EXERCISE TRAINING DOES NOT ALTER CYTOCHROME P-450 CONTENT AND MICROSOMAL METABOLISM

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Abstract:

Exercise training does not alter cytochrome P-450 content and microsomal metabolism. The purpose of this investigation was to determine whether increased endurance exercise capacity alters total hepatic cytochrome P-450 content and cytochrome P-450 (CYP1A and CYP2B) mediated hepatic microsomal mixed-function oxidase drug metabolism. Twenty adult male Sprague-Dawley rats were randomly assigned to either a control (C) or an endurance trained group (ET). ET rats were progressively trained 5 days per week for 11 weeks. Both control and endurance trained rats were administered in random order single posttraining doses of probe drugs theophylline (probe for CYP1A) and antipyrine (probe for CYP2B). Soleus muscle citrate synthase activity of ET rats was significantly greater ($P<0.01$) than for C rats (mean ± SD; C, 26.4 ± 1.3 $\mu mol.g^{-1}.min^{-1}$; ET, 46.1±2.7). In contrast, total liver cytochrome P-450 content was not significantly different ($P>0.01$) among C and ET rats (mean ± SD; C, 0.554 ± 0.055 nmol. Mg^{-1} liver protein ET, 0.604 ± 0.080). Likewise, the posttraining C and ET single-sample plasma clearances of theophylline (mean ± SD; C, 1.89 ± 0.3601. h^{-1}. kg^{-1} total liver weight; ET, 2.08 ± 0.49) and antipyrine (mean ± SD; C, 6.44 ± 1.561.h^{-1}.kg^{-1} total liver weight ; ET, 6.51 ± 1.02) were not significantly different ($P > 0.01$). Therefore, it was concluded that strenuous endurance training of 11 weeks duration did not alter total hepatic cytochrome P – 450 content or CYP1A or CY2B activity.

Keywords: Physical activity, exertion, single-sample plasma clearance, liver microsomes, drug disposition, pharmacokinetics.

Introduction

For many medical conditions, both medications and exercise are part of the treatment program. For instance, patients with angina, hypertension, or cardiac rhythm disturbances are often prescribed both exercise and drug therapy (27). Since the ultimate objective of drug

therapy is to provide a safe and effective dosing regimen for the patient, it is important to know whether drug-exercise interactions exist. In general, interactions leading to inappropriate dosing regimens can produce either subtherapeutic or toxic drug levels, which can be very unpleasant, dangerous, or even fatal to the patient (6). For example, a drug-drug interaction that diminishes the action of warfarin (an anticoagulant) may result in subtherapeutic doses and thrombus formation. In contrast, agents that enhance the action of warfarin may result in toxic doses and spontaneous bleeding. In both instances, the outcome may prove fatal, since thrombus formation can damage vital organs (e.g., the brain and heart through stroke and myocarchal infarction, respectively) and spontaneous bleeding can produce hemorrhaging (22).

To develop an appropriate dosing regimen, the rate and extent of drug absorption, protein binding, distribution, and elimination, i.e., drug disposition must be considered. Most drugs are eliminated through renal excretion and/or hepatic metabolism, with most hepatic drug metabolism involving phase I1 and / or phase II enzyme-mediated metabolic reactions (15). The phase I nonspecific cytochrome P – 450 (isoforms) are involved in the metabolism of many therapeutic agents (e.g., digitoxin, phenytoin, lidocaine, theophyline, propranolol, warfarin, tocainide, spironolactone). Additionally, cytochrome P-450 is the terminal enzyme in an electron transport system that dominates phase I mediated mixed-function oxidase (MFO) drug metabolism (15, 23). Mixed-function oxidase induced increases in the rate of drug hydroxylation, for example, are paralleled by increases in the level of cytochrome P-450. Consequently, the induction or inhibition of cytochromes P-450 may lead to changes in the metabolism of cytochromes P-450 – dependent metabolized drugs. Both internal (species, genetics, sex, age, hormones, pregnancy, disease) and external (diet, environment) factors (15) are known to alter hepatic cytochrome P-450 – dependent drug metabolism. Nevertheless very little is known about the way exercise training affects total hepatic cytochrome P – 450 content and cytochrome P – 450 mediated hepatic microsoma MFO drug metabolism.

It is presently unclear whether physical training alters the MFO metabolism of certain agents (7, 10-12) in humans. This lack of clarity may be greatly attributed to study design limitations or lack of control for possible confounding variables. Confounders have included failing to use each subject as his/her own control, using relatively highly trained individuals as subjects at the outset of the study. Failing to verify or specify the subject's cardiovascular fitness or level of endurance training, and a lack of documentation regarding host factors known to alter cytochromes P-450 (e.g., dietary habits).

In rats, chronic exercise has been shown to increase (1, 8, 13, 14), decrease (8, 9), and not change (9, 25) hepatic microsomal metabolism. Furthermore, authors examining the effects of exercise on total hepatic cytochrome P-450 content have also shown increases (8, 13), decreases (8, 9), and no changes (8, 25) in rat hepatic cytochrome P-450 levels as a result of exercise training. Training has included swimming (8, 13) as well as short term (2 weeks) strenuous (13) and mild (1, 8, 9) treadmill running.

Due to the paucity and inconclusive nature of the data presently available, the purpose of this study was to determine the effects of strenuous endurance treadmill running (11 weeks) on total hepatic cytochrome P-450 content and cytochrome P-450 mediated hepatic microsomal MFO drug metabolism in male rats.

MATERIALS AND METHODS

Animal care

Twenty adult (150-175g) male Sprague-Dawley rats (Zivic-Miller Labs. Inc. Zelienopole, PA) were housed two or three to a cage with corn cob bedding. (The Anderson industrial products Div., Maumee, OH). Corn cob bedding least affects the basal activities of rat liver microsomal cytochrome P-450 – dependent drug metabolizing enzymes (28). Bedding was changed twice weekly and rats were provided standard laboratory chow (Wayne Pet Food Div., continental Grian Co., Chicago, IL) and water adlibitum. Rats were both housed and exercised at room temperatures between 20^{o}C and 22^{o}C and were maintained a 12 hours light-dark cycle. Rats were randomly assigned to either a control (C, N = 10) or endurance trained (ET, N = 10) group. The death of two rats from natural causes resulted in a final n of 9 for both the C and ET groups. The research conducted in this investigation adhered to the policies and procedures in the “Guide for the care and use of laboratory animals”.

Training Program

Control rats were familiarized to treadmill running by exercising on a motorized treadmill at 0% incline and 15 metres per minute for 5 minutes per day, 5 days per week. This form of familiarization enabled C rats to be handled like ET rats yet prevent training adaptations in the C rats (18). ET rats were run 5 d.wk^{-1} for 11 weeks. Initially, these rats were run 10 minutes at 22m.min^{-1}, twice daily. Speed and duration were progressively increased so by week 7 rats were running continuously for 120 minutes at 35m.min^{-1}, interspersed every 10 minutes with sprints lasting 30 seconds and at 44 m.min^{-1} (18). By the end of week 1, rats were running once daily for 20 minutes at 22m.min^{-1}. During weeks 2-6,

running speed and time were increased 2-3m.min^{-1} and 20 minutes. Wk^{-1}, respectively. Soleus muscle citrate synthase activity was used to verify the effectiveness of the training program.

Citrate Synthase Activity

Citrate synthase activity was assessed according to Srere's (26), with some modification. Tissue homogenates stored at -20^{o}C were prepared for assay by thawing and freezing three times.

Drug Administration

Antipyrine and theophylline were used to determine the effect of endurance training on cytochrome P-450 mediated hepatic microsomal MFO drug metabolism. By measuring changes in total plasma clearance of probe drugs, host-factor influenced changes in the activity of cytochrome P-450-dependent isoforms can be identified (3-5). Antipyrine and theophylline were chosen as probe drugs for several reasons. One, antipyrine has been used extensively in the research setting to assess changes in cytochrome P-450 mediated hepatic microsomal MFO drug metabolism (24). Two, theophylline is often used to treat those with chronic asthma. Three, both drugs are metabolized extensively by hepatic cytochromes P-450 (2, 5, 16, 17, 20, 24). And four, I sought to determine whether endurance training might selectively affect the oxidative metabolizing capacity of some well-known cytochromes P-450 but not others. Theophylline was used as a probe of the CYPIA subfamily (2, 4, 16) and antipyrine as a probe of the CYP2B subfamily (4, 19, 29).

During weeks 8 and 10, C and ET rats, respectively, both received a randomly ordered single i.v. tail injection of antipyrine and theophylline. Appropriate solutions (4) of antipyrine (100mg.kg^{-1} body weight, dissolved as a 10% w.v. solution in 0.9% w.v. aq. Nacl) and theophylline (15mg.kg^{-1} body weight; aminophylline, lyphomed, inc., Rosemont IL) were used. Injections were separated by a 96h drug washout period. Drug doses were administered to rats approximately 48h following any exercise. The nonexercise period-helped minimize any carryover effects of acute exercise (18).

Blood Collection

Blood samples were collected 4 and 6h after the administration of antipyrine and theophylline, respectively. The 4 and 6h postdosing collection times were establsiehd by Bachmann *et al.* (4) and are considered to be optimal times for determining antipyrine and theophylline plasma clearance. Using 350μl heparinized capillary tubes, blood was collected

from the cut tail tip capillary tubes were then capped and centrifuged at high speed for 5 minutes in a table top centrifuge. The plasma was transferred to polypropylene tubes and stored at -20^{o}C until assayed for plasma concentrations of theophylline and antipyrine (see below).

Microsomal preparation and P-450 Content

Rats were euthanized by decapitation. Liver homogenates were prepared on ice and in cold (4^{o}C) rinse and buffer solutions. After being euthanized, approximately 4g of liver was removed and rinsed several times in 1.15% KCL solution, blotted dry weighed, and finely minced with sicssors in a 25% w.v. 0.01M 1.15% KCL/potassium phosphate buffer, PH 7.4. The remaining liver was removed and weighed. Liver preparations were homogenized using a polytron homogenizer (Brinkmann Instruments Inc., Westburg, NY). Tissue was homogenized for 15-20s at a speed producing minimal frothing. Approximately 15mlof liver homogenates were transferred into centrifuge tubes and centrifuged at 10,000 x g for 20 minutes at 4^{o}C in a Sorval Superspeed RC 2 – B centrifuge (Dupont Co.). To isolate microsomal fractions, 5ml of supernatant was pipette into ultracentrifuge tubes. The tubes were centrifuged for 60 minutes at 100,000 x g and 4^{o}C in a Beckman Model L – 2 ultra centrifuge (Beckman Instruments). The supernatant was removed, and the pellets were rinsed once with 1.15% KCl and then resuspended in 10ml of 0.05 M potassium phosphate buffer, PH 7.6. The microsomal suspensions were used immediately to determine total liver protein concentration and cytochrome P-450 content. Liver protein concentrations were determined using the commercially available Bio-Rad Protein Assay Kit I (Bio-Rad laboratories, Richmond, CA). total liver microsomal cytochrome P-450 content was determined as previously described (21), and the following formula (15) was used to calculate total hepatic cytochrome P-450 content; C = (AB . 1000/EC).df/OTP, where C (nmol.mg^{-1} protein) is cytochrome P-450 specific content, AB is the absorbance difference (450-490nm) between the reduced and CO complexed cytochrome P-450 (sample cuvette) and reduced cytochrome P-450 (reference cuvette), EC (91 mM^{-1} cm^{-1}) is the extinction coefficient for the wavelength couple 450-490nm, df is the dilution factor used on the original tissue sample, and OTP is the original tissue protein concentration.

Antipyrine and Theophylline Clearance

Plasma concentrations of antipyrine were measured by gas liquid chromatography (Hewlett-Packard 5840A) as previously described by Bachmann *et al.* (4, 5). All samples

were assayed within 24 hours of collection and done in the same assay. Single – sample clearance values were calculated using the following relationship (4, 5, 24): CL = [(In dose/v) – In Ct].v/t, (2) where CL is the single – sample estimate of clearance, dose (100mg.kg^{-1} body weight), v (0.66 1.kg^{-1}) is the sample mean value for volume of distribution (4), Ct is the plasma probe concentration at time t, and t (4h) is the postdose sampling time (4) clearance values were expressed as l.h^{-1}.kg^{-1} total liver weight.

Plasma concentrations of theophylline were determined by fluorescence polarization immunoassay (FPIA) using a TDx analyzer (Abbott laboratories, inving, TX) and a commercially available reagent kits. Assay procedures are explained elsewhere (4, 5). All samples were analyzed in the same assay and within 24 hours of collection. Single-sample clearance values for theophylline were also calculated using equation (2). The dose, v, and t used in calculatiosn were 15mg.kg^{-1} body weight, 0.39 l.kg^{-1}, and 6h, respectively (4). As with antipyrine, CL values for theophylline were expressed as l.h^{-1}.kg^{-1} total liver weight. The aforementioned single-sampling times have been demobstrated to accurately reflect multiple sample clearance estimates for these two probes under these two conditions (4).

Statistical Analysis

Independent t-test analysis was used to identify existing differences in soleus muscle citrate synthase activity, total hepatic cytochrome P-450 content, and postraining theophylline and antipyrine clearances between C and ET rats. The level of significance was set at $P<0.01$ for all statistical tests.

RESULTS

Soleus muscle citrate synthase activity was significantly greater for ET than for C rats (Table 1),

Table 1: Soleus Muslce Citrate Synthase activity and total hepatic microsomal cytochrome P-450 content

Group	Citrate Synthase (μmol.g^{-1}.min^{-1})	Cytochrome P-450 content (nmol.mg^{-1} liver protein)
C	26.4 ± 1.30	0.554 ± 0.055
ET	46.2 ± 2.72	0.604 ± 0.080

Values are means ± SD; * P < 0.01, ET > C

Establishing the increased endurance capacity of the ET rats. Further, no significant differences in total hepatic cytochrome P-450 content (Table 1) and posttraining single-sample plasma clearances of antipyrine and theophylline (Table 2) were observed between the C and ET rats. When euthanized, body and liver weights of C (8 weeks old) and ET (11 weeks old) rats were 577 ± 32g (mean ± SD) and 523 ± 33g (differences were significant), and 18±2.7g and 16.7 ± 1.9g (differences were not significant), respectively.

Table 2: Posttraining single-sample clearances for probe drugs antipyrine and theophylline

Variable	Control	Endurance Trianed
Antipyrine	6.44 ± 1.56	6.51 ± 1.02
Theophylline	1.89 ± 0.36	2.08 ± 0.49

Values are mean ± SD; single-sample clearance values are expressed as $1.h^{-1}.kg^{-1}$ total liver weight

DISCUSSION

It presently is unclear whether a high level of cardiovascular fitness (7, 12) or endurance training (11) alters cytochrome P-450

Hepatic microsomal drug metabolism in humans. Antipyrine and aminopyrine aminopyrine have been used extensively to assess changes in overall hepatic microsomal drug metabolism (10). Ducry et al. (11) found that the plasma clearance of aminopyrine was not different between sedentary students and well trained long-distance runners having a 44% greater maximal oxygen consumption. In a longitudinal study, Boel et al. (7) found no significant correlation between the metabolism of aminopyrine (13 % increase) and maximal oxygen consumption for subjects with a mean increase in post-training maximal oxygen consumption of 6%. Nevertheless, they did find a significant correlation between the metabolism of antipyrine (12% increase) and maximal oxygen consumption in these same subjects. In another study, Frenkl et al. (12) showed that the half-life of antipyrine was shorter in top athletes than in physical education students, and was shorter in the physical education students than in sedentary students.

In well-controlled animal studies, Frenkl et al. (13, 14) reported that female rats swum for $1h.d^{-1}$ for 6 weeks had reduced hexobarbital sleep times (14), had significantly shortened

antipyrine half-lives, and had increased antipyrine elimination rates (13) compared with sedentary controls. Day et al. (8)b showed that, in male rats, swimming increased microsomal metabolizing activity. In particular, they reported that aniline hydrrroxylase (P45011EI or CYP2EI; 29) activity was significantly elevated in aged rats, i.e., aniline metabolism increased (8, 9). Likewise, Ardies et al. (1) found that ethanol (CYP2EI metabolized) metabolism increased in female rats following chronic running exercise. Day and colleagues (8,9) have also reported that in male rats, 8 weeks of mild treadmill running (by week 4 running was done 4 $d.wk^{-1}$, 16 pr 20 $m.min^{-1}$, 60 $min.d^{-1}$) significantly decreased the microsomal metabolism of aniline and p-nitroanisole but did not affect the metabolism of ethoxyresorufin (CYPIAI metabolized; 29). In the present study, plasma clearances of antipyrine and theophylline were similar in both control and endurance trained rats (Table 2), indicating that the activities of rat liver CYP2B2 and CYPIA (in agreement with Day and Weiner (9) were unaffected by regular endurance exercise perhaps endurance exercise training selectively affects specific isoforms of P-450 since the activity of some P-450 isoforms appear to change with training while others remain unaffected.

Total hepatic cytochrome P-450 content was unaltered by increases in endurance exercise capacity (Table 1) Frenkl et al. (13), using two separate groups of female rats, found that 2 week of treadmill running (50$m.min^{-1}$, 14% incline, 30 $min.d^{-1}$) and 6 week of swim training (1$h.d^{-1}$) significantly increased total hepatic cytochrome P-450 concentration 123% and 105%, respectively. In constrast, Day et al. (8,9) demonstrated that in male rats, 8 week of mild treadmill running decreased total hepatic cytochrome P-450 content approximately 30-35% in middle-aged and aged rats. Day et al. (8) also found that 1yr of swimming, 5$d.wk^{-1}$, 2 times. d^{-1}, and 60 min. $session^{-1}$ significantly increased total hepatic cytochromes P-450 content 27% in middle-aged male rats. Nonetheless, regardless of swimming duration (i.e., no swimming (dry control), 1-min $swim.d^{-1}$ (wet control), and 120-min $swim.d^{-1}$ (exercise group)), 6 months of swim training did not change the hepatic cytochrome P-450 content of aged male rats.

Exactly why regular endurance can increase (1, 8, 13, 14), decrease (9) and not change (present study) (8, 9, 25) hepatic microsomal metabolic activity and cytochrome P-450 content currently is not well understood. Perhaps factors such as age, sex, strain, species, P-450 isoform, type and mode of training, or interactions among these factors may account for some of this disparity. Except for the findings of Saborido et al. (25), it presently appears as if regular endurance exercise increases hepatic micromal metabolic capacity and cytochrome P-450 content in female rats (1, 13, 14). This also seems to be the case for swim-

trained rats (8, 13, 14), although it may not be true for aged male rats (8) that have undergone swim training. Clearly, more work needs to be done to determine what part these and other factors may have in helping explain the effects of chronic endurance exercise on P-450-dependent drug metabolism.

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