รายงานวิจัยฉบับสมบูรณ์

โครงการผลของการออกกำลังกายแบบฉับพลันและแบบต่อเนื่องต่อการขับสารทางไต

(Effect of acute and chronic exercise on rat secretory function)

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มิถุนายน 2560

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Abstract

Title: Effect of acute and chronic exercise on rat secretory function

Background. Exercise induces certain physiological changes such as altered hemodynamic and reduced blood flow to kidneys. These changes would affect pharmacokinetics of some drugs. However, little is known about the interaction between exercise and renal excretory process. The objective of this study is to investigate the effect exercise on organic anion transporters (Oats) function, a major transporters expressed in basolateral membrane of renal proximal tubule.

Method. Male Wistar rats were randomly divided into 4 groups: non-exercise, acute exercise, exhaustive exercise and training exercise. Renal Oat1 and Oat3 function were examined by measurement of *p*-aminohippurate (PAH) and estrone sulfate (ES) uptake into rat renal cortical slices, respectively. The amount of Oats protein expression was used to examine whether the alteration of Oats function might be associated with downregulation of Oats protein.

Results. All type of exercises had no effect on PAH uptake into rat renal cortical slices, suggested that exercise did not change Oat1 function. Whereas, only exhaustive exercise reduced ES uptake into rat renal cortical slices, suggested impairment of Oat3 function. The reduced in Oat3 function was gradual recovered at 6 h after exhaustive exercise. The impairment of Oat3 function after exhaustive exercise was accompanied by decreased renal Oat3 protein expression compare with non-exercise rats. Thus, the decreased in Oat3 protein expression is sufficient to explain the reduction in Oat3 function after exhaustive exercise.

Conclusion. Exhaustive exercise decreased Oat3 function and associated with downregulation of Oat3 protein. In addition, this impairment of Oat3 function and expression was transient and gradual recovered at 6 h after exhaustive exercise. As a result, exhaustive exercise may have an impact on rat renal organic anion excretion.

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Keywords : exercise, exhaustive exercise, organic anions, transport function

Executive summary

Nowadays, physical activity or exercise is recommended for healthy individuals in order to improve their health and well-being. Moreover, exercise has been prescribed to individuals who are taking drugs for improved their health conditions and quality of life. It is well known that exercise induces several physiological changes and adaptation following exercise. During acute bout of exercise, cardiac output, heart rate, respiratory rate, metabolic enzyme activity and sympathetic nerve activity increase in order to meet energy demand (1). In addition, blood is redistributed from liver and kidneys to exercising muscles during exercise (2). Exercise training (repeated bout of exercise at least 3 time/week) induce several physiological adaptations including a decreased in heart rate, increased in plasma volume, cardiac muscle fiber size, skeletal muscle mass and muscle performance (1). These physiological changes following acute bout of exercise and exercise training may have an effect on pharmacokinetics of some drugs.

Pharmacokinetics describe the process of drug absorption, distribution, metabolism and elimination (3). Exercise has been shown to increase serum concentration of tetracycline, doxycycline, sulphamethizole and sulphadimidine (4, 5). Whereas, exercise decreased serum level of digoxin (6, 7). Taken together, exercise may have a significant effect on one or more aspects in drug pharmacokinetics. Exercise decreased blood flow to splanchnic tissues with corresponding an increased blood flow to exercising muscles (2). These changes may delay drug absorption and distribution due to decrease drug availability and delay gastric emptying (8). On the other hand, exercise increased blood flow to exercising muscle which resulted in accelerating intramuscular-injected drug absorption (8). Drug metabolism occurs in liver. Exercise decreased drug delivery to liver. Therefore, exercise may decrease drug metabolism in liver. For drug elimination, exercise suppressed urinary excretion of tetracycline and doxycycline with a corresponding an increase serum level of these drugs (4, 5). Taken together, drug elimination seems to have major clinical significant in maintaining the plasma concentration. However, little information is available about the effect of exercise on renal drug elimination.

Kidney is the primary organ that responsible for clearance of drugs from the body. The drug excretion through kidney is involved glomerular filtration, tubular reabsorption and tubular secretion. It is well documented that renal blood flow decreased during exercise resulting in decreased glomerular filtration (8). Subsequently, drug bioavailability was reduced for excretion. While tubular

reabsorption and secretion depend on renal transporters. The drug excretion via tubular reabsorption seem to be a minor clinical significant during exercise. However, little is known about the influence of exercise on renal tubular secretion.

Organic anion transporters (Oats), expressed in renal proximal tubule, has a significant role in the excretion of organic anions including drugs, endogenous substances and toxic compounds (9). Oats located at basolateral membrane of renal epithelia cells and responsible for uptake of organic anions from blood into cells across basolateral membrane (10). Subsequently, organic anions secrete from the cells into renal proximal tubule and excretion in urine. Several members of Oats have been identified. Among these, Oat1 and Oat3 have a major role in renal organic anion secretion (11). The impairment of Oat1 and Oat3 function resulted in decrease renal tubular secretion and increase serum concentration of some drugs (12, 13). The change in Oat1 and Oat3 function may affect renal drug elimination. Therefore, it is important to know that how exercise has an effect on renal secretory function. The purpose of present study is to investigate effect of exercise on Oat1 and Oat3 transport function. The significant of this finding will provide the information concerning the interaction between exercise and renal excretory process. The finding would give benefic information for healthcare professional in taking care of patients who may be at a particular high risk of drug accumulation. In addition, it would give further insight on individualization of drug dosing regimens to the patients who participate in irregular and regular exercise.

Objective

1. To examine the different types of exercise on Oat1 and Oat3 transport function
    - Acute exercise
    - Exhaustive exercise
    - Training exercise

2. To investigate underlying mechanism of effect of exercise on Oats transport function

Research methodology

Animals and experimental designs

All procedures were conducted in accordance with the Guidelines of the National Laboratory Animal Center of Thailand and approved by the Animal Care and Use Committee of Faculty of Science, Mahidol University. Male Wistar rats were housed in single standard cages in an environmental-controlled room (25 ± 2°C with 12 h-12h dark-light cycle) at Laboratory Animal Facility, Faculty of Science, Mahidol University. Rats were fed with standard rat chow and water ad libitum.

After 1-week acclimatization, male Wistar rats (age, 6 weeks; weight, 200-250g) were randomly divided into 4 groups as follows: non-exercise group (NE); exhaustive exercise group (EE); acute exercise group (AE); and training exercise group (TE).

Exercise protocols

Exhaustive exercise. On the day of experiment, rats were run on motorized treadmill at 16 m/min with 0° inclination. Running speed and inclination were adjusted to 24 m/min with 15° inclination for first 5 min. This running speed was maintained for 10 min. After 15 min, running speed was gradually increased to 38 m/min until exhaustion. Exhaustive sign was determined by loss of righting reflex. Immediately (0 h), 6 h and 24 h after exhaustive exercise, kidneys were removed for subsequently analysis.

Acute exercise. On the day of experiment, rats were run on motorized treadmill for 60 min at speed of 20 m/min, 15° inclination. Immediately after exercise, kidneys were removed for subsequently analysis.

Training exercise. Rat were introduced to familiar with treadmill running for 5 days. The first 2 days, rats were run on motorized treadmill at a lowest speed (16 m/min, 0° inclination) for 10 min. For other 3 days, rats were run at 18 m/min, 0° inclination for 20 min. Running speed, inclination and duration were gradually increased to 20 m/min, 15° inclination for 60 min/day, 5 days/ week at the end of week 2. Rats were performed exercise training following this intensity for 5 day/week and training duration was 8 weeks. During exercise, rats were stimulated by tapping at their tails or

blowing by dry air if they stop running. Rat kidneys were removed for subsequent analysis at 24 h after last exercise session to avoid effect of acute exercise.

Renal slice preparation and uptake study

The function of Oat1 and Oat3 were determined by the uptake of [^{3}H] *para*-aminohippurate ([^{3}H]-PAH) and [^{3}H] estrone sulfate ([^{3}H]-ES), respectively. At the end of exercise session, rats were euthanized by sodium pentobarbital intraperitoneal injection. Kidneys were removed, decapsulated and maintained on ice-cold modified Cross and Taggart buffer (95 mM NaCl, 80 mM mannitol, 5 mM KCl, 0.74 mM $CaCl_2$, and 9.5 mM Na_2HPO_4, pH 7.4). The renal cortical slices ($\leq$ 0.5 mm; 5-10 mg/slice) were cut using a Stadie-Rigge microtome and incubated in ice-cold buffer for 10 min. The slices were incubated in buffer containing 80 nM of [^{3}H]-PAH or 30 nM of [^{3}H]-ES at 37°C for 30 min. The uptake was stopped by washing 3 times with ice-cold buffer containing 1 mM unlabeled-PAH or 1 mM unlabeled-ES. Slices were then blotted, weight, dissolved in 1N NaOH for 24 h and neutralized with 1N HCL. Tissue samples were determined for [^{3}H]-PAH and [^{3}H]-ES using a Liquid Scintillation Analyzer (1214 Rackbeta, Wallac). The uptake was calculated as tissue per medium ratio (T/M) (dpm/mg of tissue ÷ dpm/μl uptake buffer) and expressed as a percentage of control.

Western blot analysis

The rat renal cortex was dissected and homogenized using a Polytron PT3100 homogenizer (Kinematica) in ice-cold homogenization buffer (300 mM sucrose, 25 mM imidazole, 1 mM EDTA, 1 mM PMSF, and complete protease inhibitor, pH 7.2). Tissue homogenates were centrifuged 12,000 rpm for 20 min at 4°C and supernatant was kept at -80°C for subsequent analysis. Supernatant was separated by 10% SDS-PAGE and transferred to a nitrocellulose membrane (Millipore, Billerica, MA, USA). The membranes were blocked with 5% non-fat dry milk in TBST (Tris-buffered saline, 0.1% tween 20) at room temperature for 1 h. The membranes were then incubated overnight with primary antibody (polyclonal rabbit anti-Oat3 antibody, 1:500 dilution, Cosmobio, Tokyo, Japan). After washing with TBST, the membranes were probed with horseradish peroxidase (HRP)-conjugated goat anti-rabbit IgG (1:3,000 dilution, Cell Signaling Technology, Danver, MA, USA) at room temperature for 1 h. The immunoreactivity was developed using an enhanced chemiluminescence (ECL) detection kit. The protein expression signals were quantified using Image J software (NIH, Bethesda, MD, USA).

Biochemical analysis

On the day of experiment, blood was collected using cardiac puncture technique under anesthesia. The serum was separated by centrifugation at 3,000 rpm at 4°C for 10 min. These samples were used to measure serum blood urea nitrogen (BUN) and serum creatinine as parameters indicative of renal function. BUN and serum creatinine were measured by enzymatic colorimetric techniques using commercial kits (Biotech, Bangkok, Thailand).

Materials

Chemicals were purchased from Sigma Chemical Co. (St. Louis, MO, USA) and Merck (Darmstadt, Germany). [^{3}H]-PAH and [^{3}H]-ES were purchased from Perkin Elmer (Waltham, MA, USA). Polyclonal rabbit anti-Oat3 antibody were purchased from Cosmobio (Tokyo, Japan).

Statistical analysis

All data are expressed as mean ± SE. For multiple comparison, data were analysed with one-way ANOVA followed by Bonferroni *post hoc* test. The level of significant difference was set at $P < 0.05$.

Results

Effect of different types of exercise on general characteristics

The general characteristics of non-exercise, exhaustive, acute and training rats were determined.

Table 1. General characteristics of experimental rats.

	Age (weeks)	Body weight (g)	Kidney weight (g)	Kidney/body weight ratio
Non-exercise (NE)	15	502.02 ± 12.71	1.31 ± 0.03	0.26 ± 0.01
Exhaustive exercise (EE)	15	496.47 ± 13.62	1.35 ± 0.03	0.27
Acute exercise (AE)	15	512.93 ± 11.04	1.37 ± 0.02	0.27 ± 0.01
Training exercise (TE)	15	467.01 ± 6.54*	1.37 ± 0.04	0.30 ± 0.01***

Values are means ± SEM (n = 7-12 rats per group). $^{*}P < 0.05$, $^{***}P < 0.001$ versus non-exercise group.

As shown in Table 1, all experimental rats were age-matched controls (15 weeks old). Acute and exhaustive exercise had no effect on rat body weight when compared with non-exercise rats. Training exercise had significant reduced in rat body weight compared with non-exercise rats ($p < 0.05$). All exercises had no effect on kidney weight. However, kidney/body weight ratio significantly increased in trained rats compared to non-exercised rats ($p < 0.001$). The significant increase in kidney/body weight ratio in trained rats may due to the decrease in body weight from adaptation to training.

Effect of different types of exercise on Oat1 and Oat3 function

The function of Oat1 and Oat3 were determined by measured the [^{3}H]-PAH and [^{3}H]-ES uptake in rat renal cortical slices. As shown in Fig. 1.

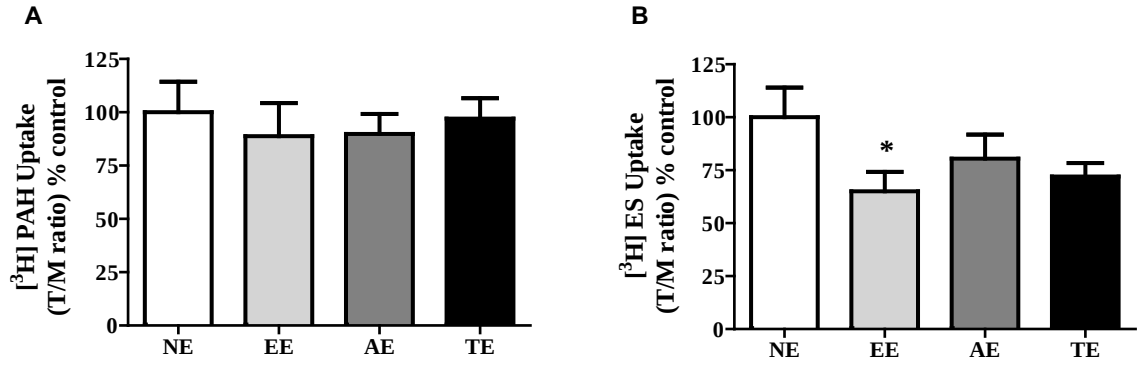

Figure 1. Effect of different types of exercise on [^{3}H]-PAH uptake and [^{3}H]-ES uptake in rat renal cortical slices. A: effect of exercise on Oat1 function. B: effect of exercise on Oat3 function. Rat renal cortical slices from NE, EE, AE and TE were incubated in medium containing [^{3}H]-PAH or [^{3}H]-ES for 30 min. The uptake was calculated as the tissue-to-medium ratio and expressed a percentage of control. Values are mean ± SEM (n= 6-10 rats per group). $^*P < 0.05$ versus non-exercise.

There was no significant difference in [^{3}H]-PAH uptake in rat renal cortical slices among non-exercise, acute, exhaustive and training exercise groups. Whereas, exhaustive exercise significantly decreased [^{3}H]-ES uptake in rat renal cortical slices compared to non-exercise ($p < 0.05$). There was no significant difference in [^{3}H]-ES uptake in rat renal cortical slices among non-exercise, acute and training exercise groups. These results indicated that exercise had no effect on Oat1 transport function. Whereas, exhaustive exercise decreased Oat3 function.

Decline in Oat3 function after exhaustive exercise was not due to reduction of renal function

To examine whether decreases in Oat3 transport activity after exhaustive exercise caused by exercise-induced renal dysfunction. Serum creatinine and BUN were used to determined renal function. As shown in Table 2, exhaustive, acute and training exercise did not affect serum creatinine and BUN when compared to non-exercise. This finding implied that the reduction in Oat3 function after exhaustive exercise was not due to exercise-induced renal dysfunction.

Table 2. Effect of different types of exercise on renal function parameters.

	Serum creatinine (mg/dl)	BUN (mg/dl)
Non-exercise (NE)	0.29 ± 0.06	37.58 ± 1.47
Exhaustive exercise (EE)	0.38 ± 0.06	38.72 ± 1.83
Acute exercise (AE)	0.24 ± 0.06	40.95 ± 2.47
Training exercise (TE)	0.25 ± 0.05	36.67 ± 1.39

Values are means ± SEM (n = 7-9 rats per group). Abbreviation: BUN, blood urea nitrogen.

The recovery of Oat3 function after exhaustive exercise

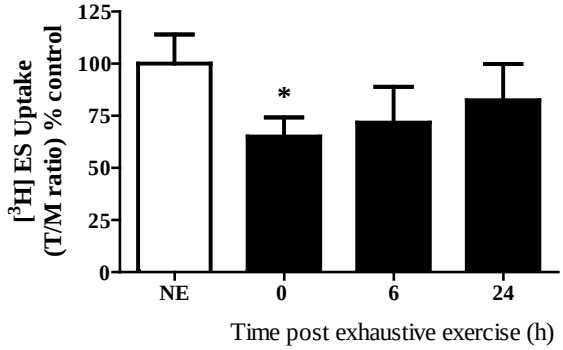

Figure 2. The recovery of Oat3 mediated-[^{3}H]-ES uptake in rat renal cortical slices after exhaustive exercise. Immediately (0 h), 6 and 24 h after exhaustive exercise, rat renal cortical slices were prepared and [^{3}H]-ES uptake was determined as described above. The uptake was calculated as the tissue-to-medium ratio and expressed a percentage of control. Values are mean ± SEM (n= 8-10 rats per group). $^{*}P < 0.05$ versus non-exercise group.

The data above showed that immediately after exhaustive exercise decreased [^{3}H]-ES uptake in rat renal cortical slices. To assess the recovery of Oat3 function after exhaustive exercise. Immediately (0 h), 6 h and 24 h after exhaustive exercise, renal cortical slices were prepared and used for measured [^{3}H]-ES uptake study. Immediately after exhaustive exercise significantly decreased [^{3}H]-ES uptake into rat renal cortical slices compared to non-exercise ($p < 0.05$) (Fig. 2). Whereas [^{3}H]-ES uptake into rat renal cortical slices gradually recovered at 6 h and 24 h after exhaustive exercise. These results supported that the decrease of Oat3 transport activity after exhaustive exercise was temporary decline and function could recover at 6 h after exhaustive exercise.

Effect of exercise on Oat3 protein expression

The amount of Oat3 protein expression in whole renal cortex extracted was used to determine the mechanism of exhaustive exercise induced-reduction of Oat3 transport function. As indicated in Fig. 3B, the relative protein expression of Oat3 was significantly decreased at immediately after exhaustive exercise compared to non-exercise ($p < 0.05$). Whereas there was no difference in protein expression after acute and training exercise.

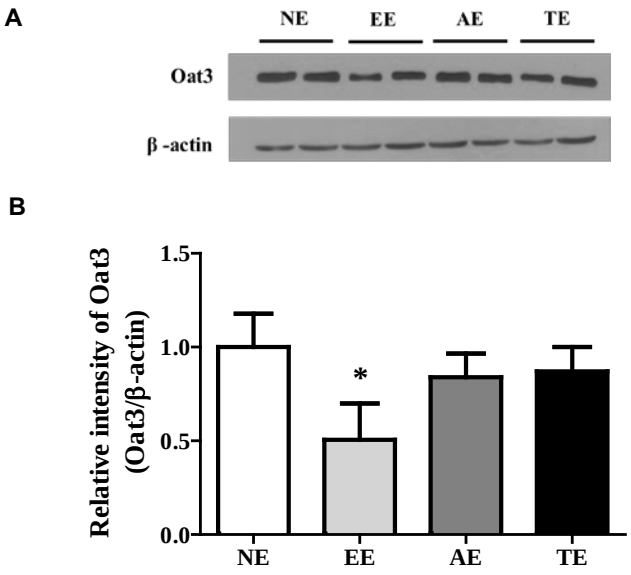

Figure 3. Effect of different types of exercise on Oat3 protein expression in renal cortex. A: representative blots. B: densitometric quantification of Oat3 protein expression. At the end of exercise session, total renal cortex proteins were extracted and incubated with anti-Oat3 antibody. Oat3 protein expression was normalizes by β-actin and expressed as relative value to non-exercise group. Values are mean ± SEM (n= 6-8 rats per group). *$P < 0.05$ versus non-exercise group.

In addition, the relative protein expression of Oat3 was gradually recovered at 6h and 24 h after exhaustive exercise compared to non-exercise (Fig. 4B). Theses finding indicated that the decrease of Oat3 transport activity after exhaustive exercise may be mediated by decrease in Oat3 protein expression.

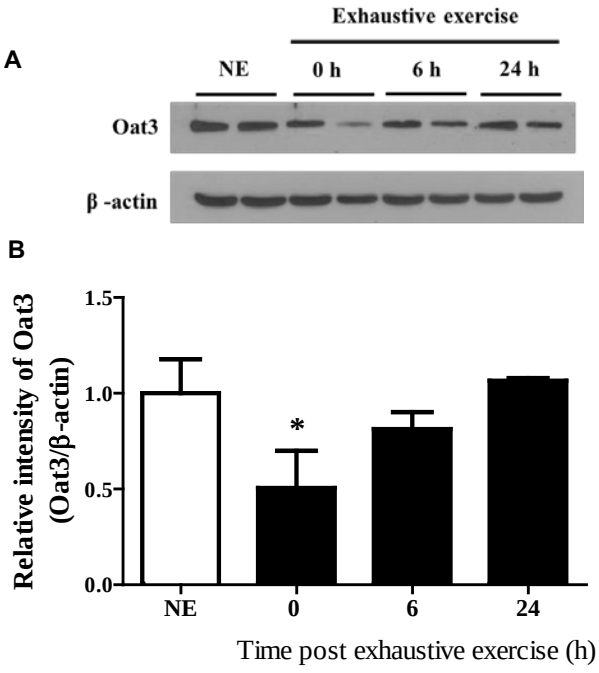

Figure 4. The recovery of OAT3 protein expression in renal cortex after exhaustive exercise. A: representative blots. B: densitometric quantification of Oat3 protein expression. Immediately (0 h), 6 and 24 h after exhaustive exercise, total renal cortex proteins were extracted and incubated with anti-Oat3 antibody. Oat3 protein expression was normalizes by β-actin and expressed as relative value to non-exercise group. Values are mean ± SEM (n= 6-8 rats per group). *P < 0.05 versus non-exercise group.

Conclusion and Discussion

To our knowledge, this is the first study to investigate effect of different types of exercise (exhaustive, acute and training exercise) on secretory function which focused on Oat1 and Oat3 transport activity. The uptake of [^{3}H]-PAH and [^{3}H]-ES into rat renal cortical slices was used to study Oat1 and Oat3 function in present study, respectively (14, 15).

In this study, we investigated whether exercise had an effect on Oat1 and Oat3 which is the rate-limiting step in organic anion secretion. The results demonstrate that exhaustive exercise decreased Oat3 transport activity in rat renal cortical slices. The reduction of Oat3 function after exhaustive exercise was accompanied by decreased Oat3 protein expression in renal cortex. However, Oat3 transport activity was gradually recovered at 6 h after exhaustive exercise. Acute and training exercise did not affect Oat3 transport function and protein expression, suggested that these types of exercise might not alter rat Oat3 secretory function. Whereas, acute, exhaustive and training exercise had no effect on Oat1 transport function in rat renal cortical slices. Previous study suggested that the urinary clearance of benzyl penicillin, which is Oat3 substrate, was suppressed by increasing physical activity or exercise (16). Taken together, exercise might not affect Oat1 transport function.

Since, Oat3 plays a significant role in renal tubular excretion of anionic drugs and endogenous substances. The impairment of Oat3 activity in the kidney resulted in the reduced renal secretion of some drugs (12, 13). The accumulation of drugs in the body could led to increase drug toxicity, especially in narrow therapeutic index drugs. Thus, the alteration of Oat3 transport activity after exhaustive exercise could affect pharmacokinetics of some drugs and plasma concentration.

Exhaustive exercise represents high intensity of exercise. In this study, sedentary rats performed treadmill running until exhausted which used the loss of righting reflex as exhaustive

index (17). Exhaustive exercise was introduced to examine effect of high intensity of exercise which performed by sedentary rat on Oats function. Acute exercise represents single episode of exercise. The intensity and duration of acute exercise were equivalent to moderate intensity of exercise. Acute exercise was induced to examine the acute physiological response on Oats function after single episode of exercise. Training exercise is the repeated episode of moderate intensity of exercise to obtain physiological adaptation including increased cardiovascular endurance and enhanced metabolic adaptation as well as control of body weight (1). Present study shows that after 8 weeks of training, trained rats had body weight lower than non-exercise rats (Table 1). This could be due to the adaptation to training on body weight.

Exhaustive, acute and training exercise did not induce any adverse effect on kidneys which indicated by the unchanged in physiological parameter (kidney weight) and renal functions (serum creatinine and BUN) (Table 2). However, the kidney weight/ body weight ratio was higher in training exercise group compared to non-exercise group (Table 1). The alteration of this ratio did not from exercise-induced kidney hypertrophy but it was adaptation effect of training on body weight.

As exhaustive exercise decreases Oat3 transport activity in rat renal cortical slices, we hypothesized whether this could be due to a downregulation of Oat3 protein expression in renal cortex decreases after exhaustive exercise. The amount of Oat3 protein expression decreased after exhaustive exercise. The reduced Oat3 protein expression is recovered 6 h after exhaustive exercise which accompanied by the restoration of Oat3 transport activity at 6 h after exercise. This is sufficient evidence to explain that the reduced Oat3 protein expression could account for the reduced Oat3 transport activity after exhaustive exercise.

It is well known that exercise disrupted body homeostasis and altered physiological parameters including changed in cardiac output, redistribution of blood flow to working muscle tissues, splanchnic tissues and kidneys (1). At rest, approximately 22% of cardiac output is distributed to kidneys. During exercise, blood flow is redistributed to working muscle tissues. Exercise decreases renal blood flow in intensity dependent manner. Approximately 10%, 3% and 1% of cardiac output is redistributed to kidneys during light, moderate and high intensity of exercise, respectively (2). Training exercise maintains greater blood flow to kidneys and splanchnic tissues during exercise (18). In present study, intensity of acute exercise is equivalent to moderate intensity whereas, exhaustive exercise is high intensity of exercise. As mentioned earlier, acute and

training exercise did not alter Oat3 transport activity whereas, only exhaustive exercise reduced Oat3 activity. Therefore, renal blood flow may contribute to reduce Oat3 transport activity and protein expression after exercise. Previous study showed the reduction of organic anion *p*-aminohippurate (PAH) secretion in ischemic acute renal failure (iARF) of rat model (12). The impairment of organic secretion after ischemia-reperfusion injury was associated with decreased mRNA and protein of Oat1 and Oat3 (12). iARF induced by bilateral clamping of renal arteries for 45 min whereas renal blood flow after exhaustive exercise is approximately 1% of cardiac output (2, 12). Present study, rats performed exhaustive exercise until exhaustion which taken approximately 21 min. Taken together, the reduced renal blood flow after exhaustive exercise may partially contribute to reduced Oat3 transport activity and protein expression.

Previous study found that activation of α_1-adrenergic receptor by phenylephrine reduces [^{3}H]-ES uptake into renal proximal tubule isolated from rabbit (19). In addition to pharmacological agonist, the α_1-adrenergic receptor was stimulated by norepinephrine and epinephrine. In response to exercise, activation of sympathetic activity produces vasoconstriction via stimulation of α_1-adrenergic receptor and results in redistribution of blood to splanchnic tissues and kidneys. During exercise, level of norepinephrine and epinephrine progressively increased in exercising skeletal muscle, heart, kidney and liver in intensity-dependent manner (20-22). During high intensity exercise, plasma norepinephrine and epinephrine were increased approximately 14 and 8 times compared with rest. Whereas, resting total and renal plasma norepinephrine spillover were reduced after 1-month endurance exercise training, indicating that exercise training reduced whole-body and renal resting sympathetic activity (23). Taken together, exhaustive exercise increases plasma norepinephrine and epinephrine which may sufficient to stimulate α_1-adrenergic receptor and contribute to reduced Oat3 transport activity and protein expression. However, the exactly mechanism of exhaustive exercise reduced Oat3 activity need to be elucidated.

In summary, we provided the first evidence that exhaustive exercise decreased Oat3 transport function by downregulation its expression in kidney. Oat3 has a critical role in excretion of variety of anionic substances including anionic drugs, xenobiotic substances or toxic compounds. Thus, exhaustive exercise will affect pharmacokinetics of mentioned compounds. The significant of this finding provides the information concerning the interaction between exercise and renal secretory process. It would give benefic information for healthcare professional in taking care of patients who may be participate in exercise and has high risk of drug accumulation. In addition, it

would give further insight on individualization of drug dosing regimens to the patients who participate in irregular and regular exercise.

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Output จากโครงการวิจัยที่ได้รับทุนจาก สกว.

1. ผลงานตีพิมพ์ในวารสารวิชาการนานาชาติ

งานวิจัยอยู่ในขั้นตอนเตรียมและแก้ไข manuscript (ตามเอกสารแนบ ภาคผนวก) เพื่อตีพิมพ์วารสารวิชาการนานาชาติ โดยคาดว่าจะส่ง manuscript เพื่อตีพิมพ์ภายในเดือนกรกฎาคม 2560 และคาดว่าจะตีพิมพ์ในวารสาร Experimental Physiology (Impact factor 2016: 2.912) หากได้รับการ accept หรือตีพิมพ์จะส่งเอกสารการตอบรับและฉบับ reprint ให้ทาง สกว. ต่อไป

2. การนำผลงานไปใช้ประโยชน์

- <u>เชิงสาธารณะ</u> คาดว่าจะนำองค์ความรู้จากงานวิจัยที่ได้รับการตีพิมพ์ในวารสารวิชาการนานาชาติไปเผยแพร่ต่อสาธารณะชนทางการเขียนบทความลงนิตยสารเกี่ยวกับด้านสุขภาพ เพื่อให้บุคคลากรทางสาธารณสุขเกิดการตระหนักถึงความสัมพันธ์ระหว่างเภสัชจลศาสตร์ของยา (pharmacokinetics) และการออกกำลังกาย โดยเฉพาะการขับยาออกทางไต ซึ่งปกติส่วนใหญ่ เรามักจะตระหนักถึงยาที่ใช้ในการลดน้ำตาลในเลือดกับการออกกำลังกาย ซึ่งองค์ความรู้ใหม่นี้ จะทำให้บุคคลากรทางสาธารณสุขตระหนักถึงการให้โปรแกรมการออกกำลังกายกับผู้ป่วยมากขึ้น อีกทั้งการเผยแพร่ความรู้สู่ประชาชน จะทำให้ประชาชนตระหนักถึงวิธีการออกกำลังกายที่ถูกต้อง โดยเฉพาะในผู้ป่วยที่ไม่เคยออกกำลังกายมาก่อน ไม่ควรออกกำลังกายอย่างหนักในครั้งแรก เพราะอาจจะทำให้มีผลต่อการขับยาออกทางร่างกาย โดยเฉพาะทางไต แต่หากค่อยๆ เพิ่มระดับการออกกำลังกายหรือออกกำลังกายระดับความหนักปานกลาง จะไม่มีผลต่อการขับยาทางไต

- <u>เชิงวิชาการ</u> คาดว่าจะนำองค์ความรู้จากงานวิจัยที่ได้รับการตีพิมพ์ในวารสารวิชาการนานาชาติไปใช้ประโยชน์ โดยใช้อ้างอิงในการเรียนการสอนทั้งในภาคทฤษฎีและภาคการฝึกปฏิบัติงานทางคลินิก เนื่องจากผู้วิจัยเป็นอาจารย์คณะกายภาพบำบัด และได้สอนในรายวิชาเกี่ยวกับการดูแลผู้ป่วยหลังการผ่าตัดทางด้านกระดูกและกล้ามเนื้อและในผู้สูงอายุ และได้นำนักศึกษาฝึกปฏิบัติงานทางคลินิกในหอ

ผู้ป่วยศัลยศาสตร์ออโธปิดิก ซึ่งในการรักษาฟื้นฟูทางกายภาพบำบัดนั้น จะใช้การออกกำลังกายเพื่อฟื้นฟูร่างกายในระบบต่างๆ เพื่อให้ผู้ป่วยสามารถกลับมาใช้ชีวิตประจำวันได้อย่างใกล้เคียงปกติที่สุด ซึ่งผู้ป่วยเหล่านั้น จะได้รับการรักษาทางยาร่วมกับการออกกำลังกายเพื่อฟื้นฟูร่างกาย ผู้วิจัยจะนำองค์ความรู้ที่ได้จากงานวิจัยมาประยุกต์ในการสอนโดยให้ นักศึกษาตระหนักถึงความสัมพันธ์ระหว่างยาและการออกกำลังกาย โดยการออกกำลังกายอาจจะมีผลต่อระดับยาในเลือด โดยสอนให้นักศึกษาพิจารณายาว่า เมื่อใช้ร่วมกับออกกำลังกายจะมีผลหรือไม่ และให้ตระหนักเกี่ยวกับการให้โปรแกรมการออกกำลังกาย โดยเฉพาะในผู้ป่วยที่ไม่เคยออกกำลังกายมาก่อน หากให้ออกกำลังกายอย่างหนัก (exhaustive exercise) จะมีผลต่อการขับยาออกทางไต โดยเฉพาะยาที่เป็นประจุบวก ดังผลการทดลองที่ได้จากวิจัย ซึ่งจะทำให้นักศึกษามองค์รวมในการรักษาผู้ป่วยมากขึ้นและวิเคราะห์การรักษาจากบุคลากรทางการแพทย์ท่านอื่นร่วมด้วย เพื่อประโยชน์สูงสุดของผู้ป่วย

ภาคผนวก

Manuscript

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Effect of Exercise on Renal Secretory Function: Role of Organic Anion Transporter 3

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Abstract

Exercise induces certain physiological changes such as altered hemodynamic and reduced blood flow to kidneys. These changes would affect pharmacokinetics of some drugs. However, little is known about the interaction between exercise and renal excretory process. The objective of this study is to investigate the effect exercise on organic anion transporter 3 (Oat3) function, a major transporters expressed in basolateral membrane of renal proximal tubule. Male Wistar rats were randomly divided into 4 groups: non-exercise, acute exercise, exhaustive exercise and training exercise. After exercise, [^{3}H] estrone sulfate (ES) uptake into rat renal cortical slices were examined to study the renal Oat3 function. Acute and training exercise had no effect on [^{3}H]ES uptake into rat renal cortical slices. Whereas, exhaustive exercise reduced [^{3}H]ES uptake into rat renal cortical slices, suggested impairment of Oat3 function. The reduced in Oat3 function was gradual recovered at 6 h after exhaustive exercise. The impairment of Oat3 function after exhaustive exercise was accompanied by decreased renal Oat3 protein expression compare with non-exercise rats. Thus, the decreased in Oat3 protein expression is sufficient to explain the reduction in Oat3 function after exhaustive exercise. These results indicated that Exhaustive exercise decreased Oat3 function and associated with downregulation of Oat3 protein. In addition, this impairment of Oat3 function and expression was transient and gradual recovered at 6 h after exhaustive exercise. As a result, exhaustive exercise may have an impact on rat renal organic anion excretion.

(word count 237)

Introduction

Nowadays, physical activity or exercise is recommended for healthy individuals in order to improve their health and well-being. Moreover, exercise has been prescribed to individuals who are taking drugs for improved their health conditions and quality of life. It is well known that exercise induces several physiological changes and adaptation following exercise. During acute bout of exercise, cardiac output, heart rate, respiratory rate, metabolic enzyme activity and sympathetic nerve activity increase in order to meet energy demand (1). In addition, blood is redistributed from liver and kidneys to exercising muscles during exercise (2). Exercise training (repeated bout of exercise at least 3 time/week) induce several physiological adaptations including a decreased in heart rate, increased in plasma volume, cardiac muscle fiber size, skeletal muscle mass and muscle performance (1). These physiological changes following acute bout of exercise and exercise training may have an effect on pharmacokinetics of some drugs.

Pharmacokinetics describe the process of drug absorption, distribution, metabolism and elimination (3). Exercise has been shown to increase serum concentration of tetracycline, doxycycline, sulphamethizole and sulphadimidine (4, 5). Whereas, exercise decreased serum level of digoxin (6, 7). Taken together, exercise may have a significant effect on one or more aspects in drug pharmacokinetics. Exercise decreased blood flow to splanchnic tissues with corresponding an increased blood flow to exercising muscles (2). These changes may delay drug absorption and distribution due to decrease drug availability and delay gastric emptying (8). On

the other hand, exercise increased blood flow to exercising muscle which resulted in accelerating intramuscular-injected drug absorption (8). Drug metabolism occurs in liver. Exercise decreased drug delivery to liver. Therefore, exercise may decrease drug metabolism in liver. For drug elimination, exercise suppressed urinary excretion of tetracycline and doxycycline with a corresponding an increase serum level of these drugs (4, 5). Taken together, drug elimination seems to have major clinical significant in maintaining the plasma concentration. However, little information is available about the effect of exercise on renal drug elimination.

Kidney is the primary organ that responsible for clearance of drugs from the body. The drug excretion through kidney is involved glomerular filtration, tubular reabsorption and tubular secretion. It is well documented that renal blood flow decreased during exercise resulting in decreased glomerular filtration (8). Subsequently, drug bioavailability was reduced for excretion. While tubular reabsorption and secretion depend on renal transporters. The drug excretion via tubular reabsorption seem to be a minor clinical significant during exercise. However, little is known about the influence of exercise on renal tubular secretion.

Organic anion transporters (Oats), expressed in renal proximal tubule, has a significant role in the excretion of organic anions including drugs, endogenous substances and toxic compounds (9). Oats located at basolateral membrane of renal epithelia cells and responsible for uptake of organic anions from blood into cells across basolateral membrane (10). Subsequently, organic anions secrete from the cells into renal proximal tubule and excretion in urine. Several members of Oats have been identified. Among these, Oat3 have a major role in renal organic anion secretion (11). The impairment of Oat3 function resulted in decrease renal tubular secretion and increase serum concentration of some drugs (12, 13). The change in Oat3 function may affect renal drug elimination. Therefore, it is important to know that how exercise has an effect on renal

secretory function. The purpose of present study is to investigate effect of exercise on Oat3 transport function. The significant of this finding will provide the information concerning the interaction between exercise and renal excretory process. The finding would give benefic information for healthcare professional in taking care of patients who may be at a particular high risk of drug accumulation. In addition, it would give further insight on individualization of drug dosing regimens to the patients who participate in irregular and regular exercise.

Methods

Animals and experimental designs

All procedures were conducted in accordance with the Guidelines of the National Laboratory Animal Center of Thailand and approved by the Animal Care and Use Committee of Faculty of Science, Mahidol University. Male Wistar rats were housed in single standard cages in an environmental-controlled room (25 ± 2°C with 12 h-12h dark-light cycle) at Laboratory Animal Facility, Faculty of Science, Mahidol University. Rats were fed with standard rat chow and water ad libitum.

After 1-week acclimatization, male Wistar rats (age, 6 weeks; weight, 200-250g) were randomly divided into 4 groups as follows: non-exercise group (NE); exhaustive exercise group (EE); acute exercise group (AE); and training exercise group (TE).

Exercise protocols

Exhaustive exercise. On the day of experiment, rats were run on motorized treadmill at 16 m/min with 0° inclination. Running speed and inclination were adjusted to 24 m/min with 15° inclination for first 5 min. This running speed was maintained for 10 min. After 15 min, running speed was gradually increased to 38 m/min until exhaustion. Exhaustive sign was determined by

loss of righting reflex. Immediately (0 h), 6 h and 24 h after exhaustive exercise, kidneys were removed for subsequently analysis.

Acute exercise. On the day of experiment, rats were run on motorized treadmill for 60 min at speed of 20 m/min, 15° inclination. Immediately after exercise, kidneys were removed for subsequently analysis.

Training exercise. Rat were introduced to familiar with treadmill running for 5 days. The first 2 days, rats were run on motorized treadmill at a lowest speed (16 m/min, 0° inclination) for 10 min. For other 3 days, rats were run at 18 m/min, 0° inclination for 20 min. Running speed, inclination and duration were gradually increased to 20 m/min, 15° inclination for 60 min/day, 5 days/ week at the end of week 2. Rats were performed exercise training following this intensity for 5 day/week and training duration was 8 weeks. During exercise, rats were stimulated by tapping at their tails or blowing by dry air if they stop running. Rat kidneys were removed for subsequent analysis at 24 h after last exercise session to avoid effect of acute exercise.

Renal slice preparation and uptake study

The function of Oat3 were determined by the uptake of [^{3}H] estrone sulfate ([^{3}H]-ES). At the end of exercise session, rats were euthanized by sodium pentobarbital intraperitoneal injection. Kidneys were removed, decapsulated and maintained on ice-cold modified Cross and Taggart buffer (95 mM NaCl, 80 mM mannitol, 5 mM KCl, 0.74 mM $CaCl_2$, and 9.5 mM Na_2HPO_4, pH 7.4). The renal cortical slices (≤ 0.5 mm; 5-10 mg/slice) were cut using a Stadie-Rigge microtome and incubated in ice-cold buffer for 10 min. The slices were incubated in buffer containing 30 nM of [^{3}H]-ES at 37°C for 30 min. The uptake was stopped by washing 3 times with ice-cold buffer containing 1 mM unlabeled-ES. Slices were then blotted, weight, dissolved in 1N NaOH for 24 h and neutralized with 1N HCL. Tissue samples were determined for [^{3}H]-

ES using a Liquid Scintillation Analyzer (1214 Rackbeta, Wallac). The uptake was calculated as tissue per medium ratio (T/M) (dpm/mg of tissue ÷ dpm/µl uptake buffer) and expressed as a percentage of control.

Western blot analysis

The rat renal cortex was dissected and homogenized using a Polytron PT3100 homogenizer (Kinematica) in ice-cold homogenization buffer (300 mM sucrose, 25 mM imidazole, 1 mM EDTA, 1 mM PMSF, and complete protease inhibitor, pH 7.2). Tissue homogenates were centrifuged 12,000 rpm for 20 min at 4°C and supernatant was kept at -80°C for subsequent analysis. Supernatant was separated by 10% SDS-PAGE and transferred to a nitrocellulose membrane (Millipore, Billerica, MA, USA). The membranes were blocked with 5% non-fat dry milk in TBST (Tris-buffered saline, 0.1% tween 20) at room temperature for 1 h. The membranes were then incubated overnight with primary antibody (polyclonal rabbit anti-Oat3 antibody, 1:500 dilution, Cosmobio, Tokyo, Japan). After washing with TBST, the membranes were probed with horseradish peroxidase (HRP)-conjugated goat anti-rabbit IgG (1:3,000 dilution, Cell Signaling Technology, Danver, MA, USA) at room temperature for 1 h. The immunoreactivity was developed using an enhanced chemiluminescence (ECL) detection kit. The protein expression signals were quantified using Image J software (NIH, Bethesda, MD, USA).

Biochemical analysis

On the day of experiment, blood was collected using cardiac puncture technique under anesthesia. The serum was separated by centrifugation at 3,000 rpm at 4°C for 10 min. These samples were used to measure serum blood urea nitrogen (BUN) and serum creatinine as

parameters indicative of renal function. BUN and serum creatinine were measured by enzymatic colorimetric techniques using commercial kits (Biotech, Bangkok, Thailand).

Materials

Chemicals were purchased from Sigma Chemical Co. (St. Louis, MO, USA) and Merck (Darmstadt, Germany). [^{3}H]-ES were purchased from Perkin Elmer (Waltham, MA, USA). Polyclonal rabbit anti-Oat3 antibody were purchased from Cosmobio (Tokyo, Japan).

Statistical analysis

All data are expressed as mean ± SE. For multiple comparison, data were analysed with one-way ANOVA followed by Bonferroni post hoc test. The level of significant difference was set at $P < 0.05$.

Results

Effect of different types of exercise on general characteristics. The general characteristics of non-exercise, exhaustive, acute and training rats were determined. As shown in Table 1, all experimental rats were age-matched controls (15 weeks old). Acute and exhaustive exercise had no effect on rat body weight when compared with non-exercise rats. Training exercise had significant reduced in rat body weight compared with non-exercise rats ($p < 0.05$). All exercises had no effect on kidney weight. However, kidney/body weight ratio significantly increased in trained rats compared to non-exercised rats ($p < 0.001$). The significant increase in kidney/body weight ratio in trained rats may due to the decrease in body weight from adaptation to training.

Effect of different types of exercise on Oat1 and Oat3 function. The function of Oat3 was determined by measured the [3H]-PAH and [3H]-ES uptake in rat renal cortical slices. As shown

in Fig. 1. There was no significant difference in [^{3}H]-ES uptake in rat renal cortical slices among non-exercise, acute and training exercise groups. Whereas, exhaustive exercise significantly decreased [^{3}H]-ES uptake in rat renal cortical slices compared to non-exercise ($p < 0.05$). These results indicated that exhaustive exercise decreased Oat3 function.

Decline in Oat3 function after exhaustive exercise was not due to reduction of renal function. To examine whether decreases in Oat3 transport activity after exhaustive exercise caused by exercise-induced renal dysfunction. Serum creatinine and BUN were used to determined renal function. As shown in Table 2, exhaustive, acute and training exercise did not affect serum creatinine and BUN when compared to non-exercise. This finding implied that the reduction in Oat3 function after exhaustive exercise was not due to exercise-induced renal dysfunction.

The recovery of Oat3 function after exhaustive exercise. The data above showed that immediately after exhaustive exercise decreased [^{3}H]-ES uptake in rat renal cortical slices. To assess the recovery of Oat3 function after exhaustive exercise. Immediately (0 h), 6 h and 24 h after exhaustive exercise, renal cortical slices were prepared and used for measured [^{3}H]-ES uptake study. Immediately after exhaustive exercise significantly decreased [^{3}H]-ES uptake into rat renal cortical slices compared to non-exercise ($p < 0.05$) (Fig. 2). Whereas [^{3}H]-ES uptake into rat renal cortical slices gradually recovered at 6 h and 24 h after exhaustive exercise. These results supported that the decrease of Oat3 transport activity after exhaustive exercise was temporary decline and function could recover at 6 h after exhaustive exercise.

Effect of exercise on Oat3 protein expression. The amount of Oat3 protein expression in whole renal cortex extracted was used to determine the mechanism of exhaustive exercise induced-reduction of Oat3 transport function. As indicated in Fig. 3B, the relative protein expression of Oat3 was significantly decreased at immediately after exhaustive exercise compared to non-

exercise ($p < 0.05$). Whereas there was no difference in protein expression after acute and training exercise.

In addition, the relative protein expression of Oat3 was gradually recovered at 6h and 24 h after exhaustive exercise compared to non-exercise (Fig. 4B). Theses finding indicated that the decrease of Oat3 transport activity after exhaustive exercise may be mediated by decrease in Oat3 protein expression.

Discussion

In this study, we investigated whether exercise had an effect on Oat3 function which is the rate-limiting step in organic anion secretion. The results demonstrate that exhaustive exercise decreased Oat3 transport activity in rat renal cortical slices. The reduction of Oat3 function after exhaustive exercise was accompanied by decreased Oat3 protein expression in renal cortex. However, Oat3 transport activity was gradually recovered at 6 h after exhaustive exercise. Acute and training exercise did not affect Oat3 transport function and protein expression, suggested that these types of exercise may not alter rat Oat3 secretory function. Previous study suggested that the urinary clearance of benzyl penicillin, which is Oat3 substrate, was suppressed by increasing physical activity or exercise (16).

To our knowledge, this is the first study to investigate effect of different types of exercise (exhaustive, acute and training exercise) on secretory function which focused on Oat3 transport activity. The uptake of [^{3}H]-ES into rat renal cortical slices was used to study Oat3 function in present study, respectively (14, 15).

Since, Oat3 plays a significant role in renal tubular excretion of anionic drugs and endogenous substances. The impairment of Oat3 activity in the kidney resulted in the reduced renal secretion of some drugs (12, 13). The accumulation of drugs in the body could led to increase drug toxicity, especially in narrow therapeutic index drugs. Thus, the alteration of Oat3 transport activity after exhaustive exercise could affect pharmacokinetics of some drugs and plasma concentration.

Exhaustive exercise represents high intensity of exercise. In this study, sedentary rats performed treadmill running until exhausted which used the loss of righting reflex as exhaustive index (17). Exhaustive exercise was introduced to examine effect of high intensity of exercise which performed by sedentary rat on Oats function. Acute exercise represents single episode of exercise. The intensity and duration of acute exercise were equivalent to moderate intensity of exercise. Acute exercise was induced to examine the acute physiological response on Oats function after single episode of exercise. Training exercise is the repeated episode of moderate intensity of exercise to obtain physiological adaptation including increased cardiovascular endurance and enhanced metabolic adaptation as well as control of body weight (1). Present study shows that after 8 weeks of training, trained rats had body weight lower than non-exercise rats (Table 1). This could be due to the adaptation to training on body weight.

Exhaustive, acute and training exercise did not induce any adverse effect on kidneys which indicated by the unchanged in physiological parameter (kidney weight) and renal functions (serum creatinine and BUN) (Table 2). However, the kidney weight/ body weight ratio was higher in training exercise group compared to non-exercise group (Table 1). The alteration of this ratio did not from exercise-induced kidney hypertrophy but it was adaptation effect of training on body weight.

As exhaustive exercise decreases Oat3 transport activity in rat renal cortical slices, we hypothesized whether this could be due to a downregulation of Oat3 protein expression in renal cortex decreases after exhaustive exercise. The amount of Oat3 protein expression decreased after exhaustive exercise. The reduced Oat3 protein expression is recovered 6 h after exhaustive exercise which accompanied by the restoration of Oat3 transport activity at 6 h after exercise. This is sufficient evidence to explain that the reduced Oat3 protein expression could account for the reduced Oat3 transport activity after exhaustive exercise.

It is well known that exercise disrupted body homeostasis and altered physiological parameters including changed in cardiac output, redistribution of blood flow to working muscle tissues, splanchnic tissues and kidneys (1). At rest, approximately 22% of cardiac output is distributed to kidneys. During exercise, blood flow is redistributed to working muscle tissues. Exercise decreases renal blood flow in intensity dependent manner. Approximately 10%, 3% and 1% of cardiac output is redistributed to kidneys during light, moderate and high intensity of exercise, respectively (2). Training exercise maintains greater blood flow to kidneys and splanchnic tissues during exercise (18). In present study, intensity of acute exercise is equivalent to moderate intensity whereas, exhaustive exercise is high intensity of exercise. As mentioned earlier, acute and training exercise did not alter Oat3 transport activity whereas, only exhaustive exercise reduced Oat3 activity. Therefore, renal blood flow may contribute to reduce Oat3 transport activity and protein expression after exercise. Previous study showed the reduction of organic anion p-aminohippurate (PAH) secretion in ischemic acute renal failure (iARF) of rat model (12). The impairment of organic secretion after ischemia-reperfusion injury was associated with decreased mRNA and protein of Oat1 and Oat3 (12). iARF induced by bilateral clamping of renal arteries for 45 min whereas renal blood flow after exhaustive exercise is

approximately 1% of cardiac output (2, 12). Present study, rats performed exhaustive exercise until exhaustion which taken approximately 21 min. Taken together, the reduced renal blood flow after exhaustive exercise may partially contribute to reduced Oat3 transport activity and protein expression.

Previous study found that activation of α1-adrenergic receptor by phenylephrine reduces [3H]-ES uptake into renal proximal tubule isolated from rabbit (19). In addition to pharmacological agonist, the α1-adrenergic receptor was stimulated by norepinephrine and epinephrine. In response to exercise, activation of sympathetic activity produces vasoconstriction via stimulation of α1-adrenergic receptor and results in redistribution of blood to splanchnic tissues and kidneys. During exercise, level of norepinephrine and epinephrine progressively increased in exercising skeletal muscle, heart, kidney and liver in intensity-dependent manner (20-22). During high intensity exercise, plasma norepinephrine and epinephrine were increased approximately 14 and 8 times compared with rest. Whereas, resting total and renal plasma norepinephrine spillover were reduced after 1-month endurance exercise training, indicating that exercise training reduced whole-body and renal resting sympathetic activity (23). Taken together, exhaustive exercise increases plasma norepinephrine and epinephrine which may sufficient to stimulate α1-adrenergic receptor and contribute to reduced Oat3 transport activity and protein expression. However, the exactly mechanism of exhaustive exercise reduced Oat3 activity need to be elucidated.

In summary, we provided the first evidence that exhaustive exercise decreased Oat3 transport function by downregulation its expression in kidney. Oat3 has a critical role in excretion of variety of anionic substances including anionic drugs, xenobiotic substances or toxic compounds. Thus, exhaustive exercise will affect pharmacokinetics of mentioned compounds. The significant

of this finding provides the information concerning the interaction between exercise and renal secretory process. It would give benefic information for healthcare professional in taking care of patients who may be participate in exercise and has high risk of drug accumulation. In addition, it would give further insight on individualization of drug dosing regimens to the patients who participate in irregular and regular exercise.

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Table 1. General characteristics of experimental rats.

	Age (weeks)	Body weight (g)	Kidney weight (g)	Kidney/body weight ratio
Non-exercise (NE)	15	502.02 ± 12.71	1.31 ± 0.03	0.26 ± 0.01
Exhaustive exercise (EE)	15	496.47 ± 13.62	1.35 ± 0.03	0.27
Acute exercise (AE)	15	512.93 ± 11.04	1.37 ± 0.02	0.27 ± 0.01
Training exercise (TE)	15	467.01 ± 6.54*	1.37 ± 0.04	0.30 ± 0.01***

Values are means ± SEM (n = 7-12 rats per group). $^{*}P < 0.05$, $^{***}P < 0.001$ versus non-exercise group.

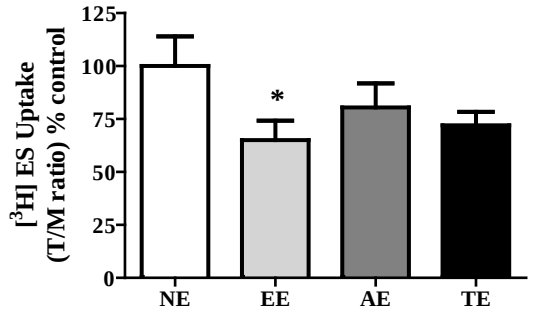

Figure 1. Effect of different types of exercise on [^{3}H]-ES uptake in rat renal cortical slices. Rat renal cortical slices from NE, EE, AE and TE were incubated in medium containing [^{3}H]-ES for 30 min. The uptake was calculated as the tissue-to-medium ratio and expressed a percentage of control. Values are mean ± SEM (n= 6-10 rats per group). *$P < 0.05$ versus non-exercise.

Table 2. Effect of different types of exercise on renal function parameters.

	Serum creatinine (mg/dl)	BUN (mg/dl)
Non-exercise (NE)	0.29 ± 0.06	37.58 ± 1.47
Exhaustive exercise (EE)	0.38 ± 0.06	38.72 ± 1.83
Acute exercise (AE)	0.24 ± 0.06	40.95 ± 2.47
Training exercise (TE)	0.25 ± 0.05	36.67 ± 1.39

Values are means ± SEM (n = 7-9 rats per group). Abbreviation: BUN, blood urea nitrogen.

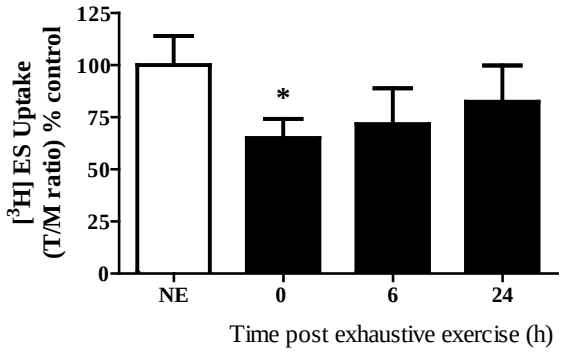

Figure 2. The recovery of Oat3 mediated-[^{3}H]-ES uptake in rat renal cortical slices after exhaustive exercise. Immediately (0 h), 6 and 24 h after exhaustive exercise, rat renal cortical slices were prepared and [^{3}H]-ES uptake was determined as described above. The uptake was calculated as the tissue-to-medium ratio and expressed a percentage of control. Values are mean ± SEM (n= 8-10 rats per group). $^*P < 0.05$ versus non-exercise group.

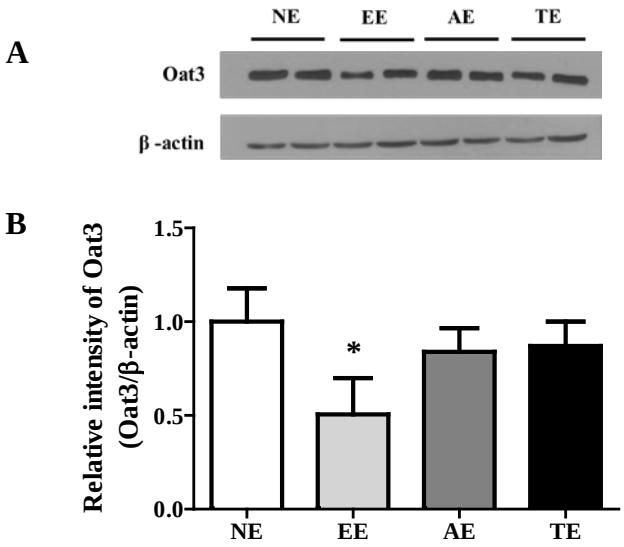

Figure 3. Effect of different types of exercise on Oat3 protein expression in renal cortex. A: representative blots. B: densitometric quantification of Oat3 protein expression. At the end of exercise session, total renal cortex proteins were extracted and incubated with anti-Oat3 antibody. Oat3 protein expression was normalizes by β-actin and expressed as relative value to non-exercise group. Values are mean ± SEM (n= 6-8 rats per group). *P < 0.05 versus non-exercise group.

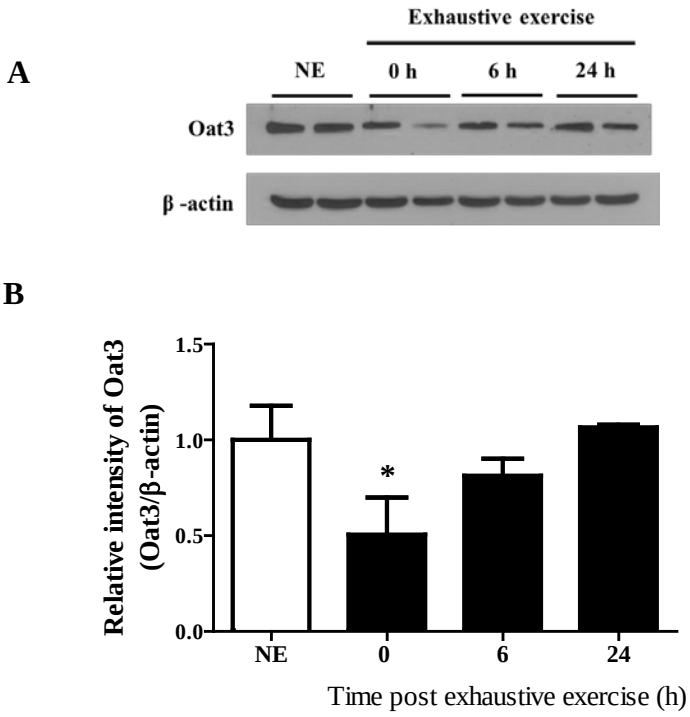

Figure 4. The recovery of OAT3 protein expression in renal cortex after exhaustive exercise. A: representative blots. B: densitometric quantification of Oat3 protein expression. Immediately (0 h), 6 and 24 h after exhaustive exercise, total renal cortex proteins were extracted and incubated with anti-Oat3 antibody. Oat3 protein expression was normalizes by β-actin and expressed as relative value to non-exercise group. Values are mean ± SEM (n= 6-8 rats per group). *$P < 0.05$ versus non-exercise group.