Downregulation of Na\textsuperscript{+}-K\textsuperscript{+}-ATPase pumps in skeletal muscle with training in normobaric hypoxia

H. GREEN,1 J. MACDOUGALL,2 M. TARNOPOLSKY, and N. L. MELISSA2

1Department of Kinesiology, University of Waterloo, Ontario N2L 3G1; and 2Department of Kinesiology and Neurology, McMaster University, Hamilton, Ontario, Canada L8S 4K1

Green, H., J. MacDougall, M. Tarnopolsky, and N. L. Melissa. Downregulation of Na\textsuperscript{+}-K\textsuperscript{+}-ATPase pumps in skeletal muscle with training in normobaric hypoxia. J. Appl. Physiol. 86(5): 1745–1748, 1999.—To investigate the effects of training in normoxia vs. training in normobaric hypoxia (fraction of inspired O\textsubscript{2} = 20.9 vs. 13.5%, respectively) on the regulation of Na\textsuperscript{+}-K\textsuperscript{+}-ATPase pump concentration in skeletal muscle (vastus lateralis), 9 untrained men, ranging in age from 19 to 25 yr, underwent 8 wk of cycle training. The training consisted of both prolonged and intermittent single leg exercise for both normoxia (N) and hypoxia (H) during a single session (a similar work output for each leg) and was performed 3 times/wk. Na\textsuperscript{+}-K\textsuperscript{+}-ATPase concentration was 326 ± 17 (SE) pmol/g wet wt before training (Control), increased by 14% with N (371 ± 18 pmol/g wet wt; P < 0.05), and decreased by 14% with H (282 ± 20 pmol/g wet wt; P < 0.05). The maximal activity of citrate synthase, selected as a measure of mitochondrial potential, showed greater increases (P < 0.05) with H (1.22 ± 0.10 mmol·h\textsuperscript{-1}·g wet wt\textsuperscript{-1}; 70%; P < 0.05) than with N (0.99 ± 0.10 mmol·h\textsuperscript{-1}·g wet wt\textsuperscript{-1}; 51%; P < 0.05) compared with pretraining (0.658 ± 0.09 mmol·h\textsuperscript{-1}·g wet wt\textsuperscript{-1}). These results demonstrate that normobaric hypoxia induced during exercise training represents a potent stimulus for the upregulation in mitochondrial potential while at the same time promoting a downregulation in Na\textsuperscript{+}-K\textsuperscript{+}-ATPase pump expression. In contrast, normoxic training stimulates increases in both mitochondrial potential and Na\textsuperscript{+}-K\textsuperscript{+}-ATPase concentration.

sodium-potassium-adenosinetriphosphatase; normoxia; mitochondria

THE CATION PUMP Na\textsuperscript{+}-K\textsuperscript{+}-ATPase (Na\textsuperscript{+}-K\textsuperscript{+} pump) functions to extrude Na\textsuperscript{+} from the cell and to pump K\textsuperscript{+} into the cell. In skeletal muscle, the pump attempts to ensure rapid recovery of ionic gradients across the sarcolemma after an action potential, a property that appears to be essential for sustained excitability and contractility (4).

Evidence is accumulating to suggest that, in addition to a variety of hormonal factors that can alter the long-term regulation of the pump (7), the level of contractile activity must also be considered (9). Voluntary training in humans, for example, results in a rapid upregulation in Na\textsuperscript{+}-K\textsuperscript{+}-ATPase concentration (11), with increases in the range of 14–16% typically reported (17, 18). Interestingly, training also induces an increase in muscle mitochondrial potential, the magnitude of which also appears to depend on training stimuli similar to that observed for the Na\textsuperscript{+}-K\textsuperscript{+} pump (10). Previous work has suggested that the Na\textsuperscript{+}-K\textsuperscript{+} pump concentration is much more closely associated with the mitochondrial potential of the muscle fiber rather than with the fiber type as determined by myosin-based histochemistry (2).

Muscle hypoxia, induced either by hypoxic gas mixtures (25) or partial ischemia (15) in combination with exercise, appears to potentiate the training increase in mitochondrial potential. Although the intracellular stimulus responsible for the altered gene expression is not clear, the greater disturbance in energy potential observed during exercise with hypoxia compared with normoxia is implicated (28). On the basis of the association previously shown between the increases in the Na\textsuperscript{+}-K\textsuperscript{+} pump and increases in mitochondrial potential with sustained activity in normoxia (10), it might also be expected that increases in Na\textsuperscript{+}-K\textsuperscript{+}-ATPase would be enhanced with training in hypoxia. Increases in the Na\textsuperscript{+}-K\textsuperscript{+} pumps could conceivably serve the same general function as postulated for mitochondria, namely, an increased ability to maintain homeostasis (i.e., ionic) in hypoxic environments or during periods of high ATP turnover (5).

In this study, our objective was to compare the response of the skeletal muscle Na\textsuperscript{+}-K\textsuperscript{+}-ATPase between training in normoxia and training in hypoxia. Contrary to what we hypothesized, we found that, although training in hypoxia potentiates the increase in mitochondrial potential, training in hypoxia down-regulates Na\textsuperscript{+}-K\textsuperscript{+}-ATPase.

METHODS

Subjects. Nine healthy men, ages 19–27 yr, served as subjects. Although all subjects were active, none had a history of regular endurance training. As required, the study was approved by the Office of Human Research at McMaster University, and all volunteers were fully informed of the procedures and risks involved before written consent was obtained.

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Experimental design. To investigate the effects of training in normoxia vs. training in normobaric hypoxia, the subjects inspired either room air (normoxia (N)) or 13.5% O2 (balance nitrogen) [hypoxia (H)] from a 350-liter Tissot gasometer. In the case of H, the O2 level was maintained by mixing nitrogen with ambient air at a controlled rate. The training program, which consisted of performing cycle exercise with one leg under N and with the other leg under H during the same training session, was performed 3 times/wk for 8 wk. During the initial 6 wk, a training session involved cycling for 60 min (30 min with one leg and 30 min with the other leg). The order of conditions was alternated with each training session. Absolute training intensity and work performed were similar for N and H, and the level was initially set at 75% of the maximal mechanical power output achieved by the leg with the lower maximal potential. On average, training intensity was increased by 2%/wk. During the final 2 wk, interval training was used. During this period, each training session began with five repetitions of 3 min of cycling at 100% of the pretraining maximal power output, interspersed with 3 min of recovery. The training session concluded with 10 min of continuous cycling at a power output equal to that used at the start of training. Further details of the training program can be obtained from a previously published paper (19). Muscle samples were obtained from the vastus lateralis by using the needle biopsy technique before training [control (C)] and after training from the N- and H-trained legs. Tissue samples were analyzed for maximal citrate synthase (CS) activity, a marker of mitochondrial potential, and for the concentration of Na\textsuperscript{+}\textsuperscript{-}K\textsuperscript{-}ATPase.

Analytic procedures. The concentration of Na\textsuperscript{+}\textsuperscript{-}K\textsuperscript{-}ATPase followed the procedures of Nørgaard et al. (21), as previously published by our group (11). The procedure, which is based on the measurement of [\textsuperscript{3}H] after ouabain binding to the Na\textsuperscript{+}\textsuperscript{-}K\textsuperscript{-}ATPase, was performed on two samples from each biopsy; each sample weighed between 2 and 8 mg. The concentrations of [\textsuperscript{3}H]ouabain (1.8 µCi/ml) and ouabain (10\textsuperscript{-6} mol/l) that were employed have been previously demonstrated to produce saturable binding with similar sample sizes (21). All determinations of [\textsuperscript{3}H]ouabain-binding capacity were multiplied by a factor of 1.05 to correct for the loss of specifically bound [\textsuperscript{3}H]ouabain (21). Corrections were also made for isotopic purity of [\textsuperscript{3}H]ouabain, which was estimated at 99% as determined by the supplier (New England-DuPont Canada) by chromatographic techniques. No correction was made for nonspecific uptake and retention of [\textsuperscript{3}H], which has been estimated at <3% in human samples (21). In control samples, we have also found that nonspecific uptake and retention is of a similar magnitude (H. Green, unpublished observations). Maximal CS activity was determined in duplicate at 23°C, according to previously published procedures (14).

RESULTS

Before training, the concentration of Na\textsuperscript{+}\textsuperscript{-}K\textsuperscript{-}ATPase in the vastus lateralis was 326 ± 17 pmol/g wet wt. Training in N resulted in an increase in Na\textsuperscript{+}\textsuperscript{-}K\textsuperscript{-} pump concentration of 45 pmol/g wet wt (13.8%; Fig. 1). In contrast, training in H induced a reduction of 44 pmol/g.

In a given analytic session, all tissue for a given subject was analyzed for either Na\textsuperscript{+}\textsuperscript{-}K\textsuperscript{-}ATPase or CS. Tissue was stored at −80°C before analyses.

Statistics. The data were analyzed by using one-way ANOVA procedures for repeated measures. Where significance was found, post hoc tests were performed by using Newman-Keuls procedures. Pearson product-moment correlations were also calculated between the changes in CS and the changes in Na\textsuperscript{+}\textsuperscript{-}K\textsuperscript{-}ATPase for both N and H. The significance level was set at P < 0.05 for all comparisons.

Fig. 1. Changes in Na\textsuperscript{+}\textsuperscript{-}K\textsuperscript{-}ATPase concentration with normoxia (N) and normobaric hypoxia (H) compared with control (C). Values are means ± SE; n = 9 subjects. *Significantly different from C (P < 0.05); †significantly different from N (P < 0.05).

Fig. 2. Changes in maximal citrate synthase activity with N and hypobaric H compared with C. Values are means ± SE; n = 9 subjects. *Significantly different from C (P < 0.05); †significantly different from N (P < 0.05).

Fig. 3. Correlation coefficients between changes in Na\textsuperscript{+}\textsuperscript{-}K\textsuperscript{-}ATPase (\(\Delta\text{Na}^{+}\text{K}^{-}\text{ATPase}\)) and citrate synthase (\(\Delta\text{CS}\)) activity with N (A) and hypobaric H (B) training. Each symbol represents 1 subject.
wet wt (13.5% of the pretraining value). Training in both N and H increased maximal CS activity (Fig. 2). With H training, the increase exceeded that observed with N training. Increases were 50.5 and 70.2% for N and H, respectively. The correlation coefficients, calculated between the change in CS and the change in Na\(^{+}\)-K\(^{+}\)-ATPase from C were not significant for either N (r = 0.301) or H (r = −0.048) (Fig. 3).

**DISCUSSION**

The major finding of this study, namely that training in normobaric hypoxia induces a downregulation in Na\(^{+}\)-K\(^{+}\)-ATPase, was unexpected. However, the increase in Na\(^{+}\)-K\(^{+}\)-ATPase with normoxic training was anticipated. The magnitude of the increase that we have found is within the range previously reported in humans (11, 17, 18). Moreover, the increase in Na\(^{+}\)-K\(^{+}\) pump may be an early adaptive phenomenon, observable soon after the onset of training (11). The fact that increases may occur early is supported by the work of Tsakiridis et al. (26), who showed that, in rats, 60 min of exercise were sufficient to increase the mRNAs of the \(\alpha_3\)-subunit in red muscle and \(\beta_2\)-subunit in white muscles. The Na\(^{+}\)-K\(^{+}\)-ATPase consists of an \(\alpha\) - and \(\beta\)-subunit, which in skeletal muscle can exist as one of two \(\alpha\) isoforms (\(\alpha_1\) and \(\alpha_2\)) or one of three \(\beta\) isoforms (\(\beta_1\), \(\beta_2\), and \(\beta_3\)) that are distributed in a fiber type-specific manner (1, 26). Given the mixed distribution of fiber types in the human vastus lateralis (12), it is not clear whether the changes noted in Na\(^{+}\)-K\(^{+}\)-ATPase were specific to a given fiber type population. In rats, swimming training resulted in increases in the Na\(^{+}\)-K\(^{+}\) pump in a variety of locomotor muscles composed of fundamentally different fiber composition (16).

A variety of different messengers has been identified in the long-term regulation of Na\(^{+}\)-K\(^{+}\) pump concentration. In general, gene expression is increased by a number of circulating hormones, including glucocorticoids, aldosterone, and thyroid hormones (3, 7), and by O\(_2\) tension (27). Of these, aldosterone and ionic imbalance (i.e., K\(^{+}\)) could be of potential significance, given the changes that occur with exercise (3, 8). However, the fact that a differential response was found between N and H in the subjects' different legs would suggest that the stimulus is localized and possibly associated with O\(_2\) tension. Although few studies are available on hypoxia, it is well established that hypoxia is a potent stimulus for the upregulation of the Na\(^{+}\)-K\(^{+}\) pump concentration in a variety of tissues (27). The regulation by hypoxia appears complex, and it is unclear whether the altered expression is due to the direct effects of O\(_2\) tension or due to the production of reactive O\(_2\) species and cell injury (27). Increased production of O\(_2\) free radicals would be expected to occur with exercise in normoxia and to be depressed with exercise in hypoxia, given the differences in oxidative phosphorylation that would occur during exercise (23, 24). Interestingly, Na\(^{+}\)-K\(^{+}\)-ATPase in skeletal muscle also appears to be downregulated in muscle of chronic heart failure patients, in whom blood flow and O\(_2\) delivery are compromised (20, 22). It remains to be determined whether O\(_2\) tension per se represents the primary stimulus or whether some disturbance secondary to hypoxia is the regulatory stimulus.

The results of the present study clearly demonstrate that increases in mitochondrial potential with training need not be coupled to increases in the Na\(^{+}\)-K\(^{+}\) pump. This is most clearly demonstrated with H training, in which increases in CS were potentiated, compared with N training, in which the opposite effect was observed for Na\(^{+}\)-K\(^{+}\)-ATPase. These results suggest that fundamentally different signals regulate mitochondrial expression compared with Na\(^{+}\)-K\(^{+}\)-ATPase expression. The potentiation of mitochondrial potential with H is entirely consistent with the hypothesis that energy imbalance is a key regulator of mitochondrial biogenesis (28). Exercise in hypoxia is known to cause a greater disturbance in cellular metabolism than is exercise in normoxia (5, 13). Given the exercise protocol employed in this study, the imbalance that occurs with H would be expected to be particularly emphasized. At present, considerable controversy exists regarding the significance of Na\(^{+}\)-K\(^{+}\)-ATPase concentration and activity in the etiology of fatigue (4, 6). Studies that use artificially induced patterns of activation in muscle are generally consistent with a loss of membrane excitability, as a result of Na\(^{+}\)-K\(^{+}\) pump limitations, in fatigue (4). However, such effects are difficult to reproduce with voluntary activity (6). In this study, the reduction of Na\(^{+}\)-K\(^{+}\)-ATPase concentration observed with H would appear to have minimal effect, because the time to fatigue during unilaterial cycling at 95% maximal O\(_2\) consumption was not different between N and H (19). It should be emphasized, however, that we only measured Na\(^{+}\)-K\(^{+}\) pump concentration. It is unclear whether maximal Na\(^{+}\)-K\(^{+}\)-ATPase activity is also upregulated.

In summary, we report, for the first time, a differential effect of N vs. H training on the expression of Na\(^{+}\)-K\(^{+}\)-ATPase in human skeletal muscle. Additional studies are now in progress to examine whether the responses differ among fiber types and whether the altered expression relates to specific Na\(^{+}\)-K\(^{+}\)-ATPase isoforms and to the factors that control altered expression.

We thank J ohn Moroz and Marg Burnett for technical assistance. This study was supported by grants from the National Sciences and Engineering Research Council of Canada.

Address for reprint requests and other correspondence: H. J. Green, Dept. of Kinesiology, Univ. of Waterloo, Waterloo, ON, Canada N2L 3G1.

Received 31 August 1998; accepted in final form 31 December 1998.

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