

Research article

## The nephrotoxicity risk in rats subjected to heavy muscle activity

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### Abstract

When the body is exposed to insults, the kidneys exhibit adaptive changes termed renal cytoresistance, characterized by cholesterol accumulation in the membranes of the tubule cells. However, heavy muscle activity has not yet been accepted as one of the stressors that could lead to cytoresistance. In order to study the renal functional characteristics of animals exposed to heavy muscle activity, rats were subjected to exhaustive treadmill exercise for 5 days and their data was compared to those of sedentary controls. It was found that in exercised rats, blood lactate, muscle citrate synthase and proximal tubule peroxynitrite levels were all elevated, suggesting the presence of oxidative stress in the proximal tubule segments. However, mean arterial pressure, renal blood flow, glomerular filtration rate, fractional excretion of sodium and potassium, and organic anion excretion remained normal. Despite unchanged blood cholesterol levels, cholesterol loading in the proximal tubule segments, especially the free form, and decreased lactate dehydrogenase release from cytoresistant proximal tubule segments indicated the development of renal cytoresistance. However, this resistance did not seem to have protected the kidneys as expected because organic anion accumulation associated with glycosuria and proteinuria, in addition to the elevated urinary cholesterol levels, all imply the presence of an impaired glomerular permeability and reabsorption in the proximal tubule cells. Therefore, we suggest that in response to heavy muscle activity the tubular secretion may remain intact, although cytoresistance in the proximal tubule cells may affect the tubular reabsorptive functions and basolateral uptake of substances. Thus, this differential sensitivity in the cytoresistance should be taken into account during functional evaluation of the kidneys.

**Key words:** Exercise, proximal tubule, cytoresistance, nephrotoxicity.

### Introduction

In recent years, lifelong physical activity has been recommended for everyone in order to improve their physiological and functional capacity. Today, even the elderly under medication are encouraged to do daily exercises (Ichikawa et al. 2000). It is known that during heavy muscle activity, the perfusion of working muscle is elevated at the expense of several uninvolved organs, including the kidneys, which undergo partial ischemia due to the reduced blood flow. Cessation of exercise causes blood re-flow to hypoxic tissues, leading to re-oxygenation and subsequent production of excessive ROS, which is similar to the ischemia/perfusion phenomenon. Therefore, physical exercise should be accepted as a stress inducer and, apart from the working muscle, many other organs should also exhibit considerable adaptive changes in response to

heavy muscle activity (Di Meo and Venditti, 2001; Gündüz and Senturk, 2003; Koçer et al., 2008; Maeda et al., 2004; Middlekauff et al., 1997; Momen et al., 2003; 2004; Podhorska-Okolów et al., 2004).

Zager et al. (1999; 2001; 2003a; 2003b; 2005; Zager and Kalhorn, 2000) studied kidney responses to several forms of injury such as renal failure, sepsis, endotoxemia, ischemia-reperfusion or oxidative stress, and they showed that proximal tubule cells undergo some adaptive changes for protection from subsequent hazards. The response of kidneys to the studied stressors is termed “acquired renal cytoresistance” and is characterized by cholesterol accumulation in the proximal tubule cells. Cholesterol accumulation in the membranes of the tubule cells is the hallmark of cellular response to stress and makes the cells resistant to further attacks. However, as mentioned above, up to the present neither heavy muscle activity nor exercise have been studied as a stress inducer.

The results of some experimental and clinical studies indicated that acute or heavy muscle activity can induce stress response and considerable pathological changes can occur, including apoptosis in the tubular cells of the kidney (Podhorska-Okolów et al., 2004). However, several results demonstrating renal dysfunction and the ROS generating effect of heavy exercise on kidneys were disregarded, and these detrimental effects of exercise on renal functions were accepted as nonpathological benign processes by some authors (Bergstein, 1999; Brown et al., 2007; Gündüz and Senturk, 2003; Khazaeinia et al., 2000; Koçer et al., 2008).

If exercise was to be accepted as a stress inducer and had a similar resistance generating effect on proximal tubule cells, the kidneys of exercisers would be more resistant to subsequent attacks and the renal functional capacity of trained athletes would be greater than those having a sedentary lifestyle. This expectation is in good accord with the studies indicating normal renal functional capacity in trained athletes (Neumayr et al., 2005). On the other hand, the high prevalence of exercise-induced proteinuria and hematuria in athletes (Bellinghien et al., 2008) undermines the clarity of the consensus about the risk or benefit of heavy muscle activity on kidney functions, and the effects of muscle activity on proximal tubule resistance remain to be studied. Considering that many people are subjected regularly or irregularly to either voluntary or involuntary (such as labourers) heavy muscle activity throughout their lifetime, it is not surprising that their proximal tubule cells would be loaded with acidic metabolites that might trigger some subsequent adaptive changes.

Previously, we observed that, as with other stress-

ors, exhaustive heavy muscle activity also induces cytoresistance, characterised by cholesterol accumulation in the proximal tubule segments. These cholesterol loaded tubule segments also exhibited resistance to further attack, such as ATP depleting stress, and had a lower LDH release response (unpublished data). In the present experiment using both in vitro and in vivo parameters, we aimed to investigate the functional characteristics of cholesterol loaded cytoresistant tubule cells of rats subjected to exhaustive muscle activity.

## Methods

In this experimental study 2.5-3 month old male Wistar rats were used. The animals were randomly divided into a sedentary (control,  $n = 20$ ) and a heavily exercised group ( $n = 20$ ). The rats were provided with food and water ad libitum. All procedures were approved by the Akdeniz University Animal Care and Usage Committee (06-12/02).

### Exercise protocol

In this study, the exhaustive exercise protocol (Gündüz & Senturk, 2003; Kocer et al. 2008) was repeated on five consecutive days to mimic heavy muscle activity carried out by labourers. Animals were exhausted on a motor-driven treadmill (MAY-TME 9805, Commat, Ankara, Turkey) for 5 days. Before the exhaustive protocol, the rats were familiarized with treadmill running for 2 days. The exhaustive protocol was started at  $20 \text{ m}\cdot\text{min}^{-1}$  and a 5% gradient for 5 min. The gradient and speed were gradually increased to 15% and  $24 \text{ m}\cdot\text{min}^{-1}$ , and running was continued until exhaustion developed. Exhaustion was defined as the inability to continue running and the loss of the straightening reflex within ten seconds when animals were placed on their back.

All animals were placed in metabolic cages for 24 hours and urine samples were collected. The exhausted rats were placed in metabolic cages on the fourth exercise day and sacrificed after the last treadmill running.

### Blood lactate concentration

Ten minutes following the last exercise session, lactate concentration was measured in blood samples obtained from the tail vein using a lactate analyzer BM-Lactate test strips (Accusport, Mannheim Boehringer, Accusport Diagnostics & Biochemicals, U.K.).

### MAP measurement

Under light ether anesthesia, MAP was determined by the tail cuff method (Biopac, BP HR200 module plus MP100 system, Goleta, CA).

### Measurement of RBF and organic anion excretion

The renal plasma flow (RPF) was calculated as a clearance of PAH, an organic anion prototype and RBF was estimated from RPF/1-Hematocrit (Agarwal, 2002; Gehrig et al., 1986). GFR and plasma PAH concentrations were used for the calculation of filtered PAH load. Secreted PAH load was calculated by subtracting excreted total PAH from filtered PAH.

After collection of urine and blood samples, the

kidneys were perfused with ice-cold Krebs phosphate buffer (118 mM NaCl, 4.8 mM KCl, 2.5 mM  $\text{CaCl}_2$ , 1.2 mM  $\text{MgSO}_4$ , 1.2 mM  $\text{KH}_2\text{PO}_4$ , 24 mM  $\text{NaHCO}_3$ , 11 mM glucose, pH:7.4) to wash off the blood. Then, both kidneys were excised, cleaned from adhesive fat tissues and decapsulated in an ice-cold buffer. The cooled kidneys were longitudinally dissected and the medullary portion was discarded. The renal cortical tissue was used for proximal tubule isolation or mitochondria isolation.

The blood and urine samples were used for protein, creatinine, electrolyte, glucose and cholesterol measurements. Protein and creatinine levels were determined using the Lowry (Lowry et al., 1951) and Jaffe methods (Newman et al., 1999) respectively. GFR was estimated by creatinine clearance. The electrolyte levels were measured by an autoanalyzer (Roche Hitachi F-800). Appropriate commercial kits were used for glucose (Quanti-Chrom™ Glucose Assay Kit, DIGL-200) and cholesterol measurements (Cholesterol Fluorometric (Red) Assay Kit, Amplex® Red from Molecular Probes, Invitrogen; A12216). Serum total cholesterol and HDL cholesterol levels, following phosphotungstic acid/ magnesium chloride precipitation, were determined using the same cholesterol kit. LDL cholesterol levels were calculated by subtracting HDL cholesterol from the total cholesterol.

### Citrate Synthase (CS) activity

CS activity was determined for the soleus muscle of each rat according to the spectrophotometric method described by Srere (1969).

### Proximal tubule isolation

As mentioned in our previous study (Cirrik & Oner, 2006), renal proximal tubules were isolated from rats based on the method of Vinay et al. (1981).

### Tissue cholesterol levels

Following isolation, proximal tubules were subjected to lipid extraction as previously described (Zager et al., 1999). Total and free cholesterol levels were then determined using the commercial kit. Cholesterol ester (CE) levels in samples were calculated by subtracting the free cholesterol from the total cholesterol. Hepatic total cholesterol levels were determined using the same commercial kit, after the same lipid extraction procedure.

### Cytoresistance

As outlined by Zager et al. (1999; 2001; 2003a; 2003b; 2005; Zager and Kalhorn, 2000), isolated proximal tubules were incubated either under basal conditions or ATP-depleted/  $\text{Ca}^{+2}$  overloaded conditions, which consisted of mitochondrial respiration and glycolysis inhibitors and  $\text{Ca}^{+2}$  ionophore ( $7.5 \mu\text{M}$  antimycin A,  $20 \text{ mM}$  2-deoxyglucose and  $10 \mu\text{M}$  A23187, respectively) for 4 hours. After 4 hours incubation, the medium LDH level was determined using a commercial kit (QuantiChrom™ Lactate Dehydrogenase Kit, DLDH-100) and LDH release was expressed as a percentage of the total LDH level.

### Tubule organic anion accumulation study

Isolated PTSs were incubated in Krebs phosphate buffer

containing 50  $\mu\text{M}$  PAH, at 37°C for 1 hour and 1 mM Probenecide (an inhibitor of OATs localized at the basolateral membrane of the tubule cells and transport PAH into the cells) was added to the incubation medium at the 45<sup>th</sup> min to prevent its reverse release during washing processes. The PAH loaded tubules were washed three times and a further 15 min incubation was carried out under the same conditions, without PAH but with 1 mM Probenecide. At the end of the incubation, tubules were sonicated and their PAH contents were measured (Gehrig et al. 1986; Agarwal, 2002).

### Tubule peroxynitrite level

Peroxyntite levels in the PTSs incubated at 37°C for one hour were determined by a spectrophotometric method described by Beckman et al. (1992).

### Mitochondrial isolation

Mitochondria were isolated from renal cortical tissue using a method defined by Weinberg et al. (1982). Isolated mitochondria were used for inorganic phosphate determination.

### Inorganic phosphate

Inorganic phosphate levels in the mitochondria were determined according to the spectrophotometric method described by Katewa and Katyare (2003).

### Statistical analysis

All values are presented as means  $\pm$  SEM. Statistical comparisons were performed by unpaired Student's t-test.  $P < 0.05$  was considered as statistically significant.

## Results

### Functional parameters

In this study, heavy muscle activity-induced renal cytoresistance and its functional importance were examined. The PTSs of sedentary and exhausted rats were isolated and tubular cholesterol and LDH release were determined. In the exhausted group, the blood lactate level was in-

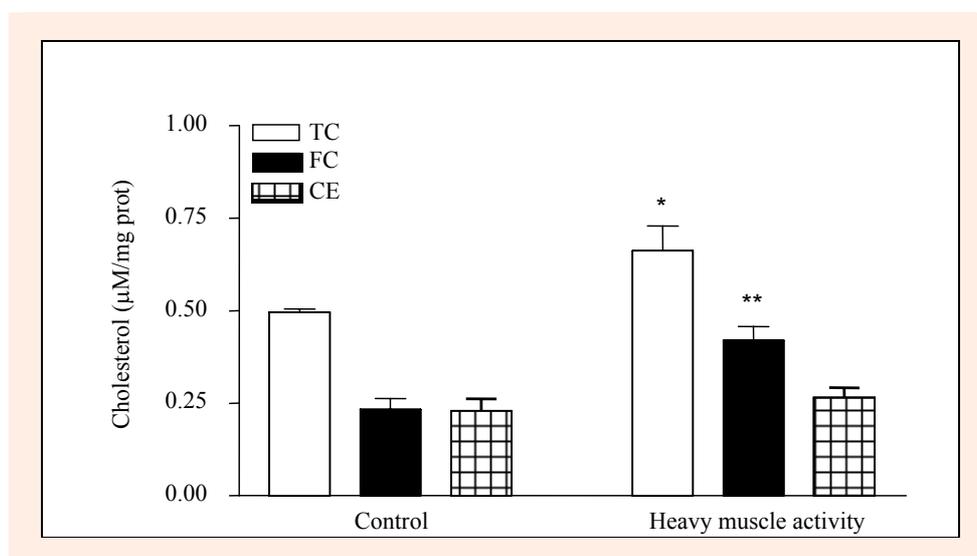
creased two fold (from  $1.87 \pm 0.12$  to  $4.36 \pm 0.39$  mM,  $p < 0.001$ ) and CS activity in the soleus muscle was elevated significantly from  $65.14 \pm 6.81$  to  $123.54 \pm 10.14$   $\mu\text{M}\cdot\text{g ww}^{-1}\cdot\text{min}^{-1}$  ( $p < 0.001$ ).

As seen in Table 1, five days of exhaustive muscle activity had no significant effect on either MAP, GFR or RBF. Kidneys displayed normal functions according to routine laboratory tests. Plasma electrolytes were within normal limits, blood total, LDL and HDL cholesterol levels did not alter significantly with respect to the control group. However, although the fractionated urinary  $\text{Na}^+$  ( $\text{FE}_{\text{Na}^+}$ ) and  $\text{K}^+$  excretion ( $\text{FE}_{\text{K}^+}$ ) remained unaltered, animals subjected to heavy muscle activity exhibited a significant proteinuria and glycosuria associated with low serum protein and glucose levels. Urinary cholesterol excretion was also increased.

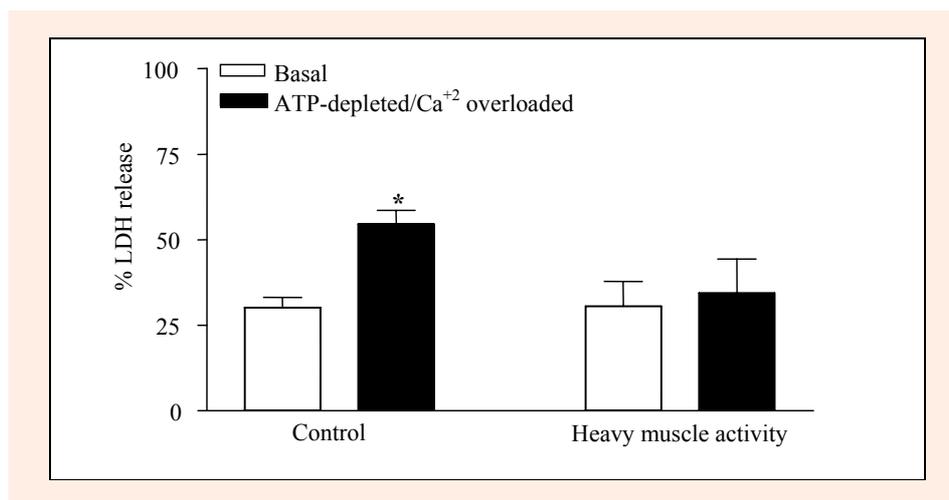
Although the serum cholesterol levels did not exhibit a significant difference between the control and the exhausted groups, there was a marked increase in the total cholesterol in the PTSs of exhausted group with respect to the controls ( $0.662 \pm 0.067$   $\mu\text{M}\cdot\text{mg}^{-1}$  protein vs  $0.499 \pm 0.008$   $\text{M}\cdot\text{mg}^{-1}$  protein,  $p < 0.05$ ) (Figure 1). Interestingly, the elevation was prominent particularly in the free cholesterol fraction ( $0.420 \pm 0.037$   $\mu\text{M}\cdot\text{mg}^{-1}$  protein vs  $0.234 \pm 0.029$ ,  $p < 0.01$ ), while the mean cholesterol ester level remained unchanged between the groups ( $0.265 \pm 0.027$   $\mu\text{M}\cdot\text{mg}^{-1}$  protein vs  $0.230 \pm 0.032$   $\mu\text{M}\cdot\text{mg}^{-1}$  protein). While studying the cholesterol loading effects of heavy muscle activity we found that, in addition to the kidney, the liver was also affected. Similar to the findings in proximal tubule cells, total cholesterol was significantly elevated in the hepatic cells of the exhausted group with respect to the control group ( $0.428 \pm 0.023$   $\text{mg}\cdot\text{g}^{-1}$  ww. vs control value of  $0.221 \pm 0.020$   $\text{mg}\cdot\text{g}^{-1}$  ww,  $p < 0.01$ ).

### Proximal tubule cytoresistance; LDH release

At basal conditions, after a 4-hour incubation at 37 °C, LDH release into the incubation medium from isolated PTSs of sedentary and 5-day exhausted rats was similar ( $30.59 \pm 7.27\%$  and  $30.2 \pm 7.07\%$  respectively). In



**Figure 1.** Total cholesterol (TC), free cholesterol (FC) and ester cholesterol (EC) levels in proximal tubules isolated from sedentary control and 5-day exhausted animals. Statistically different from sedentary control; \*  $p < 0.05$ , \*\*  $p < 0.01$ .



**Figure 2.** LDH release from proximal tubules isolated from control and 5-day exhausted rats. LDH release was determined in the basal and ATP-depleted/ Ca<sup>2+</sup> overloaded (Antimycin A, 7.5  $\mu$ M, 2-deoxyglucose, 20 mM and A23187, 10  $\mu$ M) conditions and expressed a percentage of total LDH. Statistically different from individual basal conditions; \*  $p < 0.001$ .

response to ATP-depleted/ calcium-overloaded conditions, used as a second attack, LDH release increased significantly ( $54.6 \pm 8.2$  %,  $p < 0.001$ ) in the PTSs of sedentary control rats, while LDH release ( $34.41 \pm 9.99$  %) in the PTSs of exhaustive rats remained unaltered (Figure 2).

#### Urinary PAH (organic anion) excretion

An organic anion prototype PAH excretion was found to be  $32.34 \pm 7.11$   $\mu$ g·g kw<sup>-1</sup>·min<sup>-1</sup> in the control sedentary rats and did not change significantly in the exhaustive animals ( $49.37 \pm 5.75$   $\mu$ g·g kw<sup>-1</sup>·min<sup>-1</sup>). It was estimated that  $84.81 \pm 2.31$ % and  $78.13 \pm 6.52$ % of the excreted PAH originated from the tubular secretion of control and exhausted animals, respectively (Figure 3). Thus, heavy muscle activity did not cause a significant effect on tubular PAH secretion.

#### PAH (organic anion) accumulation in the proximal tubule segments

PAH loaded PTSs were incubated at 37 °C for 15 min in the Krebs Phosphate buffer containing 1 mM probenecide

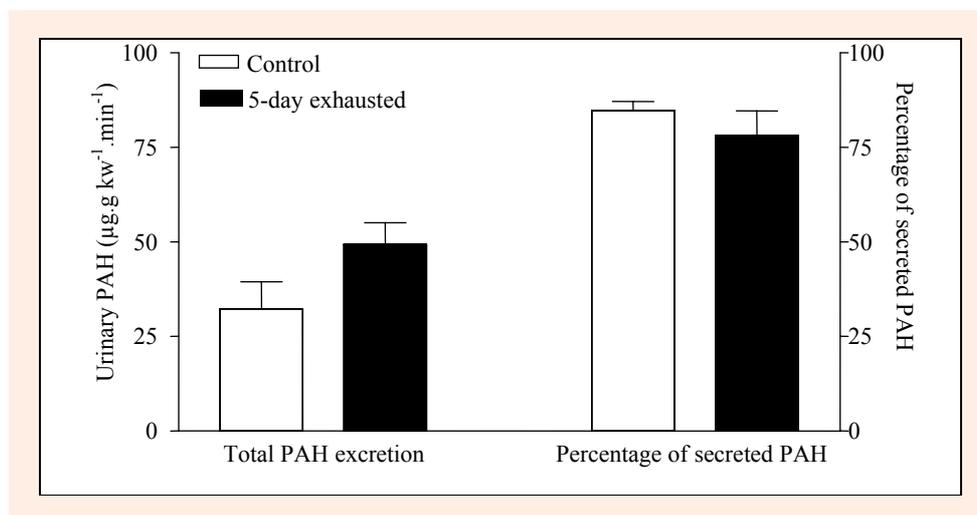
and time dependent PAH release was studied. The amount of PAH released into the medium in control and exhausted groups was similar ( $10.74 \pm 0.47$  and  $9.04 \pm 1.08$   $\mu$ g·mg<sup>-1</sup> protein, respectively). However, following the 15 min release period, the remaining unreleased PAH, which is accepted as the accumulated amount, in the PTS of exhaustive rats was significantly higher than that of sedentary rats ( $1.77 \pm 0.27$  and  $4.54 \pm 1.00$   $\mu$ g·mg<sup>-1</sup> protein in control and exercised animals respectively,  $p < 0.001$ ).

#### Peroxynitrite levels in the proximal tubule segments

Peroxynitrite, an NO oxidation product, increased dramatically in the isolated tubules of exhausted rats relative to the values obtained from the control group ( $100.87 \pm 26.60$  vs  $1213.43 \pm 19.45$  nM·mg<sup>-1</sup> protein,  $p < 0.01$ ).

#### Inorganic phosphate level

Isolated mitochondria of the renal cortex from control and exhausted animals were used to study the F<sub>0</sub>/F<sub>1</sub> ATPase activity, based on inorganic phosphate release during the 10 min incubation period. The inorganic phosphate



**Figure 3.** Total PAH excretion and percentage of secreted PAH in control and 5-day exhausted rats.

**Table 1.** Studied parameters in rats from sedentary control and 5-day exhausted groups.

Groups	Sedentary Control	Heavy muscle activity
MAP (mmHg)	91.2 (1.8)	86.75 (3.69)
GFR (ml·dk <sup>-1</sup> )	.572 (2.1)	.659 (.11)
RBF (ml·dk <sup>-1</sup> )	5.45 (1.03)	7.61 (.99)
Urinary glucose (mg·ml <sup>-1</sup> )	.003 (.003)	.293 (.070) *
Urinary protein (mg·dL <sup>-1</sup> )	.054 (.072)	.486 (.071) **
Urinary cholesterol (mg·dL <sup>-1</sup> )	.032 (.002)	.038 (.002) ***
FE <sub>Na</sub> <sup>+</sup>	.151 (.040)	.111 (.040)
FE <sub>K</sub> <sup>+</sup>	52.52 (9.32)	57.16 (3.37)
Serum total chol (mg·mL <sup>-1</sup> )	.97 (.04)	1.17 (.11)
Serum HDL-chol (mg·mL <sup>-1</sup> )	.28 (.03)	.30 (.05)
Serum LDL-chol (mg·mL <sup>-1</sup> )	.671 (.034)	.869 (.090)
Serum glucose (mg·mL <sup>-1</sup> )	1.46 (.06)	.95 (.07) ***
Serum protein (g·dL <sup>-1</sup> )	1.52 (.02)	1.46 (.02) *
Plasma [Na <sup>+</sup> ] mmol·L <sup>-1</sup>	140.25 (1.13)	141.25 (.73)
Plasma [K <sup>+</sup> ] mmol·L <sup>-1</sup>	3.58 (.05)	3.63 (.14)

Statistically different from sedentary control; \* p < 0.05, \*\* p < 0.01, \*\*\* p < 0.001.

releasing effect of F0/F1 ATP synthase in the mitochondria of control and exercised rats was  $3.69 \pm 0.23 \mu\text{g}\cdot\text{mg protein}^{-1}\cdot\text{min}^{-1}$  and  $3.85 \pm 0.39 \mu\text{g}\cdot\text{mg protein}^{-1}\cdot\text{min}^{-1}$ , respectively.

## Discussion

As demonstrated previously in our laboratory (Gündüz and Senturk, 2003; Kocer et al., 2008), exhaustive muscle activity leads to oxidative damage in the kidneys. In accordance with these studies, in the present experiment, exhaustive activity, as proved by elevated blood lactate and CS activity of the soleus muscle, is associated with significant elevation of tubular peroxynitrite levels, which is an oxidation product of nitric oxide. This exhaustive physical activity induced some degree of renal dysfunction such as proteinuria and glycosuria (Table 1). However other tests used to evaluate the functional integrity of the kidneys, such as GFR, urinary electrolytes and organic anion (PAH) excretions, remained within normal limits. The functional interpretation of these parameters may be subjective. To some authors who accept proteinuria and glycosuria as a benign exercise induced process (Asgar et al., 2007; Bergstein, 1999; Neumayr et al., 2005), the kidney functions of the exhausted rats in the present experiment can be totally normal, whereas for others, proteinuria, glycosuria and elevated urine cholesterol may be the criteria of impaired kidney functions in exhausted rats (Di Meo and Venditti, 2001; Maeda et al., 2004; Middlekauff et al., 1997; Momen et al., 2003; 2004; Podhorska-Okolów et al., 2004). Therefore, our results supported most of the previous studies, indicating both positive and negative effects of exhaustive exercise on kidney functions (Asgar et al., 2007; Poortmans, 1984; Poortmans and Labilloy, 1988; Poortmans and Vancalck, 1978; Neumayr et al., 2005). The authors who consider glomerular and tubular structures as the most sensitive areas are supported by the significant increase in urinary protein, glucose and cholesterol excretion in the exhausted animals (Poortmans, 1984; Poortmans and Labilloy, 1988; Poortmans and Vancalck, 1978). The elevated glomerular permselectivity to macromolecules and other tubular adaptive changes in exercisers are reported to be transient and decline rapidly after exercise and their aetiology remains unclear (Poortmans, 1984; Poortmans and

Vancalck, 1978; Poortmans and Vanderstraeten, 1994). We are unable to say that elevated protein and glucose excretion return to normal levels at a certain postexercise period, since we have not studied time dependent functional changes. However, Schneider et al. (2007) reported that the most sensitive test for kidney functions is the organic anion excretion rate, and therefore we also measured an organic anion prototype PAH excretion rate both in vivo and in vitro in our study. Neither PAH excretion nor its tubular secretory portion changed significantly in the exhausted animal group (Figure 3). This in vivo result is also verified by the in-vitro excretion study, and the PAH secretion rate in the isolated PTSs from exhausted rats remained unchanged.

Our data, together with the literature findings, imply that while most secretory functions of the nephron are within normal limits during heavy muscle activity, some functions depending on glomerular permeability and tubular reabsorption seem to be vulnerable (Table 1). This difference in the sensitivity of the kidney functions of exercising animals may arise from the characteristics of adaptive changes in the proximal tubule cells occurring in response to heavy muscle activity.

It has been reported previously that several forms of stress in the body produce some adaptive changes in the kidney proximal tubules by stimulating self-defence mechanisms. These changes are denoted as “acquired renal cytoresistance” (Zager et al., 1999; 2001; 2003a; 2003b; 2005; Zager and Kalhorn, 2000). Although Zager et al. (1999; 2001; 2003a; 2005; Zager and Kalhorn, 2000) did not include heavy muscle activity as a stressor, the results of the present study clearly indicated that, like other stressors, strenuous physical activity renders the kidneys more resistant to subsequent attacks, because the isolated PTSs from exhausted rats showed resistance to ATP depletion and released less LDH into the medium (Figure 2).

Cholesterol accumulation is accepted as a hallmark of stress-induced cytoresistance in the proximal tubule cells, and its mechanisms have been investigated intensively (Zager et al., 1999; 2001; 2003a; 2005; Zager and Kalhorn, 2000). As with other renal cytoresistance inducers, heavy muscle activity caused a cellular cholesterol load in the proximal tubule cells. To our knowledge, there are no direct studies on the effects of heavy muscle activ-

ity on tubule cholesterol homeostasis, and our study is the first to determine the changes in cholesterol homeostasis in the proximal tubule cells.

Under physiological conditions, free cholesterol biosynthesis and cholesterol influx and efflux are balanced, and cellular cholesterol levels remain normal (Chawla et al., 2001; Weber et al., 2004). In the present study, it is obvious that, like other stressors, heavy muscle activity leads to cholesterol accumulation and cytoresistance in the tubule cells by impairing either elimination/esterification of free cholesterol or its influx pathways. Previous reports show a predominance of esterified cholesterol elevation in tubule cells as a response to other stressors (Zager et al., 1999; 2001; 2003a; 2005; Zager and Kalhorn, 2000). However, the free cholesterol related increase indicates the involvement of some other unknown mechanisms in the present experiment which cannot be fully explained with our present data. Nevertheless, elevated peroxynitrite levels may be one of the causative factors, and reduced elimination of free cholesterol by nitrosylated Apoprotein A1 with elevated peroxynitrite (Shao et al., 2005) may account for the increased free cholesterol in the tubule cells of exhausted rats.

Despite an obvious cholesterol elevation, most excretory kidney functions, including organic anion excretion rate, are not influenced by the free cholesterol accumulation in proximal tubule cells, whereas some reabsorptive functions such as protein endocytosis and glucose reabsorption by the apical membrane appear to be vulnerable. We have not measured tubular prostaglandin E2 levels, but previous reports regard the reduced tubular prostaglandin E2 production as responsible for the diminished apical endocytosis in exercisers (Mittleman and Zambraski, 1992; Llorente et al., 2000; Zambraski et al., 1986).

Intriguingly, in the present study an unaltered PAH secretion was associated with significant accumulation in cholesterol loaded isolated tubule cells. This shows that heavy muscle activity induced cholesterol loading, by impairing the balance between influxers and effluxers, thus facilitating organic anion (as well as xenobiotics) accumulation in the cholesterol loaded proximal tubule cells. Both organic anions and xenobiotics in the blood use the same organic anion transporters (OATs) for entry into proximal tubule cells and they then exit these cells through ATP dependent ABCC2 (MRP2) and ABCC4 (MRP4), cassette transporters located at the luminal membrane of the cells (Sekine et al., 2006). Neither organic anions nor xenobiotics accumulate in the tubule cells while their entrance and exit are balanced. PAH accumulation in the tubule cells of exhausted rats shows impairment of this delicate balance. Altered PAH entry may be responsible for this imbalance, since the tubular PAH secretory rate remained unchanged in the PTSs of the exhausted rats. There are no other studies related to MRPs changes in the tubular cells of exercisers, but unaltered ATP generation in the PTSs of exhausted rats seems to support the functional integrity of these ATP dependent MRPs. Also, additional data from the literature indicating unaltered ABCA1 expression, another member of ATP dependent MRPs family in cholesterol loaded cytoresis-

tant tubule cells (Zager et al., 2003b), is also supportive evidence for our unchanged PAH secretion result.

Despite the lack of direct evidence related to organic anion transporter (OAT) activity in the cholesterol loaded kidney cells of the exercisers, some indirect data such as basolateral preference of cholesterol accumulation (Imai et al., 1992) and different action of membrane cholesterol enrichment on carrier mediated transport processes (Levi et al., 1990), suggest that OAT1 and OAT3 activities localized at the basolateral membrane, which mediate the entries of several endogenous and exogenous substances into the proximal tubule cells, can be influenced earlier by cholesterol accumulation than the effluxing transporters localized apically. Furthermore, the elevated number of OAT1/bile acid transporters in the liver of chronic exercisers (Wilund et al., 2008) supports our view concerning the sensitivity of OATs activity to exercise induced cholesterol elevation in the cells. Contrary to Zager et al. (2001), who maintain that hepatic cholesterol does not change during cytoresistance, we found a similar cholesterol elevation in the liver of exhausted rats. Since both organs are located in the same splanchnic area and are subjected to reduced perfusion during each period of exercise, similar stress induced cholesterol accumulation would be a normal result, not a paradox. Therefore, the similar changes of OATs activities in both organs loaded with cholesterol are a logical expectation.

The biological significance of increased organic anion transport without alteration in its luminal secretion rate in animals or humans subjected to heavy muscle activity can be a topic for further study. The impaired balance between entrance and exit of xenobiotic/organic anions is important data, implying the elevated susceptibility to nephrotoxicity in humans/animals subjected to heavy muscle activity. This may have clinical significance, especially in those exposed simultaneously to heavy muscle activity and xenobiotics, pollutants, heavy metals and antibiotics which are eliminated by the kidneys through the same transporters. However, this hypothesis requires further studies for proof.

## Conclusion

The present data clearly demonstrated that (i) like other studied stressors, heavy muscle activity induces renal cytoresistance, (ii) organic anion accumulation as well as failure of the absorptive capacity of the tubule cells suggest the presence of some biochemical changes and increased vulnerability of kidneys to nephrotoxic agents in rats subjected to heavy muscle activity and (iii) when considering the broad substrate spectrum of OATs, this vulnerability to several endogenous and exogenous substances is of vital importance in exercisers or workers who are subjected to several chemicals, environmentally or therapeutically. Clinically, this vulnerability to nephrotoxicity in workers is very important and must be evaluated by further experimental and clinical studies.

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### Key points

- The cholesterol loading and decreased LDH release from PTs isolated from exhausted rats indicate the heavy muscle activity induced renal cytoresistance.
- Heavy muscle activity-induced renal cytoresistance did not preserve the kidney functions.
- Organic anion accumulation as well as failure in the absorptive capacity of the tubule cells suggest the presence of some biochemical changes and elevated vulnerability of kidneys against nephrotoxic agents in rats subjected to heavy muscle activity.

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