^{172} (Figure 3D). However,

Table 2. Muscle fibre composition (Type I, IIa, IIb) and capillary supply of lean and obese Zucker rats in the control (CON), exercise training (EX), hypoxia (HYP), and exercise training with hypoxia recovery (EX+HYP) groups. Fibre composition was measured as numerical density (in%). C/F: Capillaries/fibre; CD: Capillary density.

	CON	EX	HYP	EX+HYP
Lean				
Type I	13.5±1.2	16.5±1.3	14.5±2.2	14.6±1.7
Type IIa	29.6±1.7	34.0±1.5*	31.0±2.3	31.6±4.3
Type IIb	56.8±1.5	49.4±1.5*	54.3±1.7	53.6±3.4
C/F	1.45±0.06	1.70±0.04*	1.55±0.05	1.61±0.04*
CD	594.6±28.8	701.6±31.8*	643.3±53.7	674.6±51.2
Obese				
Type I	14.6±2.5	14.1±1.5	16.1±1.8	14.5±1.7
Type IIa	28.9±1.7	35.6±2.0*	25.6±2.3	29.7±2.6
Type IIb	56.3±1.2	50.1±3.1	58.1±1.5	55.7±3.1
C/F	1.47±0.05	1.69±0.06*	1.43±0.04	1.60±0.07*
CD	665.3±62.2	819.0±62.9*	718.3±72.0	818.3±40.6*

* denotes significance against the CON group, $P < 0.05$.

Table 3. Fibre cross-sectional area (FCSA) and muscle weight of plantaris muscle of lean and obese Zucker rats in the control (CON), exercise training (EX), hypoxia (HYP), and exercise training with hypoxia recovery (EX+HYP) groups.

FCSA (μm^2)	CON	EX	HYP	EX+HYP
Lean				
Type I	1167.5±67.7	1282.2±96.3	1105.5±95.1	1160.1±104.4
Type IIa	1267.8±110.5	1339.7±62.6	1076.9±82.1*	1195.5±66.3
Type IIb	2304.5±89.4	2272.8±109.2	1955.3±161.2*	2165.0±78.4
Weight (mg)	253.4±10.3	245.8±19.9	227.8±18.7	236.9±20.9
Obese				
Type I	1312.1±79.9	1337.8±83.8	1069.0±44.2*	1145.5±67.7
Type IIa	1261.3±51.6	1255.7±42.6	1058.7±52.9*	1103.2±61.6
Type IIb	2072.0±124.1	2056.4±140.2	1686.2±100.8*	1964.2±128.3
Weight (mg)	208.6±19.1†	197.0±8.7	191.4±11.5	195.2±15.3

* denotes significance against the CON group, $P < 0.05$; † denotes significance against the lean group, $P < 0.05$.

in the obese rats, no significant differences in AMPKα and phoshpo-AMPKα Thr^{172} were found among the four groups (Figure 3C and 3D).

Discussion

Although altitude training is generally thought as a more potent training method than exercise training at sea-level, exercise training under hypoxia (15% O_2, 60 min a day, 3 days a week) failed to produce additional benefits in reversing the hyperinsulinemia compared to exercise training alone (Wiesner et al., 2010). This is probably related to the reduced exercise training intensity under hypoxic environment. One way to avoid this shortcoming would be to train an individual under normoxic condition but to recover under an extended period of moderate hypoxia. Such training method is effective to improve the whole-body glucose tolerance for lean individuals (Chen et al., 2010). Yet, it is unknown whether such a training method produces greater metabolic benefits for genetically obese animals than exercise training alone. In the study, we provide the evidence that exercise training with prolonged hypoxia recovery is superior in suppressing the metabolic abnormality than exercise training alone for genetically obese Zucker rats. In particular, body weight, fasting glucose, fasting insulin, GAUC and IAUC were significantly lower in the EX + HYP group than CON group. Without hypoxia, only GAUC was significantly lower in the EX group compared to the CON group.

The additional metabolic benefit of exercise training with prolonged hypoxia recovery is associated with its potent effect on reducing body weight. It is presently clear that high BMI is a major risk factor for the development of insulin resistance and type-2 diabetes (Kopelman, 2000). Overweight and obesity are characterized by elevated fasting blood insulin level and exaggerated insulin response to an oral glucose challenge (Leonard et al., 2005). Numerous intervention studies have already reported the improvement of insulin sensitivity and metabolic condition for individual with reduced body weight (Knowler et al., 2002). The result of the study in conjunction with the above evidence pointed to the possibility that effective weight-reducing by exercise training with prolonged hypoxia recovery is the main reason accounting for the finding of suppressed hyperinsulinemia and inadequate glycemic control of obese Zucker rats.

Under insulin-stimulated condition, skeletal muscle is the major tissue for glucose disposal (DeFronzo et al., 1981). However, alterations in skeletal muscle properties assessed in the study are less likely to explain the additional metabolic benefit of exercise training with prolonged hypoxia recovery in suppressing hyperinsulinemia and inadequate glycaemic control for the obese rats. Capillaries in skeletal muscles are vital for the delivery of substrates and insulin to skeletal muscles for fuel storage (Ivy et al.1999; Lillioja et al., 1987). In this study, we found no additional effect of exercise training with prolonged hypoxia recovery in increasing capillary density compared with exercise training alone.

Exercise training increased the proportion of type IIa and decreased the proportion of type IIb muscle fibres in the lean rats. Type IIb muscle fibres have substantially lower capillary supply than type IIa muscle fibres (Ripoll et al., 1979; Waters et al., 2004), and therefore exhibit a low rate of insulin-stimulated glucose uptake (Kriketos et al., 1996). However, exercise training effect on muscle fibre transformation from type IIb to IIa was completely inhibited by moderate hypoxia exposure. This result is similar, to some extent, with the recent study by Kawada & Ishii (2008), who reported an opposing result of fibre transformation from type IIa to IIb by total vascular occlusion in plantaris muscle. Apparently, the additional metabolic benefits of exercise training with prolonged hypoxia recovery over exercise training alone cannot be explained by muscle fibre type transformation.

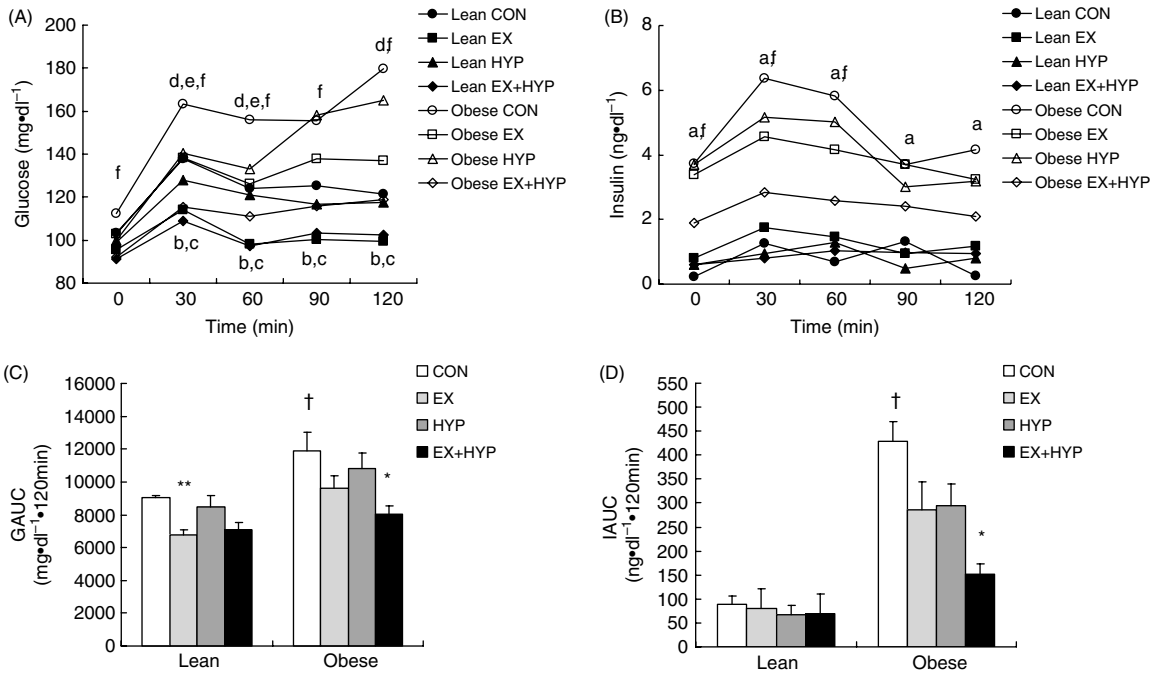

Figure 2. Glucose tolerance and insulin response. Curves for glucose and insulin responses of lean and obese Zucker rats in control (CON), exercise training (EX), hypoxia (HYP), and exercise training with hypoxia recovery (EX + HYP) groups are illustrated in (A) and (B), respectively. Area under curve of glucose (GAUC) and insulin (IAUC) are illustrated in (C) and (D), respectively. * denotes significance against the CON group, $P < 0.05$; † denotes significance against the lean group, $P < 0.05$. Significance symbol on values for oral glucose tolerance curve: lean versus obese CON (a); lean CON versus EX (b); lean CON versus EX + HYP (c); obese CON versus EX (d); obese CON versus HYP (e); obese CON versus EX + HYP (f), $P < 0.05$.

Chronic hypoxia can induce muscle fibre transformation in normal animals. However, previous reports presented contradictory results. Both Deveci et al. (2001) and Itoh et al. (1990) reported significant muscle fibre transformation in soleus (I to IIa) and EDL (IIb to IIa) muscles of normal rats, which were continuously exposed to constant 12% O_2 for more than 3 weeks. In contrast, we found no significant effect in muscle fibre type transformation for obese Zucker rats with 8 h intermittent hypoxia at 14% O_2 for 6 weeks. Our results are similar to those of Takahashi, Kikuchi and Nakayama (1992), who found no difference in the fibre type composition of plantaris muscle following four weeks of normobaric hypoxia (10% O_2) exposure. This discrepancy may be associated with muscle specificity in response to hypoxia.

Unlike the lean rats, GLUT4 protein expression was not altered by exercise training with prolonged hypoxia recovery in the obese Zucker rats. The amount of GLUT4 protein is positively associated with postprandial glucose disposal (Ivy & Kuo, 1998; Leturque et al., 1996; Saengsirisuwan et al., 2001; Wallberg-Henrikss & Zierath, 2001). Many studies reported that GLUT4 protein level in obese Zucker rats was significantly increased by chronic treadmill running (Brozinick, Etgen, Yaspelkis, & Ivy, 1992; Saengsirisuwan et al., 2001; Torgan, Etgen, Kang, & Ivy, 1995). Since rats in this study performed swimming training, the exercise regimen may be associated with different responses to exercise training.

The independent effect of hypoxia on increasing GLUT4 protein (Dill, Chadan, Li, & Parkhouse, 2001; Xia, Warshaw, & Haddad, 1997) and capillary density (Deveci et al., 2001) has been documented previously in lean rats. However, in the present study, the moderate hypoxia protocol (14% oxygen for 8 h daily) was unable to produce comparable results in obese Zucker rats. This difference could be related to the fact that most of the previous studies were performed in normal-weight rats under a longer duration of exposure (24 h a day) and more severe hypoxic levels (Dill et al., 2001; Xia et al.1997). An alternative possibility is that the hypoxia-induced effect gradually diminished over time, which has been reported in a previously study (Dill et al., 2001).

Exercise training and hypoxia are the two documented methods to modulate muscle phenotype (such as GLUT4 expression, angiogenesis and muscle fibre type). The involvement of AMPK-PGC-1α signalling in such adaptations has previously been proposed (Lin et al., 2002; Röckl et al., 2007). In this study, moderate hypoxia and swimming training also independently increased the AMPKα and PGC-1α proteins in plantaris muscle of lean rats, but such changes were not noted in the obese Zucker rats. Therefore, it is not surprising for the obese Zucker

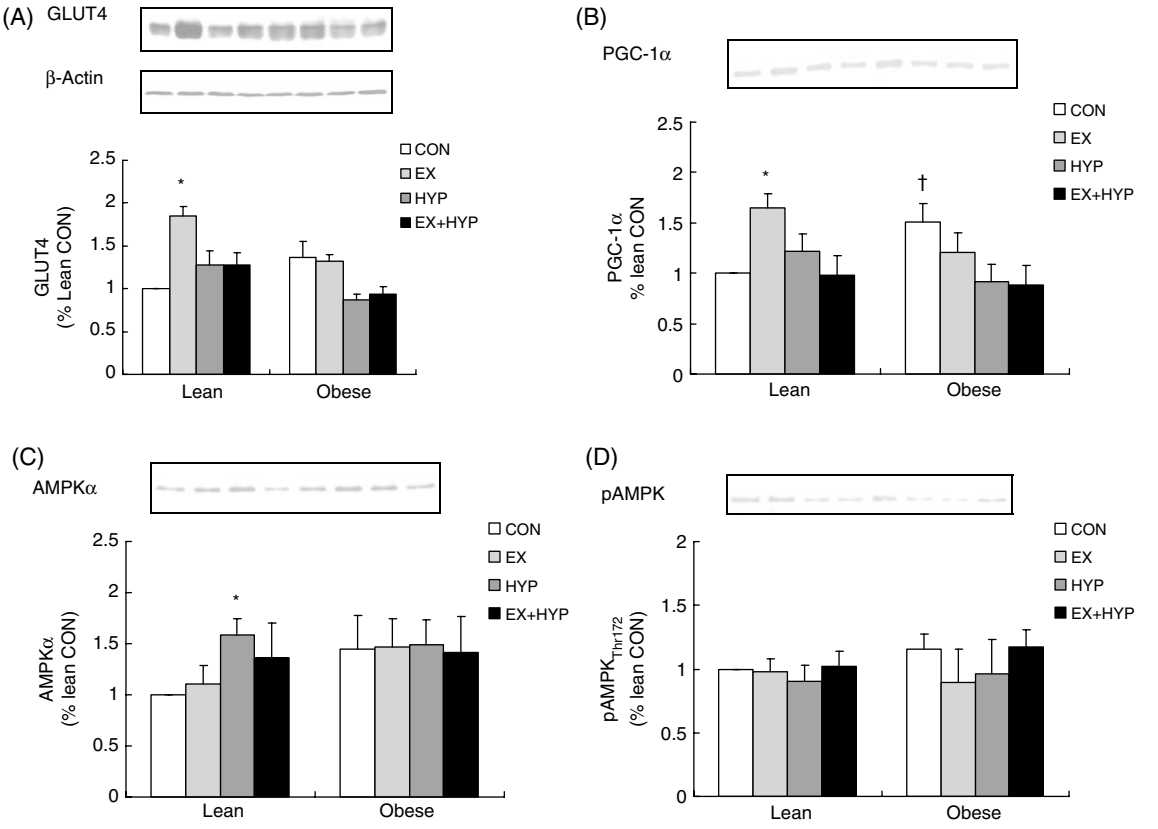

Figure 3. Protein levels of AMPK-PGC-1α signalling pathway in plantaris muscle. Represented autoradiographs of Western blots for plantaris muscle of lean and obese Zucker rats in control (CON), exercise training (EX), hypoxia (HYP), and exercise training with hypoxia recovery (EX + HYP) groups were illustrated above histograms. GLUT4 protein (A), PGC-1α (B), AMPKα (C), and phoshpo-AMPK Thr^{172} protein (D), respectively. ⋆ denotes significance against the CON group, $P < 0.05$; † denotes significance against the lean group, $P < 0.05$.

rats to have abnormal AMPK-PGC-1α signalling in skeletal muscles. In this study, we found that PGC-1α was significantly higher in obese Zucker rats than their lean littermates. Evidence from Barnes et al. (2002) also showed that contraction failed to increase the AMPKα1 activity in the skeletal muscles of obese Zucker rats, whereas a 2.3-fold increase in AMPKα1 activity was found in lean littermates under the same experimental conditions. Furthermore, AMPK activation with 5-aminoimidazole-4- carboxamide 1-β-D-ribofuranoside (AICAR) did not increase the whole-body glucose disposal in the obese Zucker rats, whereas, the same treatment caused a 2-fold increase in whole-body glucose disposal in the lean littermates (Bergeron et al., 2001). Exercise mode or the magnitude of hypoxia stress may also influence the outcome of observation in muscle AMPK-PGC-1α pathway. In our study, swimming protocol (7 d/wk for 6 wk) and moderate hypoxia were unable to alter the muscle AMPK-PGC-1α pathway. However, in contrast to our study, Sriwijitkamol et al. (2006) reported that the abnormalities of the muscle AMPK-PGC-1α pathway in obese Zucker rat were reversed by treadmill training.

Conclusions

The present study found that exercise training with prolonged hypoxia recovery offers better metabolic benefits than exercise training alone for the obese Zucker rats. This improvement was mainly associated with its potent effect on weight reduction. Despite skeletal muscle playing an important role in postprandial glucose homeostasis, the metabolic benefits of exercise training with prolonged hypoxia recovery were apparently not mediated by the alteration of skeletal muscle properties in capillary density, GLUT4 expression and muscle fibre transformation.

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