

Exercise Differentially Regulates Renalase Expression in Skeletal Muscle and Kidney

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Renalase is a newly discovered amine oxidase and may lower blood pressure by metabolizing catecholamines. We have hypothesized that exercise and training may regulate renalase expression to control blood pressure. In this study, we investigated changes in renalase expression after exercise and training in white and red portion of the gastrocnemius muscle, kidney, and serum in rats. Rats were either untrained or subjected to six weeks of endurance training, which predominantly recruits red fibers. Rats from each group were sacrificed before ($n = 10$), immediately after ($n = 10$), or three hours ($n = 10$) following exercise. Renalase mRNA and protein levels were measured by RT-PCR and ELISA, respectively. There were no significant changes in renalase expression after prolonged training or acute exercise in the serum or red muscle of rats. However, in white muscle, renalase mRNA and protein levels decreased after acute exercise in untrained rats, whereas, in trained rats, its protein level remained unchanged, despite a decrease in mRNA. Thus, exercise influenced renalase expression only in white muscle fibers that are not predominantly recruited during exercise. The reduction of renalase protein in white muscle suggests that renalase may contribute to blood redistribution between contracting and non-contracting fibers during exercise. In the kidney, renalase protein levels decreased after training, while mRNA levels increased. The reduction in renalase protein levels may contribute to functional kidney hypoperfusion, which has been observed after training. In conclusion, exercise differentially regulates renalase expression in skeletal muscle and kidney.

Keywords: blood pressure; exercise; kidney; myokines; renalase

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Introduction

Skeletal muscle was recently identified as an endocrine organ that produces and releases a wide range of cytokines and peptides, collectively named myokines (Pedersen 2013). Myokines can influence metabolism and drive adaptive changes to both acute bouts of exercise and prolonged training. The biological activity of myokines may also explain the beneficial effects of physical activity in preventing non-communicable diseases (Petersen and Pedersen 2006; Li et al. 2012).

Adaptive changes to exercise and training comprise cardiovascular modifications, besides others. During acute bouts of exercise, heart rate and cardiac output increase, together with blood pressure. Immediately after this acute exercise, blood pressure is lower than before the exercise; it returns to basal level after 7-22 hours (Pescatello et al. 2004; Mota et al. 2009). After prolonged training, heart

rate is decreased, and blood pressure is lowered, in comparison to the pre-training period (Kokkinos et al. 2009). The average training-related reduction in blood pressure differs between investigated groups, and depends on many factors, including the intensity, duration, and type of training (Haruyama et al. 2009; Manfredini et al. 2009; Chen et al. 2010). In normotensive groups, the mean decrease in resting systolic and diastolic blood pressure after endurance training was 3 mmHg and 2.4 mmHg, respectively (Fagard 2006).

The mechanism underlying the training-related reduction in blood pressure is elusive, and definitive conclusions regarding this phenomenon cannot be made at this time. It is generally accepted that training affects a number of mechanisms, resulting in changes in cardiac output and total peripheral resistance. These mechanisms include regulations of autonomic nervous system activity, plasma level of catecholamines (norepinephrine and epinephrine),

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plasma renin activity, nitric oxide production and its bioavailability for vascular smooth muscle cells, and antioxidant levels (Pescatello et al. 2004; Fagard 2006; Kokkinos et al. 2009; Manfredini et al. 2009).

Recent data indicate that the cardioprotective effect of exercise training, including decreased blood pressure, is unlikely to be mediated by changes in resting sympathetic activity (Alex et al. 2013). Plasma levels of catecholamines are decreased after physical training, however (Kokkinos et al. 2009). The plasma level of catecholamines depends on the rate of production and release from both the sympathetic nervous system and suprarenal glands, as well as the rate of their degradation. Increased degradation of catecholamines may play a role in the exercise-related decrease in blood pressure.

A novel amine oxidase, renalase, which metabolizes catecholamines, was recently discovered (Xu et al. 2005; Desir et al. 2012b). Through its regulation of catecholamines, renalase lowers blood pressure. It also regulates

sodium and phosphate exertion, and displays other cardioprotective effects through currently unknown mechanisms (Milani et al. 2011). Plasma renalase is activated by moderate increases in blood pressure and brief surges of plasma catecholamines, which also lead to increased renalase production and secretion (Desir 2009).

Renalase is detected predominantly in the kidney, but also in skeletal muscle, heart, plasma, testicles, small intestine, peripheral nerves, and brain (Wang et al. 2008; Desir 2009, 2011; Hennebry et al. 2010). The serum level of renalase is inversely correlated with the glomerular filtration rate (GFR) (Desir et al. 2012a). Another cardiovascular adaptation to exercise comprises functional renal hypoperfusion, which results from blood redistribution to supply adequate oxygen and energy substrates to working muscles (van Wijck et al. 2011). It is especially interesting that skeletal muscles express high levels of renalase, suggesting that this enzyme may be a myokine, which plays an important role in regulating local catecholamine concentration

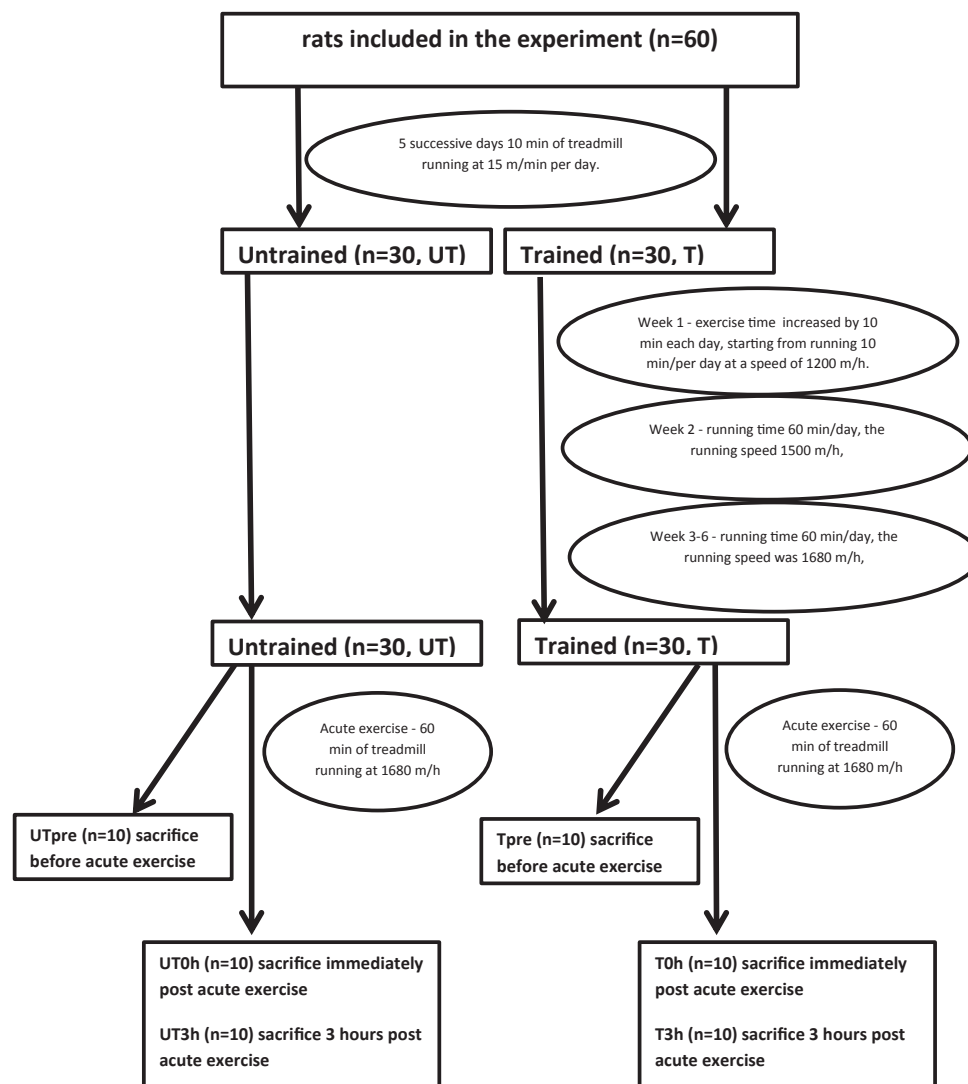


Fig. 1. Experimental scheme.

and blood flow.

We hypothesized that renalase contributes to the effect of lowering blood pressure after exercise and training. The goal of the present study was to investigate the influence of acute bouts of exercise on renalase expression, at both the mRNA and protein levels, in red and white skeletal muscle and serum in trained and untrained rats. Additionally, we investigated the influence of prolonged endurance training on renalase expression in red and white skeletal muscle, kidney, and serum.

Materials and Methods

All procedures used in this study were approved by the Ethical Committee of the Medical University in Białystok, and were

performed in accordance with EU regulations regarding the humane treatment of laboratory animals.

The experimental protocol was as previously described (Czarkowska-Paczek et al. 2009, 2010, 2011). Briefly, 60 male Wistar rats were randomly assigned to an untrained (UT, $n = 30$) or trained (T, $n = 30$) group. The rats had access to water ad libitum, were fed Labofeed B, and were maintained in a 12 h light/12 h dark cycle during the entire period of the study.

During the first five successive days of the experiment, rats from both groups were subjected to exercise adaptation, which consisted of 10 min of treadmill running at 15 m/min per day. After the adaptation, the T group was subjected to six weeks (5 days/week) of endurance training with a progressive work-load. During the first week, the exercise time was increased by 10 min each day, starting from running for 10 min/day at a speed of 1,200 m/h. During

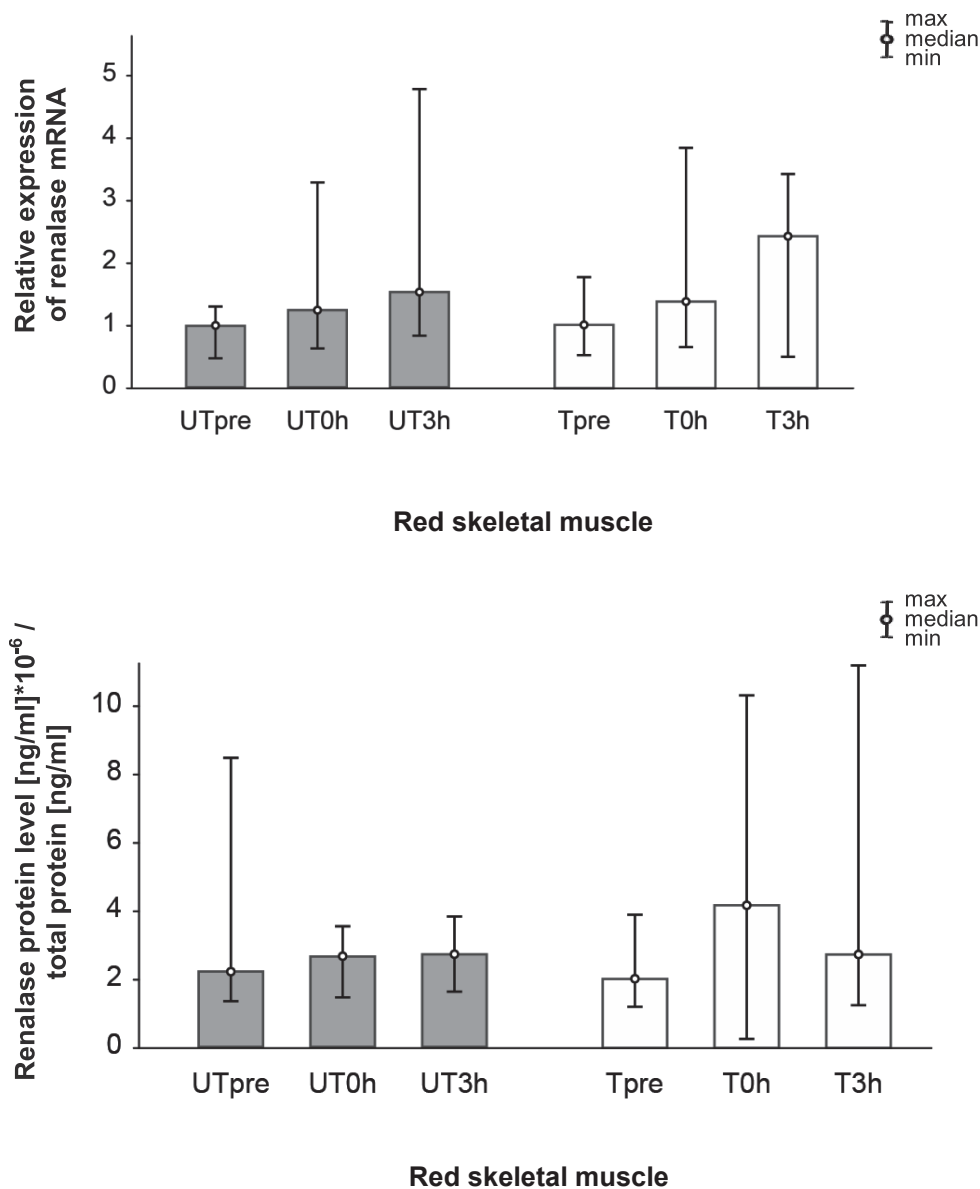


Fig. 2. There are no changes in renalase expression in red skeletal muscle after an acute bout of exercise. Timepoints include: pre-exercise for trained and untrained rats (Tpre and UTpre, respectively), immediately after exercise (T0h, UT0h), and three hours post exercise (T3h, UT3h). Relative changes in mRNA and protein levels are shown.

subsequent weeks of training, running time was a constant 60 min/day. During week two, the running speed was 1,500 m/h, and then it was increased to 1,680 m/h during weeks 3-6. No additional stimulus was applied to enhance running.

The UT group remained at rest during the training period. At 24 h after the last training session of trained group was completed, the rats from both groups were subjected to an acute bout of exercise consisting of 60 min of treadmill running at 1,680 m/h. Each group (UT and T) was randomly divided into three subgroups. Ten rats from each group (UTpre, $n = 10$ and Tpre, $n = 10$) were sacrificed under anaesthesia (intraperitoneal chloral hydrate, 1 mL/100 mg body mass), while the remaining rats were subjected to an acute bout of exercise, described above. The rats from both groups were then sacrificed immediately post exercise (UT0h, $n = 10$, and T0h, $n = 10$)

or 3 hours post exercise (UT3h, $n = 10$, and T3h, $n = 10$). Immediately after sacrifice, samples from the gastrocnemius (red and white portions) muscle, kidney, and serum were collected and stored at -80°C for subsequent analyses. The experimental scheme is presented in Fig. 1.

mRNA isolation

About 50 mg of skeletal muscle (red and white portions of the gastrocnemius) and kidney tissues were homogenized in a TissueLyser bead mixer (Qiagen, Germany) at a frequency of 25 Hz for 5 min. Total mRNA isolation was performed using an EZ1 RNA Universal Tissue Kit and Biorobot EZ1 (Qiagen, Germany), according to the manufacturer's instructions. Total RNA concentrations were measured at 260 nm, using the ND-1000 Spectrophotometer

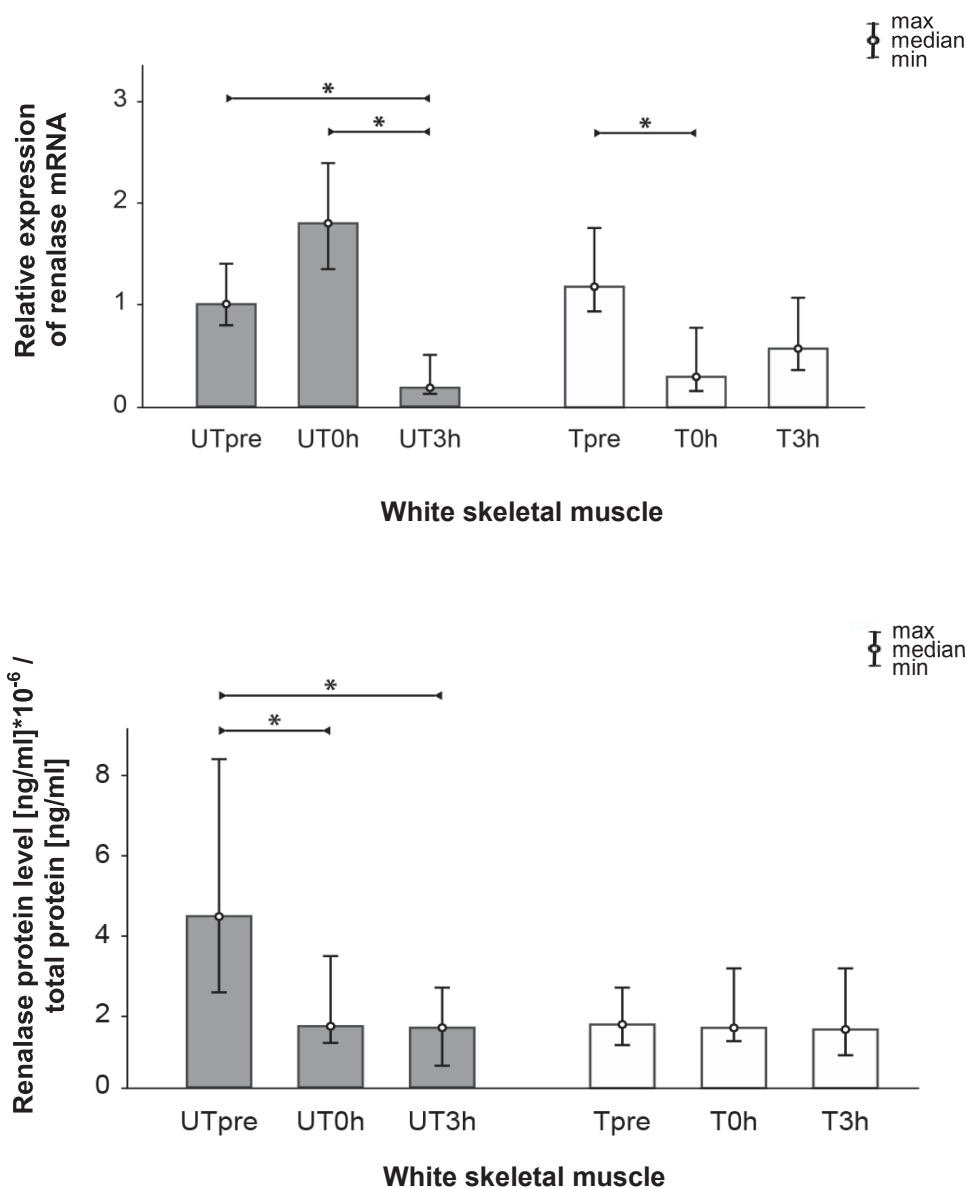


Fig. 3. Acute exercise differentially affects renalase mRNA and protein levels in white skeletal muscle in trained and untrained rats. Timepoints include: pre-exercise for trained and untrained rats (Tpre and UTpre, respectively), immediately after exercise (T0h, UT0h), and three hours post exercise (T3h, UT3h). *Statistically significant difference, $p < 0.01$.

(NanoDrop Technologies, Inc., USA). Samples were then frozen and stored at -80°C for further analyses.

Reverse transcription

Reverse transcription of total mRNA into cDNA was performed using the SuperScript III First-Strand Synthesis System for RT-PCR (Invitrogen, USA), according to the manufacturer's instructions.

Real-time PCR to quantify renalase mRNA expression

Detection of mRNA was performed using an ABI-Prism 7500 Sequence Detection System (Applied Biosystems, USA). Specific primers, probes, and TaqMan Universal Master Mix were all purchased from Applied Biosystems. The amount of specific mRNA was normalized to rat glyceraldehyde-3-phosphate dehydrogenase (GAPDH) gene. According to Mahoney et al. (2004), GAPDH is the most stably expressed gene in skeletal muscle, following endurance exercise. The relative expression of mRNA was calculated using the $2^{-\Delta\Delta C_t}$ method (Livak and Schmittgen 2001).

Protein quantification in skeletal muscle, kidney, and serum

The evaluation of renalase concentrations in serum and tissue homogenates was performed using the enzyme-linked immunosorbent assay (ELISA). Each sample of skeletal muscle and kidney was homogenized in a TissueLyser bead mixer (Qiagen, USA), and centrifuged at 10,000 rpm for 10 min, 4°C . The supernatants were collected and frozen at -80°C until analysis. Renalase protein levels in serum and supernatants from tissue homogenates were measured by ELISA, according to the manufacturer's instructions (USCN, Life Science, China).

Total protein concentration in serum and supernatants was measured at 562 nm on a Bio-Tek Power Wave XS spectrophotometer (Bio-Tek Instruments, USA), using the bicinchoninic acid (BCA) Protein Assay Reagent (Pierce, Holland), according to the manufacturer's instructions. The results are presented as the ratio of renalase concentration/protein concentration ($\times 10^{-6}$) or ($\times 10^{-5}$), depending on the tissue.

Statistical analyses

Results are provided as the median and range, or as relative fold changes. Differences between investigated groups were analyzed using a non-parametric test (U-Mann-Whitney test). The differences in multiple comparisons for relative renalase mRNA expression and the renalase protein fraction out of total protein were considered statistically significant when the p -value was less than 0.016 (Bonferroni correction) and 0.05, when two groups (UTpre and Tpre) were compared.

Results

The mean body mass of rats on the day of acute exercise was 271 ± 11.6 g and 283.17 ± 24.67 g in UT and the T groups, respectively. After an acute bout of exercise, renalase mRNA and protein levels were similar in red skeletal muscle between UT and T groups, and between time points (Fig. 2).

In white muscle, renalase mRNA levels decreased after an acute bout of exercise in the UT3h group, compared with the UTpre and the UT0h groups ($p = 0.002$ and $p = 0.001$, respectively, Fig. 3). In the white muscle of T rats,

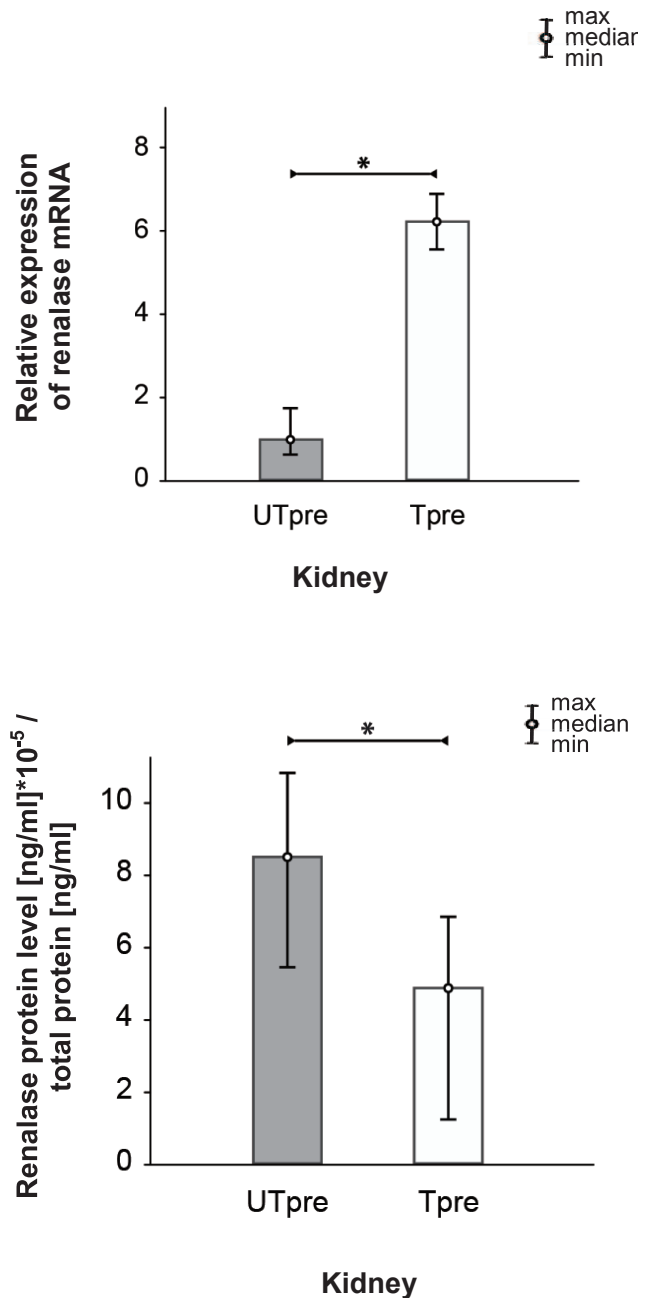


Fig. 4. Renalase expression in the kidney of trained (T) and untrained (UT) rats. Relative changes in mRNA and protein levels are shown. *Statistically significant difference, $p < 0.05$.

there was a significant decrease in renalase mRNA in the T0h group compared with the Tpre group ($p = 0.004$). The trend toward increased levels appeared three hours post exercise; however, this increase was not significantly different compared with earlier time points. Renalase protein levels were decreased in the UT0h group and the UT3h group compared with the UTpre group ($p = 0.003$ and $p = 0.001$, respectively).

Renalase mRNA increased after prolonged training in the kidney ($p = 0.001$) following prolonged endurance

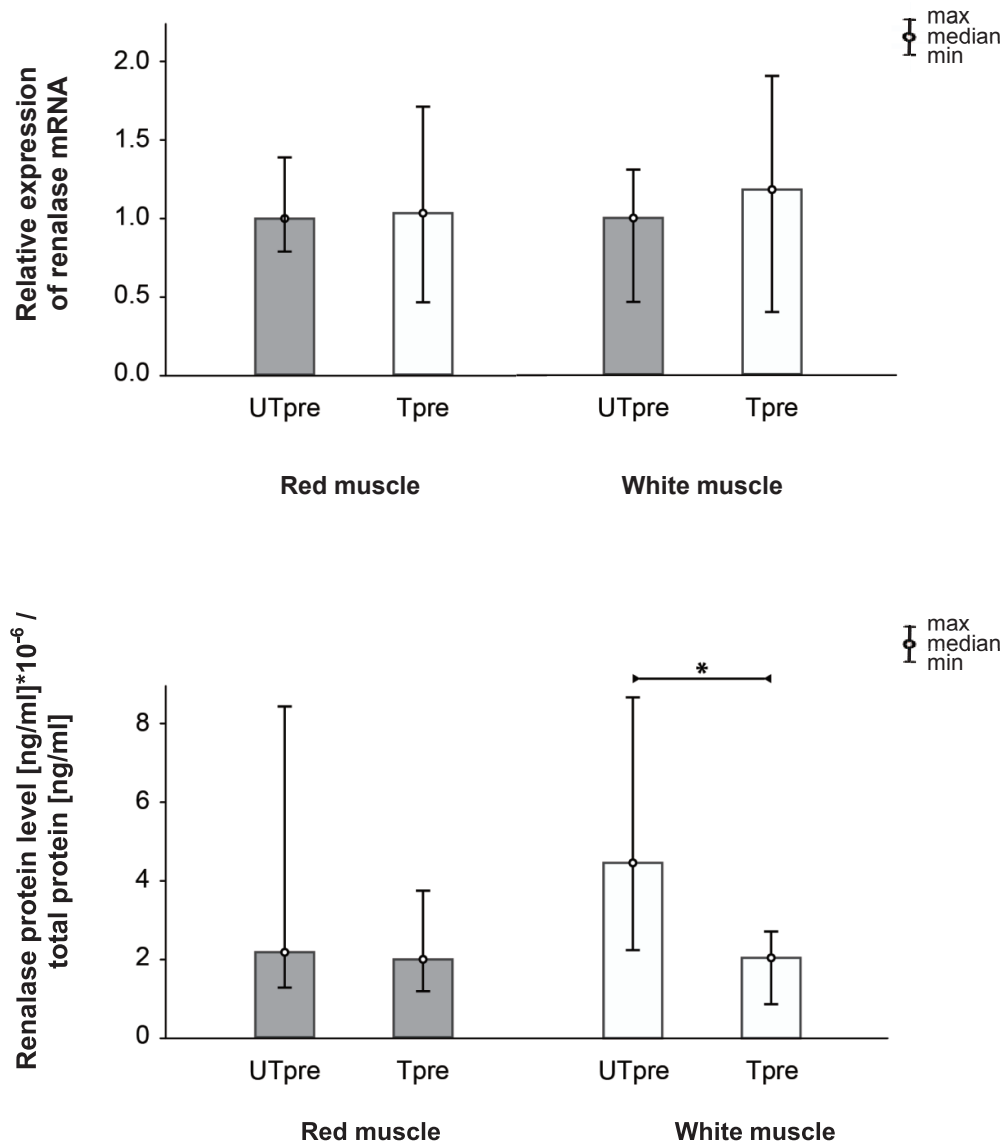


Fig. 5. Expression of renalase in skeletal muscle (red and white) after prolonged endurance training. Relative changes in mRNA and protein levels in trained (T) and untrained (UT) rats are shown. *Statistically significant difference, $p < 0.05$.

training, while its protein levels decreased ($p = 0.003$) (Fig. 4). After prolonged endurance training, renalase protein levels also decreased in white skeletal muscle ($p = 0.0003$), but did not change in red muscle (Fig. 5). In fact, there were no changes in relative renalase mRNA in either muscle tissue after prolonged training.

Serum renalase levels did not change after prolonged training or after an acute bout of exercise in either UT or T rats (Fig. 6).

Discussion

In this study, neither acute exercise nor prolonged training influenced serum levels of renalase. Similarly, no changes in renalase expression were observed in red mus-

cle. However, there were significant changes in renalase expression in the kidney and white muscle.

In white muscle, an acute bout of exercise decreased mRNA and protein expression of renalase in untrained rats. Since renalase metabolizes catecholamines, which are strong vasoconstrictors, this decrease could result in a higher level of vessel constriction and lower level of blood flow. In trained rats, the protein level of renalase remained stable, however, despite decreased mRNA expression.

The different, and sometimes opposing, effects of exercise training on renalase mRNA and protein expression suggest alternative mechanisms for the regulation of renalase protein levels than solely through its mRNA expression. According to Pradet-Balade et al. (2001), the

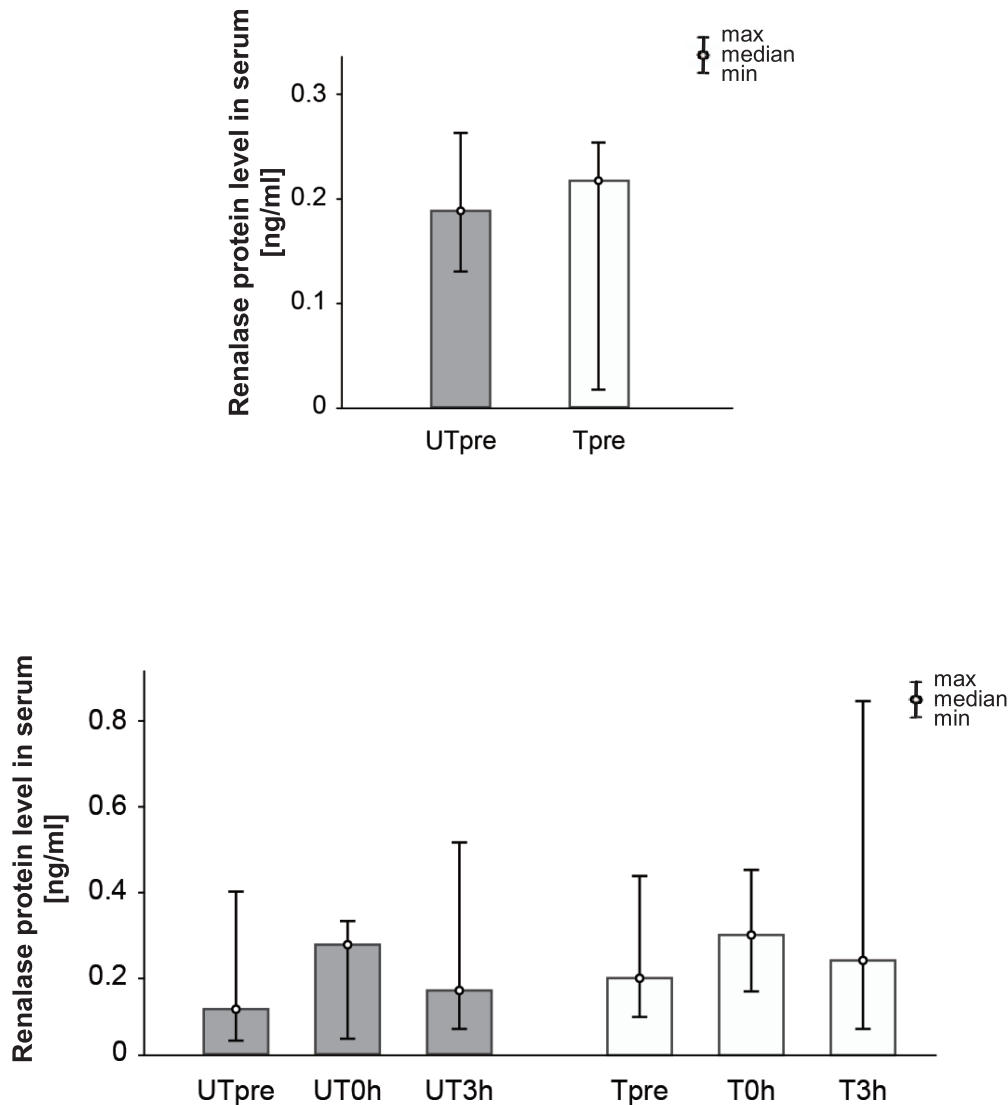


Fig. 6. Serum levels of renalase do not change after endurance training or acute exercise. Timepoints include: pre-exercise for trained and untrained rats (Tpre and UTpre, respectively), immediately after exercise (T0h, UT0h), and three hours post exercise (T3h, UT3h).

correlation coefficient between mRNA and protein levels in mammalian cells is less than 0.5. The mechanisms regulating this phenomenon are complicated, and also depend on the post-transcriptional period.

Because of the speed of running used in our experiment, the exercise was moderate, and primarily recruited red muscle fibers (Lambert and Noakes 1989).

We hypothesize that, in the fibers that are not recruited to the acute exercise (e.g., white fibers), the signal to decrease the expression of renalase mRNA appears, and results in lower levels of renalase protein, especially in untrained animals. This result could contribute to blood redistribution between contracting and non-contracting fibers, allowing for better oxygen supply to the former during acute exercise. These small changes, observed after

an acute bout of exercise, may add up after prolonged training, and may result in lower renalase protein level in trained rats, thus, explaining why the expression of renalase mRNA did not change in white muscles after training, but the protein level decreased significantly.

In our experiment, renalase protein levels decreased in the kidney of trained rats, while its mRNA increased. Kocer et al. (2011), recently showed that the constrictive response of renal resistance arteries to norepinephrine in rats increased following exercise training. In a study by Moninga et al. (2013), the authors showed that endurance training exacerbates the fall in GFR and renal blood flow following acute kidney injury. The results of Conboy et al. (2010), showed that endurance training reduced vasoconstriction during the orthostatic reaction, and could contrib-

ute to training-induced orthostatic intolerance. Our results are in a way consistent with the results from these studies: lower levels of renalase could result in higher baseline levels of norepinephrine, which may result in non-adequate increase in catecholamine during the orthostatic reaction and the training-induced orthostatic drop of blood pressure (Conboy et al. 2010).

In conclusion, exercise influenced renalase expression, but only in the muscle fibers that are not predominantly recruited during the exercise. After training, the changes at the protein level persisted. The reduction of renalase protein in white muscle, in comparison to red muscle, suggests that renalase may contribute to blood redistribution between contracting and non-contracting fibers during exercise. In the kidney, renalase protein decreased after training, and may contribute to functional kidney hypoperfusion, which is observed after training. Furthermore, no changes occurred in serum levels of renalase after exercise or training, suggesting that renalase acts locally.

Acknowledgements

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Conflict of Interest

The authors declare no conflict of interest.

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