

EXPRESSION PROFILES OF CYTOCHROME P450S FOLLOWING SWIMMING EXERCISE IN AGING RATS

By Bang sub Lee¹, Wi-Young So¹, Sang-Hoon Kim², Jin-Ho Yoon³

¹Sports and Health Care Major, College of Humanities and Arts, Korea National University of Transportation, Chungju-si 27469, Republic of Korea.

²Korea Athletic Training Research Institute, Seoul 05392, Republic of Korea.

³Department of Sport and Leisure Studies, Far East University, Chungbuk 27601, Republic of Korea.

Corresponding Author Jin-Ho Yoon: tkd97@daum.net

Submitted: July 6, 2017. Accepted: September 25, 2017. Published: October 18, 2017.

Abstract

Background and Objective

Cytochrome P450s (CYPs) are the major drug-metabolizing enzymes responsible for the clearance of approximately 75% of all drugs in clinical use. Drug clearance may be reduced with aging, resulting in increased drug toxicity in the elderly. Here, we evaluated changes in CYP expression following exercise in an aging rat model.

Materials and Methods

Sixteen male Sprague-Dawley rats were grouped into control ($n = 5$), short-term exercise (SE) ($n = 4$), and long-term exercise (LE) ($n = 7$) groups and changes in CYPs and nuclear receptors (NRs) were measured with aging.

Results

CYP2C22, *CYP3A1*, and *CYP2C11* mRNAs were up-regulated, whereas *CYP26B1* was downregulated in the SE and LE groups compared with the control group. Moreover, mRNA levels of the NRs, constitutive androstane receptor, retinoid X receptor α , peroxisome proliferator-activated receptor, and pregnane X receptor, were significantly increased in the LE group compared with those in the control group. As an indicator of more long-term changes, protein levels of CYP2C11, CYP2B, CYP1A, CYP2C, and CYP2C22 were significantly up-regulated in the LE group compared with the control group. Overall, our data show that CYP and NR expression increased in rats with forced long-term exercise during aging.

Conclusion

Therefore, we propose that regular swimming exercise may increase CYP levels, resulting in enhanced drug clearance and thereby reducing age-related drug toxicity in elderly individuals.

Keywords: aging, cytochrome P450s, exercise, nuclear receptors, rat, swimming

Cytochrome P450s (CYPs) are found in most living organisms and are mainly localized in the endoplasmic reticulum. However, some CYPs are also found in other subcellular compartments, such as the mitochondria and Golgi body. Although CYPs are present in various tissues of the body, including the gut and heart, most CYPs are found in the liver.¹⁻³ Approximately two-thirds of human CYPs metabolize endogenous substrates, mediate the biosynthesis of steroids and eicosanoids, and control the degradation of vitamin D. Some CYPs (members of the CYP1-4 families) regulate oxidative reactions of xenobiotics such as drugs and environmental pollutants.^{4,5} For the metabolism of both endogenous factors and xenobiotics, CYPs require interaction with NADPH-P450 reductase and/or cytochrome b₅ as electron donors.⁶

CYPs can be induced or inhibited by specific dietary components and xenobiotic compounds, including drugs and alcohol; altered CYP expression affects the biotransformation rate of enzymatic substrates.⁷ For example, grapefruit inhibits the expression of CYP3A4, which is involved in the metabolism of 60-80% of clinically used drugs.^{8,9} Therefore, drugs metabolized by CYP3A4 may cause adverse effects when taken with grapefruit. Moreover, aging alters the pharmacokinetics of drugs by reducing blood flow to the kidney and liver (20-30%) and through changes in liver mass. Drug metabolism in aging humans is associated with drug toxicity.¹⁰ Additionally, aging may alter the expression of many enzymes in the liver as well as that of hepatic NADPH cytochrome P450 reductase and polymeric immunoglobulin receptor. In rats, aging affects CYP2B1 and CYP2B2 levels.¹¹ Aging has also been shown to result in decreased antipyrine clearance by increasing the half-life of antipyrine in the plasma.¹²

CYP expression is also affected by endogenous compounds, including hormones, growth factors, and cytokines.¹³ The levels of many endogenous compounds are altered by aging. Indeed, reduced drug metabolism capacity in elderly people may be a consequence of alterations in lean body mass, body fat, muscle mass, and total body water and reductions in plasma albumin levels and blood flow. Moreover, drug-metabolizing enzymes, particularly CYPs, exhibit varying expression in different age

groups.¹⁴⁻¹⁶ Additionally, aerobic exercise activity increases the hepatic oxidative metabolism of drugs.¹⁷ For example, physical exercise enhances the clearance rate of spironolactone and hexobarbital.¹⁸ CYP levels were also found to be increased in rats after 14 days of exercise compared with that in sedentary rats.^{19,20} Similarly, physical activity in elderly individuals shows beneficial effects on oxidative metabolism in the liver,²¹ and regular exercise contributes to the health of aged humans. Additionally, aerobic exercise activity increases the hepatic oxidative metabolism of low-clearance drugs.¹⁷ Regular physical activity can effectively reduce the risk of age-related disease and mitigate age-related drug toxicity by improving drug clearance.²²

Therefore, we hypothesized that regular exercise may contribute to proper drug clearance in elderly individuals. In the current study, we examined whether physical activity in elderly rats could improve drug toxicity.

METHODS

Animal Care and Overview of the Experimental Design

Sixteen male Sprague-Dawley rats (4-weeks old) were purchased from the Korea National Sport University, in Seoul, Republic of Korea and housed two per cage in environmentally controlled conditions (temperature, 22°C; relative humidity, 51%), with a 12-hour light-dark cycle (lights on 7:00 am to 7:00 pm). The rats were randomly assigned to one of three groups: control (no exercise, evaluated at 50 weeks of age, *n*=5), swim short-term exercise (exercise for 4 weeks beginning at 46 weeks of age, *n*=4), and swim long-term exercise (exercise for 20 weeks beginning at 30 weeks of age, *n*=7; see Table 1). All rats were fed a standard irradiated chow diet (Purina Mills Inc., Gray Summit, MO, USA). Before experimentation, the animals were familiarized to the laboratory conditions by swimming for 10 min/day for 5 days. The study protocol was approved by the institutional ethics review board of the Korea National Sport University.

Exercise Training

Two rats at a time were trained to swim in a 55-gallon container filled with water to a depth of 3 feet.

TABLE 1 Schematic Diagram of the Experimental Design

Animals	Sprague-Dawley rats	Age from 4 weeks to 50 weeks
Groups	Control ($n = 5$)	50-weeks old
	Short-term exercise ($n = 4$)	4 weeks exercise (46th week exercise start)
	Long-term exercise ($n = 7$)	20 weeks exercise (30th week exercise start)
Exercise Methods	5 days/week swimming (33°C~35°C)	1 hour/day (09:00~11:00 am)

The temperature of the water was maintained at 36°C. On the first day, the swimming time was 10 min/day, and the time was increased by 10 min each day to a maximum of 1 hour. The groups were trained to swim for 1 hour at the same time (9:00–11:00). Short-term swimming exercise was performed for 4 weeks starting at 46 weeks of age, whereas long-term swimming exercise was performed for 20 weeks starting at 30 weeks of age. After exercise, the rats were dried and placed back into their cages. The rats were sacrificed at 24 hours after the final exercise period.

Liver Microsome Preparation

The rats were anesthetized with an intraperitoneal injection of pentobarbital sodium (60 g/kg body weight [BW]). Rat livers were then harvested, weighed, homogenized in homogenizing buffer (0.1 M potassium phosphate buffer, pH 7.4, containing 0.125 M potassium chloride, 1.0 mM ethylenediaminetetraacetic acid [EDTA], and a protease inhibitor cocktail [Sigma, St. Louis, MO, USA]), and centrifuged at $13,000 \times g$ for 20 minutes at 4°C, yielding the supernatant (S9 fraction). To obtain the microsomal fraction, the post-mitochondrial fraction (S9 fraction) was centrifuged for 45 min at $250,000 \times g$. The resulting microsomal pellet was suspended in 10 mM Tris acetate buffer (pH 7.4) containing 0.1 mM EDTA and 23% glycerol and stored at –80°C. Protein concentrations were determined with a bicinchoninic acid protein assay kit (Thermo Scientific, Inc., Rockport, IL, USA) with bovine serum albumin as the protein standard.

Immunoblotting

Liver microsomes were separated by sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE), and CYP proteins were identified by western blotting with chemiluminescent detection,

as previously described by Lee et al., with minor modifications.²³ Briefly, equal amounts of microsomal protein (32 µg) were separated by SDS-PAGE. Primary antibodies (rat anti-CYP2C11, 1:5,000; rat anti-CYP2B from Dr. James Halpert [University of California at San Diego, CA, USA], 1:10,000; rat anti-CYP1A [Daitchi Pure Chemicals], 1:5000; rat anti-CYP2C, 1:20,000; rat anti-CYP4A, 1:5,000; rat anti-CYP3A from Dr. Halpert, 1:5,000; rat anti-CYP2E1, 1:5,000; rat anti-CYP2D, 1:5,000; rat anti-CYP2C22, 1:3,000; anti-GAPDH [Millipore, Billerica, MA, USA], 1:10,000) were incubated overnight at 4°C. After washing, the blots were incubated with horseradish peroxidase-conjugated goat anti-rabbit IgG for 1 hour at room temperature. The signals were detected with ECL substrate (Thermo Scientific, Inc.) on x-ray film. All assays were performed within a linear range, and the intensity of the stained bands was measured using Bio-Rad imaging software (Image Lab ver. 4.0; Hercules, CA, USA).

RNA Extraction, cDNA Synthesis, and Quantitative Real-Time Polymerase Chain Reaction (qPCR)

Total RNA was extracted with RNA-Bee reagent (Tel-Test, Friendswood, TX, USA) according to the manufacturer's instructions. Total RNA (1 µg) was used for cDNA synthesis with a High Capacity cDNA Archive kit (Applied Biosystems, Foster City, CA, USA), and qPCR was performed with SYBR Green PCR Master Mix (Applied Biosystems) using an Eppendorf Mastercycler ep Realplex PCR instrument with 45 cycles (Eppendorf, NY, USA). *GAPDH* mRNA was used as a normalization control. The primer sets used in the qPCR are listed in Table 2. qPCR results were analyzed by the $\Delta\Delta C_t$ method, as described by Livak and Schmittgen.²⁴

TABLE 2 Primer Sets used for qPCR

Genes	Forward	Reverse
CYP1A2	TTCCAGGACAGCTTCGTACAC	GCTGGGTAGAGACCTCCTCA
CYP2B1/2	AGTCGAACCACCACACAGAG	GCGGTCATCAAGGGTTGGTA
CYP2C11	GAATTCGCATGGATCCAGCCCTAGTCC	GTCGACAATCAGATGAGAGCTTAG
CYP2C22	TCACACCGGCTACCAACCCT	CTGTGGGTCATGGAGAGCTG
CYP3A1	GGAAATTCGATGTGGAGTGC	AGGTTTGCCTTTCTCTTGCC
CYP3A2	TACTACAAGGGCTTAGGGAG	CTTGCCTGTCTCCGCCTCTT
CYP4F4	CCGTCGTTGTACCCAAGACA	CAGTGCCACCTTCATCTCGT
CYP26A1	GTGCCAGTGATTGCTGAAGA	GGAGGTGTCCTCTGGATGAA
CYP26B1	TTGAGGGCTTGGAGTTGGT	AACGTTGCCATACTTCTCGC
CAR	ACCAGTTTGTGAGTTCAGG	CTTGAGAAGGGAGATCTGGT
PXR	GACGGCAGCATCTGGAACACTAC	TGATGACGCCCTTGAACATG
RXR	ATGCGGATGGATAAGTCG	GAGGTGTTCCAGGCATTTTC
RXR	GCTGGTGTGCGAAGATGCGTGAC	GGTACTTGTGTTTGCAGTACG
PPAR	GCCATCTTCACGATGCTGTCC	CCTCGATGGGCTTCACGTTTCAG
AhR	CTCCCTCCACAGTTGGCTTTGTTTG	GATTCTGCGCAGTGAAGCATGTCAG
GAPDH	TGCCAAGTATGATGACATCAAGAAG	AGCCAGGATGCCCTTTAGT

Statistical Analysis

All of the descriptive data are expressed in terms of the mean \pm standard deviations. Kruskal-Wallis tests were used to examine differences in characteristics between groups, and post-hoc tests (Tukey tests using ranks) were used to determine differences related to groups. All analyses were performed using SPSS version 20.0 (SPSS, Chicago, IL, USA). $p < 0.05$ was considered significant.

RESULTS

Effects of Exercise on CYP mRNA Expression in Rat Livers

First, we evaluated changes in the hepatic expression of CYP mRNAs in rats with or without short- or long-term swimming exercise to determine whether regular exercise contributed to enhanced drug clearance in aged rats. Our qPCR data (Figure 1) showed that both short- and long-term exercise significantly up-regulated *CYP2C22*, *CYP3A1*, and *CYP2C11* mRNAs and significantly decreased *CYP26B1* mRNA compared with the levels in the control. *CYP1A2*, *CYP4F4*, *CYP2B1*, and *CYP26A1* mRNA expression was slightly increased compared with that in controls;

however, these differences were not significant. Additionally, the expression levels of *CYP2B2*, *CYP26B1*, and *CYP3A2* mRNAs in livers from rats subjected to swimming exercise were similar to or lower than those of the control. Notably, the expression levels of *CYP2B1*, *CYP3A1*, *CYP4F4*, and *CYP2C11* mRNAs were higher in rats after long-term exercise than in rats subjected to short-term exercise. In general, most CYPs examined in this study showed increased expression in both exercise groups.

Effects of Exercise on the mRNA Expression of Nuclear Receptors (NRs) in Rat Livers

NRs are important in the regulation of many CYPs and other phase I and phase II drug-metabolizing enzymes (DMEs). Therefore, we next measured the mRNA levels of NRs. The expression of constitutive androstane receptor (*CAR*), retinoid X receptor α (*RXRMediua*), peroxisome proliferator-activated receptor (*PPAR*), and pregnane X receptor (*PXR*) was significantly higher in rats subjected to long-term exercise than in rats in the control group (Figure 2). In addition, *CAR*, *RXR α* , and *PPAR α* mRNAs were also significantly up-regulated in rats subjected to short-term exercise compared to the control group. The

FIG. 1 mRNA expression of CYPs in the exercise groups. Sprague-Dawley rats were grouped and subjected to short- or long-term exercise (or no exercise in the control). Hepatic mRNA levels of CYPs were measured by qPCR. The error bars represent the standard errors. Statistical comparisons among the three different groups were made using one-way ANOVA followed by Kruskal-Wallis tests. * $p < 0.05$ compared with control rats.

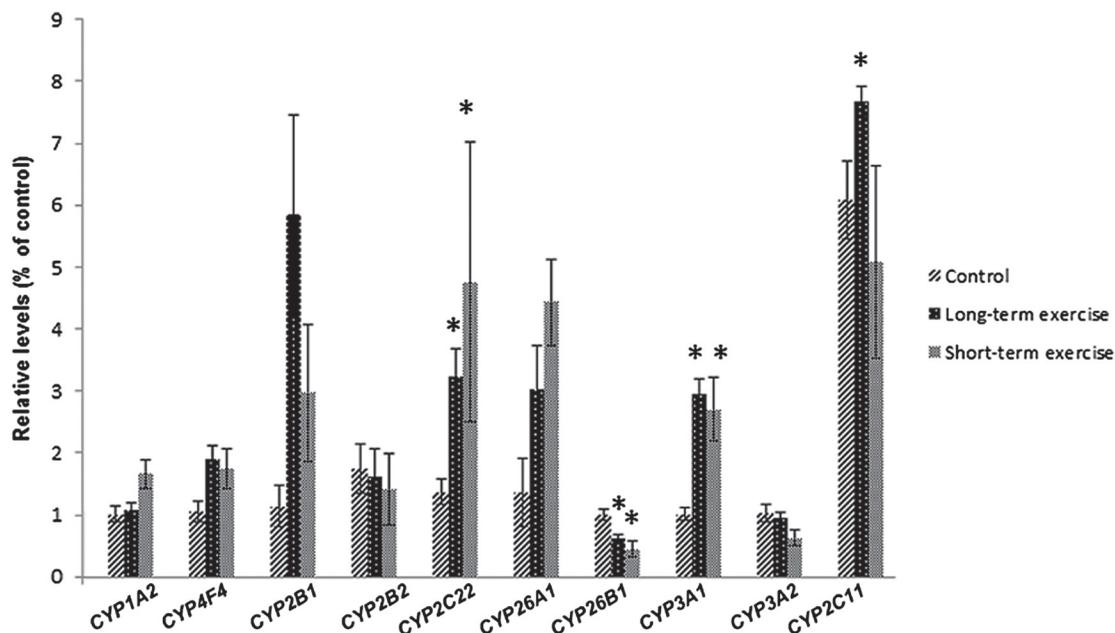
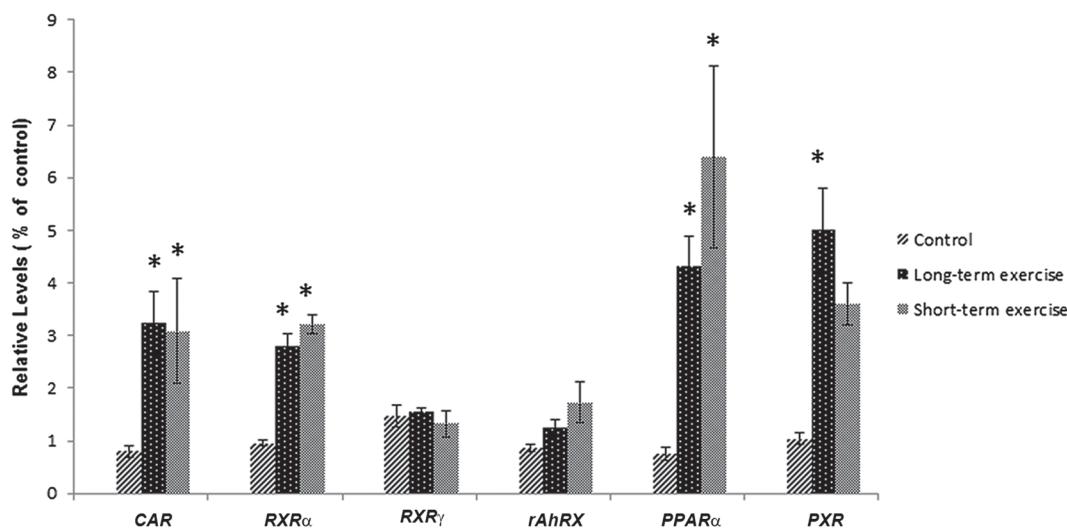


FIG. 2 mRNA levels of nuclear receptors in rats. Hepatic mRNA levels of NRs were measured by qPCR. The error bars represent standard errors. Statistical comparisons among the groups were made using one-way ANOVA followed by Kruskal-Wallis tests. * $p < 0.05$ compared with control rats.



mRNA levels of aryl hydrocarbon receptor (*AhR*) and *RXR* were not altered in either of the exercise groups compared to the control group. Notably, there were no significant differences in NR expression between short- and long-term exercise groups.

Changes in CYP Expression in the Aging Rat Group

Changes in hepatic CYP protein levels in the three groups are shown in Figure 3A and 3B. CYP2C11, CYP2B, CYP1A, CYP2C, and CYP2C22 protein

levels were significantly increased in the long-term exercise group compared to the control group ($p < 0.05$). CYP4A, CYP3A, and CYP2E1 protein levels were slightly increased in the long-term exercise group compared to the control group; however, the differences were not statistically significant. Interestingly, CYP2D protein levels were slightly reduced in the long-term exercise group compared with the control group. CYP2C11, CYP2C, and CYP2C22 protein levels were significantly increased in the short-term exercise group compared with the control group ($p < 0.05$). In contrast, the protein levels of CYP4A and CYP2E1 were not changed, and that of CYP2B was significantly reduced in the short-term exercise group compared with the long-term exercise group. CYP1A, CYP3A, and CYP2D were slightly up-regulated in the SE group compared with the control group; however, these differences were not statistically significant. In general, CYPs were expressed at higher levels in the long-term exercise group than in the control and short-term exercise groups.

DISCUSSION

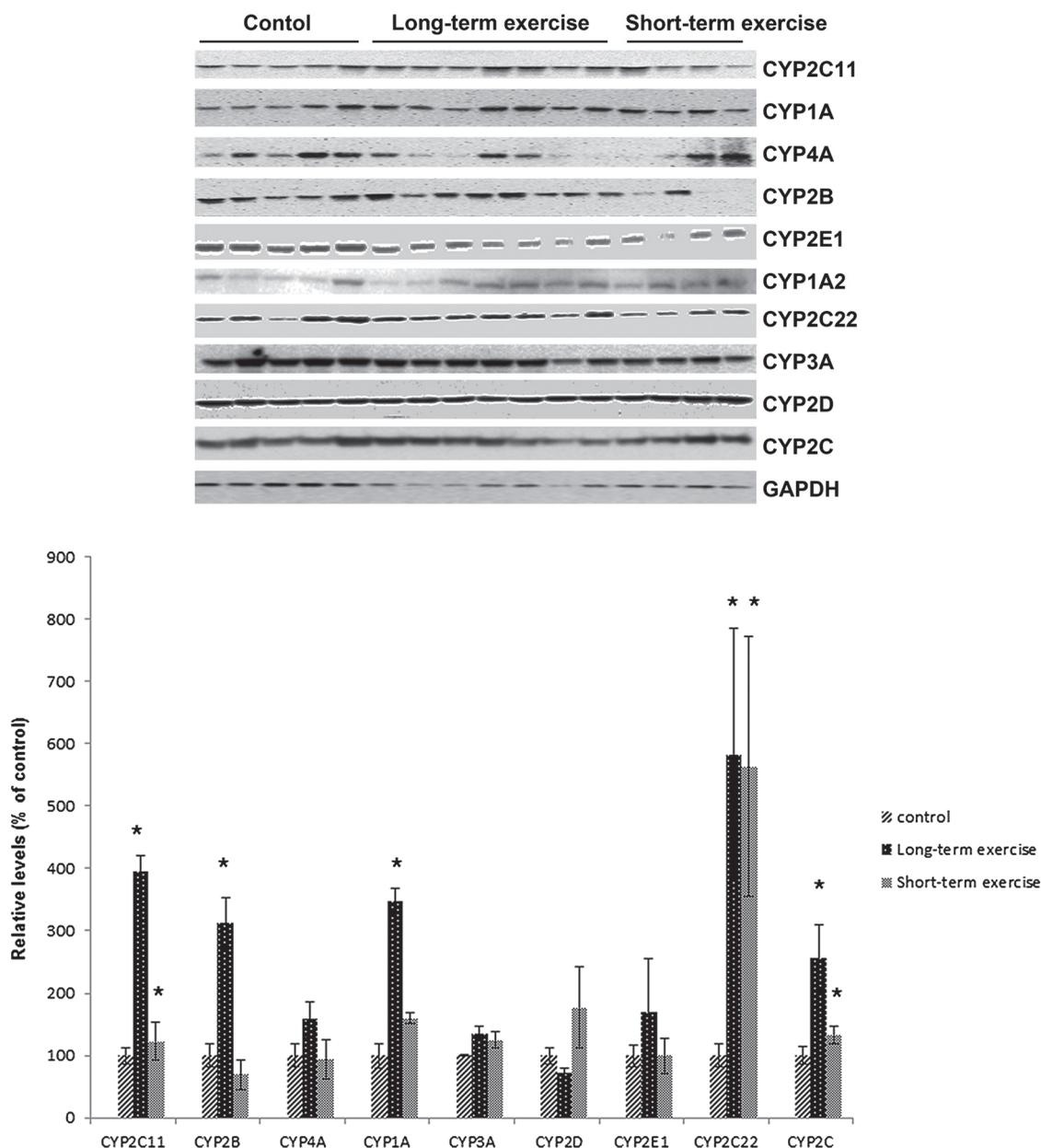
Exercise controls steroid hormone levels. Both acute and regular exercise alters steroid hormone levels. Exercise is important in preventing and treating age-related diseases and sarcopenia.²⁵ The expression and activity of many enzymes, including DMEs, usually decrease with aging.^{26,27} Such reduced levels of DMEs can lead to reduced levels of drug clearance, causing enhanced drug toxicity in the elderly.^{28,29} Aminopyrine N-demethylase activity was previously shown to be increased by 130% with increased age.³⁰ In this paper, we show that the expression levels of some CYPs increased with swimming exercise. Other studies have reported that swimming exercise increases antipyrine clearance by excretion.^{31,32} It was previously reported that CYP450 27B1, which metabolizes vitamin D, was significantly up-regulated between 1 and 3 hours after the end of exercise. Resistance exercises that improve vitamin D metabolism in skeletal muscle increased the levels of CYP450 27B1.³³ CYP450 levels were increased in young and middle-aged rats with 8 weeks of moderate-intensity running exercise. Exercise has been shown to increase deacetylation of the toxic metabolite p-aminophenol in the kidney

by 54% in young rats and by 26% in middle-aged rats.³⁴ Exercise has also been shown to improve renal phase I drug metabolism without affecting the phase II processes.³⁴ In addition, regular exercise increases the concentrations and activity of CYPs in the liver,^{6,35} and cytochrome P450s, cytochrome b5, and NADPH cytochrome reductase are induced during exercise.²⁰ The half-life of antipyrine in serum is significantly reduced in top athletes compared with that in physical education students.⁶ Additionally, sprinters and long-distance runners show significantly increased antipyrine clearance than sedentary subjects.^{31,36} Maximal exercise has been shown to alter the activity of GH, IGF-1, and CYP1A2. High-intensity exercise in young ice hockey players increased the GH concentration, with a concomitant increase in CYP1A2 mRNA levels. The expression of CYP1A2 is related to the amount of exercise in athletes.³⁷ These changes may be the result of increased blood flow throughout the body or increased expression of some CYPs in the liver, as observed for CYP2Cs, CYP2Bs, and CYP1A proteins in our study. Moreover, although CYP1A2 protein levels have been shown to decrease with aging,³⁸ we found that CYP1A protein levels were increased in the swimming exercise group compared with the control group.

A previous analysis of the hepatic expression and activity of CYPs in 3-, 12-, 26-, and 104-week-old rats showed that the levels of total CYPs, microsomal proteins, and cytochrome b5 changed with age in rats.³⁸ For example, CYP1A2, CYP2B1, and CYP2E1 levels were increased in 3-week-old rats compared with those in 12-, 26-, and 104 week-old rats. Moreover, CYP2C11 and CYP3A2 expression has been shown to decrease with age.^{39,40} Similarly, our study confirmed that long-term regular exercise could delay the age-related decreases in some CYPs and DMEs.

The present results show that *CAR*, *PXR*, *RXR*, and *PPAR* mRNA levels were increased in the exercise groups compared with the control group. Phase I and phase II DMEs are regulated by *CAR* and *PXR*, which have important roles in the metabolism of fatty acids, lipids, and glucose in energy metabolism.⁴¹ Exercise training increased *Cyp7a1* and *LXR* mRNA expression in hepatic tissue. Regular aerobic exercise increases *Cyp7a1* levels, increases the effective control

FIG. 3 Expression of CYPs after exercise. A. Immunoblots of CYPs from hepatic microsomes. B. Quantification of immunoblots. Statistical comparisons among the groups were made using one-way ANOVA followed by Kruskal-Wallis tests. * $p < 0.05$ compared with control rats.



of cholesterol in the liver, and prevents atherosclerosis by increasing HDL levels.⁴² PXR plays an important role in the regulation of CYP3A4, a major DME in humans.⁴³ In a previous study, swimming exercise for 8 weeks was found to increase mRNA and protein expression of PPAR in elderly mice, and the expression

of 3-hydroxyacyl CoA dehydrogenase and carnitine palmitoyl transferase-I, target genes of PPAR, was also increased in the heart.⁴⁴ Thus, our findings support that exercise may prevent the increased drug toxicity observed in elderly individuals.

CONCLUSION

In summary, our findings suggest that regular swimming exercise increases CYP expression during aging, resulting in enhanced levels of drug clearance. Thus, our data imply that exercise might reduce age-related drug toxicity in elderly individuals. However, expression of CYP450 isoforms was detected only in the liver in this study. Thus, further studies are required to assess changes in CYP450 expression in different organs with exercise in elderly individuals.

CONFLICTS OF INTEREST

The authors declare no conflicts of interest.

REFERENCES

- Guengerich FP, Shimada T, Yun CH, et al. Interactions of ingested food, beverage, and tobacco components involving human cytochrome P4501A2, 2A6, 2E1, and 3A4 enzymes. *Environ Health Perspect* 1994; 1029:49–53.
- Stupans IMM, Kirlich A, Tuck KL, Hayball PJ. Inactivation of cytochrome P450 by the food-derived complex phenol oleuropein. *Food Chem Toxicol* 2001;39:1119–24.
- Martignoni M, Groothuis G, de Kanter R. Comparison of mouse and rat cytochrome P450-mediated metabolism in liver and intestine. *Drug Metab Dispos* 2006;34:1047–54.
- Smith DA, Abel SM, Hyland R, Jones BC. Human cytochrome P450s: selectivity and measurement in vivo. *Xenobiotica* 1998;28:1095–128.
- Wang P, Mason PS, Guengerich FP. Purification of human liver cytochrome P-450 and comparison to the enzyme isolated from rat liver. *Arch Biochem Biophys* 1980;199:206–19.
- Frenkl R, Gyore A, Szeberenyi S. The effect of muscular exercise on the microsomal enzyme system of the rat liver. *Eur J Appl Physiol Occup Physiol* 1980;44:135–40.
- Ware WR. Nutrition and the prevention and treatment of cancer: association of cytochrome P450 CYP1B1 with the role of fruit and fruit extracts. *Integr Cancer Ther* 2009;8:22–8.
- Takizawa D, Kakizaki S, Horiguchi N, et al. Constitutive active/androstane receptor promotes hepatocarcinogenesis in a mouse model of non-alcoholic steatohepatitis. *Carcinogenesis* 2011;32:576–83.
- Cuciureanu M, Vlase L, Muntean D, et al. Grapefruit juice--drug interactions: importance for pharmacotherapy. *Rev Med Chir Soc Med Nat Iasi* 2010;114:885–91.
- Schmucker DL, Ohta M, Kanai S, et al. Hepatic injury induced by bile salts: correlation between biochemical and morphological events. *Hepatology* 1990; 12:1216–21.
- Van Bezooijen RL, Wang RK, Lechner MC, Schmucker DL. Aging effects on hepatic NADPH cytochrome P450 reductase, CYP2B1&2, and polymeric immunoglobulin receptor mRNAs in male Fischer 344 rats. *Exp Gerontol* 1994;29:187–95.
- Jorquera F, Almar MM, Pozuelo M, et al. Effects of aging on antipyrine clearance: predictive factors of metabolizing capacity. *J Clin Pharmacol* 1995;35:895–901.
- Liptrott NJ, Penny M, Bray PG, et al. Owen A. The impact of cytokines on the expression of drug transporters, cytochrome P450 enzymes, and chemokine receptors in human PBMC. *J Pharmacol* 2009;156:497–508.
- Staskin DR. Overactive bladder in the elderly: a guide to pharmacological management. *Drugs Aging* 2005;22:1013–28.
- De Stefano F, Zambon S, Giacometti L, et al. Obesity, Muscular Strength, Muscle Composition and Physical Performance in an Elderly Population. *J Nutr Health Aging* 2015;19:785–91.
- Wauthier V, Verbeeck RK, Calderon PB. The effect of ageing on cytochrome p450 enzymes: consequences for drug biotransformation in the elderly. *Curr Med Chem* 2007;14:745–57.
- Kvernmo HD, Osterud B. The effect of physical conditioning suggests adaptation in procoagulant and fibrinolytic potential. *Thromb Res* 1997;87:559–69.
- Frenkl R, Szeberenyi S. Enzyme inducing effect of muscular exertion in the rat. *Acta Med Acad Sci Hung* 1976;33:95–100.
- Boel J, Andersen LB, Rasmussen B, et al. Hepatic drug metabolism and physical fitness. *Clin Pharmacol Ther* 1984;36:121–6.
- Frenkl R, Gyore A, Meszaros J, Szeberenyi S. A study of the enzyme inducing effect of physical exercise in man. The “trained liver”. *J Sports Med Phys Fitness* 1980;20:371–6.
- Villa JG, Cuadrado G, Bayón JE, González-Gallego J. The effect of physical conditioning on antipyrine clearance. *Eur J Appl Physiol Occup Physiol* 1998;77:106–11.
- Paterson DH, Jones GR, Rice CL. Ageing and physical activity: evidence to develop exercise recommendations for older adults. *Can J Public Health* 2007; 98:S69–108.

23. Lee CM, Kim BY, Li L, Morgan ET. Nitric oxide-dependent proteasomal degradation of cytochrome P450 2B proteins. *J Biol Chem* 2008;283:889–98.
24. Livak KJ, Schmittgen TD. Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) Method. *Methods* 2001;25:402–8.
25. Sato K, Iemitsu M. Exercise and sex steroid hormones in skeletal muscle. *J Steroid Biochem Mol Biol* 2015;145:200–5.
26. Fu ZD, Klaassen CD. Short-term calorie restriction feminizes the mRNA profiles of drug metabolizing enzymes and transporters in livers of mice. *Toxicol Appl Pharmacol* 2014;274:137–46.
27. Lee JS, Ward WO, Wolf DC, et al. Coordinated changes in xenobiotic metabolizing enzyme gene expression in aging male rats. *Toxicol Sci* 2008;106:263–83.
28. Testa B, Kramer SD. The biochemistry of drug metabolism—an introduction: Part 3. Reactions of hydrolysis and their enzymes. *Chem Biodivers* 2007;4:2031–122.
29. Testa B, Kramer SD. The biochemistry of drug metabolism—an introduction: Part 2. Redox reactions and their enzymes. *Chem Biodivers* 2007;4:257–405.
30. Piatkowski TS, Day WW, Weiner M. Increased renal drug metabolism in treadmill-exercised Fischer-344 male rats. *Drug Metab Dispos*. 1993;21:474–9.
31. Villa JG, Cuadrado G, Bayon JE, Gonzalez-Gallego J. The effect of physical conditioning on antipyrine clearance. *Eur J Appl Physiol Occup Physiol* 1998;77:106–11.
32. Fabbri A, Bianchi G, Zoli M, et al. Effect of physical exercise on one-sample antipyrine clearance. *Ital J Gastroenterol* 1991;23:74–6.
33. Makanae Y, Ogasawara R, Sato K, et al. Acute bout of resistance exercise increases vitamin D receptor protein expression in rat skeletal muscle. *Exp Physiol* 2015;100:1168–76.
34. Piatkowski TS, Day WW, Weiner M. Increased renal drug metabolism in treadmill-exercised Fischer-344 male rats. *Drug Metab Dispos* 1993;21:474–9.
35. Mauriz JL, Taberero B, García-López J, et al. Physical exercise and improvement of liver oxidative metabolism in the elderly. *Eur J Appl Physiol* 2000;81:62–6.
36. Orioli S, Bandinelli I, Birardi A, et al. Hepatic antipyrine metabolism in athletes. *J Sports Med Phys Fitness* 1990;30:261–3.
37. Kochańska-Dziurawicz AA, Janikowska G, Bijak A, et al. The effect of maximal physical exercise on relationships between the growth hormone (GH) and insulin-like growth factor 1 (IGF-1) and transcriptional activity of CYP1A2 in young ice hockey players. *J Sports Med Phys Fitness* 2015;55:158–63.
38. Yun KU, Oh SJ, Oh JM, et al. Age-related changes in hepatic expression and activity of cytochrome P450 in male rats. *Arch Toxicol* 2010;84:939–46.
39. Warrington JS, Greenblatt DJ, von Moltke LL. Age-related differences in CYP3A expression and activity in the rat liver, intestine, and kidney. *J Pharmacol Exp Ther* 2004;309:720–9.
40. Vyskočilová E, Szotáková B, Skálová L, et al. Age-related changes in hepatic activity and expression of detoxification enzymes in male rats. *Biomed Res Int* 2013;2013:408573.
41. Wada T, Gao J, Xie W. PXR and CAR in energy metabolism. *Trends Endocrinol Metab* 2009;20:273–9.
42. Pinto PR, Rocco DD, Okuda LS, et al. Aerobic exercise training enhances the in vivo cholesterol trafficking from macrophages to the liver independently of changes in the expression of genes involved in lipid flux in macrophages and aorta. *Lipids Health Dis* 2015;14:109.
43. Habano W, Gamo T, Terashima J, et al. Involvement of promoter methylation in the regulation of Pregnane X receptor in colon cancer cells. *BMC Cancer* 2011;11:81.
44. Iemitsu M, Miyauchi T, Maeda S, et al. Aging-induced decrease in the PPAR-alpha level in hearts is improved by exercise training. *Am J Physiol Heart Circ Physiol* 2002;283:H1750–60.