

## Sub-Maximal Exercise Alters the Prednisolone Absorption Pattern

Kuei-Yu Chien<sup>1</sup>, Ting-Ting Chen<sup>2</sup>, Jia Hsu<sup>3</sup>, Ryh-Nan Pan<sup>4</sup>, Jih-Heng Li<sup>5</sup>, Chia-Hua Kuo<sup>6</sup>, Mei-Chieh Hsu<sup>3\*</sup>

<sup>1</sup> Department of Geriatric Health Promotion, Kainan University, Taoyuan, Taiwan.

<sup>2</sup> Graduate Institute of Coaching Sciences, National Taiwan Sport University, Taoyuan, Taiwan.

<sup>3</sup> Graduate Institute of Sports Science, National Taiwan Sport University, Taoyuan, Taiwan.

<sup>4</sup> Department of Disaster Management, Taiwan Police College, Taipei, Taiwan.

<sup>5</sup> College of Pharmacy, Kaohsiung Medical University, Kaohsiung, Taiwan.

<sup>6</sup> Laboratory of Exercise Biochemistry, Taipei Physical Education College, Taipei, Taiwan.

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**ABSTRACT – Purpose.** Prednisolone has been widely used due to its relatively short pituitary inhibition and its moderate potency. Exercise is one of the non-pharmacological interventions for glucocorticoid-induced osteoporosis prevention. However, the effects of exercise on the pharmacokinetics of prednisolone are unclear. The purpose of this study was to evaluate the effects of sub-maximal exercise on the pharmacokinetics of prednisolone. **Methods.** A randomized, cross-over experimental design was used. Subjects needed to undertake two trials: a non-exercise trial (NE) and an exercise trial (E). After the first blood sampling, the subjects were administered orally 5mg of prednisolone with 100 mL of water and then took a rest for 30 min. For the E trial, subjects cycled at 70%  $VO_{2max}$  intensity until exhaustion. For the NE trial, they remained seated for the duration of the experiment. Serial blood samples were collected at 0.25, 0.5, 0.75, 1, 1.25, 1.5, 2, 3, 4, 5, 6, 8, 10 and 12 hr after prednisolone administration. Prednisolone and cortisol concentration were analyzed with a validated high performance liquid chromatographic method. **Results.** This study indicated that the maximum concentration of the E trial was significantly lower than the NE trial ( $p < .05$ ). However, the area under the plasma concentration-time, half life, the time-averaged total body clearance, and the apparent volume of the distribution showed no significant difference between two trials. The mean percentage change of cortisol of E trail was significantly higher than NE trail ( $p < .05$ ). **Conclusions.** The results suggested that sub-maximal exercise altered the prednisolone absorption pattern.

## INTRODUCTION

Glucocorticoids (GCs) are used in a wide variety of acute disorders and chronic treatment, including inflammation asthma, rheumatoid arthritis, acne flare-up, and other diseases (1-3). GC-induced osteoporosis is one of the most common forms of secondary osteoporosis (1) as well as one of the most serious complications (4). Moreover, muscle weakness and atrophy are the potential consequences of oral GC therapy (5). Prednisolone was the GC derivative chosen because of its relatively short pituitary inhibition and its moderate potency (6,7). Epidemiological studies have found increases in the risk of fracture in prednisolone users; even daily doses of 2.5 mg prednisone have been associated with the increases in the risk of fractures (5). In order to minimize the systemic side effects of GCs, the antedugs of GCs have been developed. Studies revealed that the antedugs of steroidal  $\beta$ -isomers exhibited much faster

hydrolysis rates than those of their corresponding  $\alpha$ -isomers (8,9,10). It is well known that exercise increases bone mineral density and muscle strength. Therefore, exercise may serve as an alternative approach to decrease systemic side effects of GCs.

Exercise alters cardiac output and blood distribution in skeletal muscles, skin, digestive organ, kidney, and liver, that consequently may alter the pharmacokinetics of a drug (11,12). One study showed that the absorption of midazolam was reduced by moderate exercise (13).

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**Corresponding Author:** Mei-Chieh Hsu, Graduate Institute of Sports Science, National Taiwan Sport University, 250, Wen Hua 1st Road, Kueishan, Taoyuan, Taiwan 33301. Email address: [meichich@gmail.com](mailto:meichich@gmail.com)

Contrarily, the rates of absorption of tetracycline, doxycycline and sulphamethizol were enhanced during a basketball game (14). A recent study showed that swimming before the administration of metformin significantly improved the rate of metformin absorption (15). These clearly revealed that the pharmacokinetic effects by exercise were unexpected.

Exercise can increase the endogenous cortisol concentration (16). The competitive inhibition of prednisolone protein binding by cortisol has been shown (17,18). Five milligram of prednisolone provided approximately 30 % suppression of salivary cortisol secretion and 10 % suppression of plasma cortisol (19). Thus, when subjects perform exercise and subsequently are administered prednisolone, the levels of prednisolone and cortisol would be changed. To our best knowledge, the effect of exercise on the pharmacokinetics of prednisolone has not yet been clarified. This study was designed to investigate the effects of 70 %  $\dot{V}O_{2max}$  exercise on the pharmacokinetics of prednisolone in men. The change of cortisol concentration in regard to the effect of prednisolone pharmacokinetics was also examined.

## METHODS

### Materials

Prednisolone and betamethasone were purchased from Sigma-Aldrich Chemical (St Louis, MO, USA). Potassium dihydrogen phosphate, potassium hydroxide, and triethylamine were obtained from Merck (Darmstadt, Germany). Diethyl ether and HPLC-grade acetonitrile were purchased from J.T. Baker (Phillipsburg, NJ, USA). Doubly deionized water was obtained from a Millipore Direct-Q5 system (Bedford, MA, USA) and was used throughout the study.

### Subjects

Eight healthy male volunteers with a mean age ( $\pm$  standard deviation, SD) of  $20 \pm 2.6$  yr, height of  $170.1 \pm 6.9$  cm, weight of  $65.6 \pm 5.6$  kg, and maximal oxygen consumption ( $\dot{V}O_{2max}$ ) of  $39.5 \pm 3.9$  mL/kg/min participated in this study. Written voluntary consent was obtained from all participants after informing them the protocols. This study received the approval of the ethics committee of National Sport University. Medical histories of subjects were examined, and those with a history of cardiac or liver disease were excluded

based on a physical examination. Subjects were asked to maintain consistent exercise and diet. They were not allowed to smoke, drink alcohol, and take other drugs or supplements throughout the period of experiment. The subjects were refrained from any moderate to high intensity exercise at least 24 hr and required overnight fasting for at least 8 hr before the experiment testing.

### Study design

A randomized, cross-over experimental design was used. Subjects were randomized into either non-exercise trial (NE) or exercise trial (E). All subjects participated in both trials. Two trials were separated by a period of one week. Testing was performed at same time of day (8:00~10:00) for the two trials to account for the diurnal variation in hormone responses.

An incremental test was used to determine  $\dot{V}O_{2max}$ . All exercise testing was conducted on a mechanically braked cycle ergometer (Monark 828E, Stockholm, Sweden). Pedal frequency was maintained at a constant 60 rpm for all cycle tests. The  $\dot{V}O_{2max}$  was determined from breath-by-breath on a SensorMedics Vmax 29c Metabolic Measurement Cart (Yorba Linda, CA, USA). Before each test, the  $O_2$  and  $CO_2$  analysis systems were calibrated using ambient air and a gas of known  $O_2$  and  $CO_2$  concentrations according to the manufacturer's instructions. Subjects performed incremental exercise (3-min stages) to volitional exhaustion, to determine  $\dot{V}O_{2max}$ . The subjects began the test at 1.0 kp, increasing in power increments of 0.5 kp at each stage. Each subject was encouraged to give his maximum effort. The attainment of the  $\dot{V}O_{2max}$  was at least two of the three following criteria: (1) Respiratory exchange ratio was greater than 1.1; (2) Subject could not maintain a cadence 60 rpm; and (3) Subject's rating of perceived exertion scale was above 18 or 19. After 1 week, the effect of exercise on the prednisolone pharmacokinetics study was determined at 70 %  $\dot{V}O_{2max}$  constant work load.

### Blood sampling

After the first blood sampling via an indwelling antecubital catheter, the subjects were orally administered 5mg of prednisolone (Taiwan Biotech Co., Taiwan) with 100 mL of water and then sat for 30 min. In the E trial, subjects cycled until exhaustion at 70 %  $\dot{V}O_{2max}$  intensity with 60 rpm pedal rates. For the NE trial, the subjects remained

seated for the duration of the experiment. All of the subjects drank 100 mL water every 15 min during the 45-90 min period following prednisolone administration. Then they drank 300 mL water per 2 hr, from 2 to 12 hr. The meals ( $\approx 800$  kcal) were provided to subjects at 4 hr and 8 hr after drug administration. Serial blood samples were collected at 0.25, 0.5, 0.75, 1, 1.25, 1.5, 2, 3, 4, 5, 6, 8, 10, and 12 hr after prednisolone administration.

#### Determination of prednisolone and cortisol concentrations

Blood prednisolone and cortisol concentrations were determined by an HPLC method modified from Majid et al. (20) and Volin (21). Briefly, the blood samples were immediately centrifuged at 3500 rpm for 10 min. The plasma samples were deproteinated by 50  $\mu$ L NaOH (250 mM) with vortex for 3 min. Then, the samples were extracted twice with 4 mL of diethyl ether. The samples with diethyl ether were shaken for 10 min, and centrifuged at 3500 rpm for 5 min. The upper layers (2.7 mL) were transferred to a new tube. The mixtures of the two upper layers were evaporated to dryness under nitrogen stream at 40°C. The remaining residues were dissolved in 120  $\mu$ L of mobile phase A and then injected into an HPLC system. The HPLC system (Shimadzu, Kyoto, Japan) consisted of SIL-10A autoinjector, SPD-M10AVP photodiode-array detector, LC-10AD pump, DGU-4A degasser, CTO-10A oven, and CBM-10A communications bus module. This method used betamethasone as the internal standard. A Lichrospher C<sub>18</sub> reversed-phase column (25 cm x 4 mm i.d., 5 $\mu$ m particle size, Merck, Darmstadt, Germany) was used. A mixture of acetonitrile and 0.714 mM potassium dihydrogen phosphate (30: 70, v/v) was used as mobile phase A. A mixture of acetonitrile and 1.67 mM potassium dihydrogen phosphate (70: 30, v/v) was used as mobile phase B. The pH of buffer was adjusted to 6 by triethylamine (TEA) in mobile phases both A and B. A gradient elution program is presented in Table 1. The chromatographic separation was performed at 38°C and monitored with UV detection at 234 nm. All of the concentrations were calculated from standard curve of prednisolone obtained from spiked plasma samples.

#### Method validation

The limits of quantification (LOQ) for prednisolone in plasma was established as the lowest concentration that the method can detect with a consistent response to actual solution concentration. The signal-to-noise ratio for limits of detection (LOD) was greater than 3. Accuracy and precision of this method were evaluated by preparing samples with known concentrations of prednisolone. Intra-day and inter-day precision were assessed and expressed in terms of RSD (relative standard deviation), whereas accuracy was expressed in terms of DFA (difference from the actual value). The intra-day and inter-day precision of the analytical method was studied with six replicates within one day and once daily for six days. The recoveries were calculated by comparing observed peak areas in extracted plasma to those of non-process standard solution.

**Table 1. Gradient elution program**

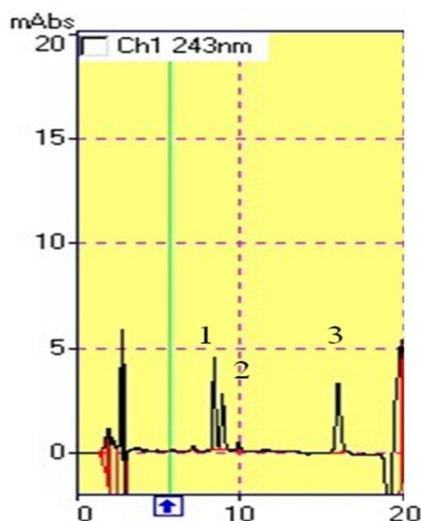
Time (minutes)	Flow rate (mL/min)	Mobile phase A (%)	Mobile phase B (%)
0-15	1	100	0
16-24	1.5	0	100
25-31	1.5	100	0
32-35	1	100	0

#### Statistical analysis

Pharmacokinetic data were analyzed using the WinNonlin program (version 1.1, Pharsight Corp., Mountain View, CA, USA) by a non-compartmental analytical method. The concentration-time profiles were used to determine the peak plasma concentrations ( $C_{max}$ ) and the time to peak plasma concentration ( $T_{max}$ ). The area under the concentration-time curve from  $t=0$  to the last measured concentration ( $AUC_{0-10}$ ) was calculated by the linear trapezoidal rule. The  $AUC_{0-\infty}$  was determined by extrapolation of  $AUC_{0-10}$  to infinity. The half-life ( $t_{1/2}$ ), apparent volume of the distribution ( $V_{d/F}$ ), and the time-averaged total body clearance ( $Cl_F$ ) of prednisolone were derived from the AUC and  $k$  values. All of the values were expressed as the mean  $\pm$  SD. The numerical data were analyzed using a paired t-test. A level of  $p < .05$  was set as significant on all tests. The statistical software used was SPSS 11.0 Statistics Program (Chicago, IL, USA).

## RESULTS

A typical chromatogram of prednisolone, cortisol, and betamethasone in plasma is shown in Figure 1. Standard curve for prednisolone was linear across the range of plasma assayed (15-250 ng/mL). The correlation coefficient of the standard curve was 0.9996. LOQ and LOD were 15 ng/mL and 5 ng/mL, respectively. The average extraction recoveries ranged from 69 to 75% (Table 2). The linear range of cortisol was 15-250 ng/mL with a regression coefficient of 0.9999.



**Figure 1.** HPLC chromatogram of prednisolone (peak 1), cortisol (peak 2), and betamethasone (peak 3).

Table 2 also shows the precision, accuracy, and recovery of the analytical method for the quantification of prednisolone. The intra-day variability was determined by analyzing six replicate controls prepared in blank urine spiked at 15, 100, 250 ng/mL on a single day. The intra-day precision and accuracy was 3.59 % to 6.44 % and -6.42 % to -1.69 %, respectively. The inter-day variability was determined in 6 different days using the same concentrations. The inter-day precision and accuracy was 0.91 % to 10.72 % and -1.77 % to -0.26 %, respectively. These results demonstrated that this method was suitable for quantification of prednisolone in plasma with satisfactory precision and accuracy.

## DISCUSSION

To our knowledge, this is the first human clinical

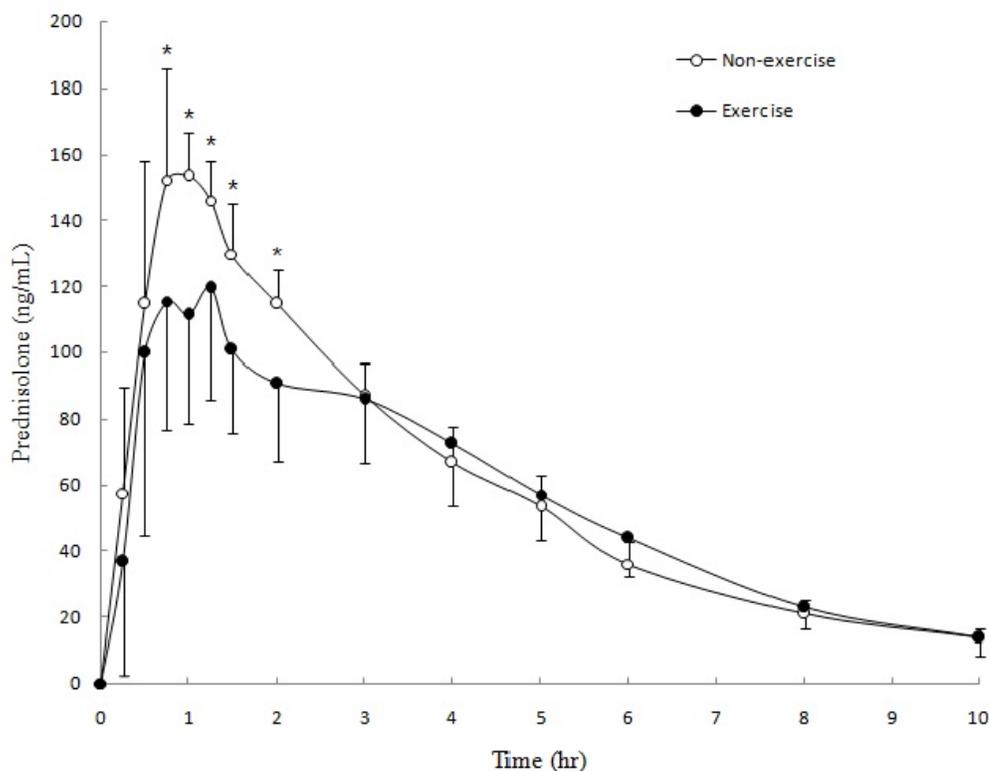
trial investigating the effect of exercise on the pharmacokinetics of prednisolone. The major finding of this study was that sub-maximum exercise (70%  $\dot{V}O_{2max}$ ) decreased the  $C_{max}$  of prednisolone (Table 3). Orally ingested prednisolone is almost completely absorbed by the intestine (22). It is clear that the stomach-emptying rate, gastric intestinal mobility, and blood flow to the absorption site all affect the rate and extent of drug absorption. Exercise may have effects on drug absorption (12). A published study showed that exercise intensity above 70%  $\dot{V}O_{2max}$  reduced the stomach-emptying rate, which probably delayed drug absorption (23). Moreover, blood flow is important in carrying absorbed drugs via systemic circulation. It is shunted away from the viscera (i.e. the stomach, liver, etc.) towards the muscles during moderate exercise (24). Our study showed that the  $C_{max}$  of the E trial was significantly lower than the NE trial. We ascribe the possible reasons to the exercise reduced gastric emptying rate and blood flow in intestine.

A lower peak level of total prednisolone concentration occurred in the E trial, and there was a tendency for the levels to fall more slowly than in the NE trial (Figure 2). This suggests a different pattern of absorption. The area under the curve was similar in the two trials (NE:  $599.2 \pm 16.6$  hr . ng/mL; E:  $562.3 \pm 30.9$  hr . ng/mL) even though the prednisolone concentration-time curves were lower in five out of eight subjects in the E trial when compared with those in the NE trial (Table 3). A previous study showed the physical activity increased drug concentration variation (25). The higher variability of prednisolone AUC was observed in E trail (% coefficient of variation = 15.55 %) versus NE trail (% coefficient of variation = 7.83 %). Thus, a larger change in prednisolone AUC would have been necessary in order to identify a significant difference in prednisolone AUC in the E trial. Area under the plasma concentration-time curve is a reflection of the total amount of drug absorption. Previous studies showed the efficacy of drug depends on total exposure rather than  $C_{max}$ . (26,27). On the other hand, a study demonstrated that drug side effects may be reduced by lowering the peak blood level of drug concentration (27). The increased exposure to prednisolone increased side effects may be apparent at low dosage (28). The long-term use predisonlone side effects occurred more than short- term use with the same dose (29).

**Table 2.** Precision, accuracy and recovery of prednisolone in plasma.

Spiked, ng/mL	Intra-day (n=6)			Inter-day (n=6)			%Recovery, n=3 ( )
	Mean±SD	%RSD	%DFA	Mean±SD	%RSD	%DFA	
15	14.75±0.95	6.44	-1.69	14.82±1.59	10.72	-1.21	69.0±7.5
100	95.37±3.43	3.59	-4.63	98.23±2.95	3.00	-1.77	69.1±3.9
250	233.95±9.87	4.22	-6.42	249.36±2.26	0.91	-0.26	74.5±4.1

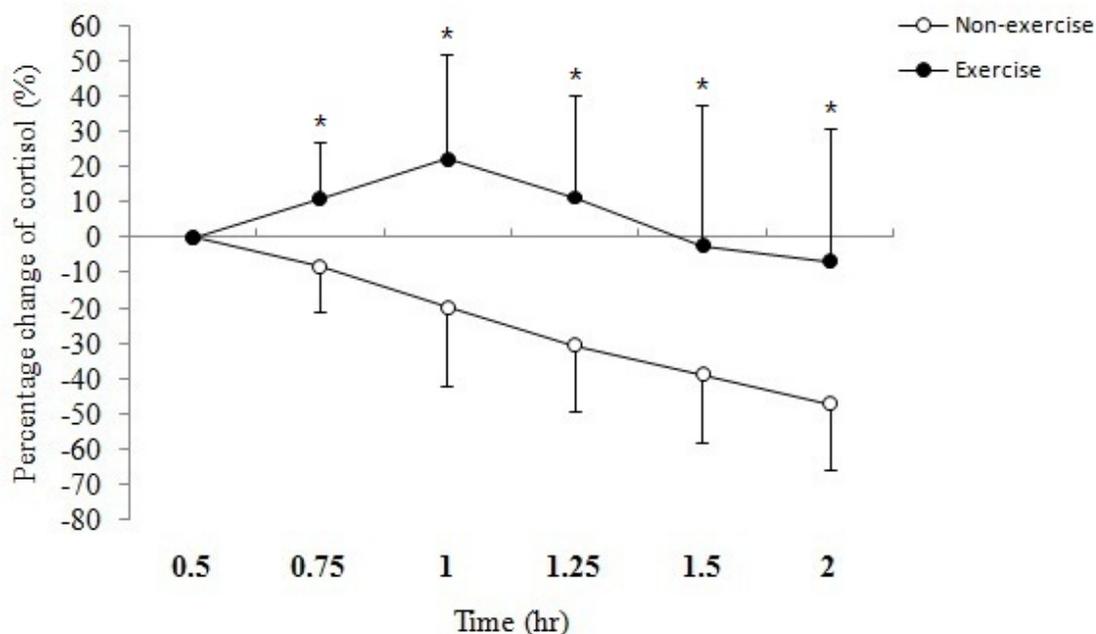
RSD, relative standard deviation; DFA, difference from the actual value.



**Figure 2.** Mean plasma prednisolone concentration-time profile after oral administration of 5mg of prednisolone. Data are shown as the mean  $\pm$  SD. \*Significantly different from the other group ( $p < .05$ )

The concentrations of prednisolone in the E trial were significantly lower than those in the NE trial at 0.75, 1, 1.25, 1.5 and 2 hr following prednisolone administration (Figure 2).  $C_{max}$  in the E trial was lower than in the NE trial. However, there were no significant differences in  $AUC_{0-10}$ ,  $k$ ,  $t_{1/2}$ ,  $Cl_F$  and

$V_{d/F}$  between the two trials (Table 3). The mean percentage change of cortisol was significantly different between the E and NE trials during the period of 0.75 to 2 hr, and E trial was significantly higher than NE trial ( $p < .05$ ) (Figure 3).



**Figure 3.** Mean percentage change of cortisol after prednisolone administration. Data are shown as the mean  $\pm$  SD. \*Significantly different from the other group ( $p < .05$ )

**Table 3.** Pharmacokinetic parameters

Parameters	Group	Subject 1	Subject 2	Subject 3	Subject 4	Subject 5	Subject 6	Subject 7	Subject 8	Mean $\pm$ SD
$T_{max}$ (hr)	NE	1.00	0.75	1.25	1.25	0.75	0.75	1.00	0.5	$0.91 \pm 0.27$
	E	3.00	0.75	0.75	1.25	1.25	0.75	1.25	0.5	$1.19 \pm 0.79$
$C_{max}$ (ng/mL)	NE	149.4	166.6	161.2	156.6	208.5	156.7	144.6	188.1	$166.5 \pm 21.5$
	E	97.1	143.5	127.0	95.9	157.2	151.5	154.9	200.0	$140.9 \pm 34.2^*$
$k$ ( $hr^{-1}$ )	NE	0.27	0.29	0.29	0.35	0.25	0.22	0.23	0.26	$0.27 \pm 0.04$
	E	0.25	0.27	0.28	0.41	0.29	0.23	0.35	0.29	$0.30 \pm 0.06$
$t_{1/2}$ (hr)	NE	2.54	2.40	2.38	1.99	2.72	3.19	3.07	2.69	$2.62 \pm 0.39$
	E	2.81	2.55	2.48	1.68	2.37	3.07	1.97	2.42	$2.42 \pm 0.44$
$AUC_{0-10}$ (hr $\cdot$ ng/mL)	NE	668.1	613.97	592.85	571.90	645.1	523.9	560.2	617.7	$599.2 \pm 46.9$
	E	512.5	685.1	570.6	468.1	704.3	527.2	539.8	490.8	$562.3 \pm 87.4$
$V_{d/F}$ (mL)	NE	24997	26323	26673	24057	28085	39200	34917	29148	$29175 \pm 5247$
	E	34247	24401	28577	25018	22308	37676	25303	33640	$28896 \pm 5605$
$Cl_{i/F}$ (mL/hr)	NE	6827	7588	7906	8371	7154	8508	7878	7489	$7715 \pm 572$
	E	8460.0	6644.1	7977.6	10328.6	6516.6	8496.1	8846	9640	$8364 \pm 1324$
MRT (hr)	NE	4.5	4.0	4.0	3.6	4.00	4.5	4.8	3.9	$4.16 \pm 0.40$
	E	5.79	4.51	4.64	3.81	3.63	4.50	3.88	3.45	$4.28 \pm 0.76$

\* Significantly different from non-exercise trial ( $p < .05$ ).

NE, non- exercise group; E, exercise group;  $T_{max}$ , the time to maximum concentration;  $C_{max}$ , the maximum concentration;  $t_{1/2}$ , the time to half life concentration; AUC, area under the plasma concentration-time curve;  $V_{d/F}$ , apparent volume of the distribution; and  $Cl_{i/F}$ , the time-averaged total body clearance; MRT, mean residence time.

GCs have anti-inflammatory effects via binding to intracellular glucocorticoid receptors (GRs) to inhibit the transcriptional activity of nuclear factor  $\kappa$ B (NF- $\kappa$ B) (30). It has been shown that exercise reduces activation of NF- $\kappa$ B (31). Moderate aerobic exercise can serve as a non-pharmacologic therapeutic method in the treatment of inflammation (32). Although we did not find a clear difference in the total amount of prednisolone absorbed, the finding of reduced  $C_{max}$ , following its administration with exercise, indicated that exercise may decrease side effects of prednisolone administration for long term use. It may require further investigation on the side effect markers for prednisolone administration in the future.

Prednisolone is a highly protein-bound drug. The extent of protein binding may affect the pharmacokinetic factors such as drug concentration, volume of distribution and elimination rate (33). Drug distribution is dependent upon the delivery of the drug to tissues, the passage of the drug through tissue membranes, and the binding of the drug to plasma proteins and other tissue components (34). Cortisol level and dose affect the protein binding of prednisolone (35). It is well known that aerobic exercise increases cortisol level (16). The increasing of cortisol level is dependent on the intensity of the exercise. Peak cortisol value may significantly increase at moderate/high intensity exercise (36). Furthermore, exercise-induced increasing cortisol concentration decreased the protein binding of prednisolone (18, 19). When administering low-dose prednisolone, cortisol is able to displace prednisolone from the binding sites. However, cortisol does not displace high dose prednisolone from the plasma binding sites (19). Our study revealed that exercise together with the administration of prednisolone significantly increased cortisol concentration in the E trail (Figure 3). Since the subjects received low dose of prednisolone (~0.077 mg/kg), the displacement of prednisolone from the plasma binding sites by cortisol might occur in the E trail. Consequently, the free form of prednisolone concentration is expected to increase and then enhance the distribution. However, our result showed that the  $V_{dF}$  was not altered. The possible reason may be that prednisolone is neither a flow-limited nor high extraction ratio drug (37). Exercise diverts blood flow away from the liver and could decrease the clearance of drugs, particularly those highly

extracted by the liver or flow-limited drugs (38, 39). Therefore, the metabolism of prednisolone is less likely to be influenced by changes in blood flow during exercise.

Prednisolone is substantially excreted via the kidneys (40). Drugs that are unchanged form as they get excreted by the kidney are most likely affected by exercise (12). However, only 11%–24% of a given dose of prednisolone can be recovered in urine as unchanged form (41). Thus, it may be the reason that the elimination rate constant (k) and half-life ( $t_{1/2}$ ) of prednisolone were not altered by exercise in this study.

## CONCLUSION

Reducing the side effects is an important issue for the long-term use of GCs. A lower peak blood level of drug concentration may reduce side effects. This study revealed that exercise following administration of prednisolone can reduce  $C_{max}$  and the absorption pattern. In addition to the antedrug, exercise may serve as a potential approach to decrease systemic adverse effects of prednisolone. The results of this study may provide valuable information to the subjects undergoing long term administration of prednisolone.

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