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CHRONIC PHYSICAL ACTIVITY: HEPATIC HYPERTROPHY AND INCREASED TOTAL BIOTRANSFORMATION ENZYME ACTIVITY

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Abstract—Does chronic voluntary physical activity alter hepatic or intestinal capacities for xenobiotic biotransformation? This question was investigated by comparing biotransformation enzyme activities in liver and small intestine of active and sedentary rats. Male rats allowed unlimited access to a running wheel and fed *ad lib.* for 6 weeks were weight-matched to sedentary controls; the active rats ate 22% more food than the sedentary rats ($P < 0.05$). Active rats ran 2.8 ± 0.6 miles/day. Liver weights were higher in the active rats (11.2 ± 0.2 vs 9.8 ± 0.2 g; $P < 0.05$), as were total liver protein, and liver microsomal and cytosolic protein ($P < 0.05$). As a result of liver hypertrophy, the active rats showed higher total liver activity of several biotransformation enzymes, including 2-naphthol sulfotransferase, styrene oxide hydrolase, benzphetamine *N*-demethylase, ethacrynic acid glutathione *S*-transferase and morphine UDP-glucuronosyltransferase ($P < 0.05$). In contrast, there was no detectable difference in total liver *N*-acetyltransferase activity toward *p*-aminobenzoic acid, 2-naphthylamine, and 2-aminofluorene as well as, relative hepatic enzyme activity (expressed per g liver or per mg protein) and total and relative intestinal enzyme activity. We conclude that chronic voluntary physical activity, accompanied by an increased food intake, results in liver hypertrophy and potentially increases total hepatic capacity to biotransform certain xenobiotic chemicals.

Hepatic and intestinal biotransformation enzymes protect the body by either directly altering the chemical structure of an endo- or xenobiotic, or by conjugating it with an endogenous compound to render it more water soluble. Because elimination of a drug often depends largely on the rate of biotransformation, individual differences in drug-metabolizing capacity may dramatically influence the therapeutic outcome of drug treatment. Factors that have been demonstrated to influence biotransformation include age, gender, diet, many drugs and environmental chemicals, and various disease states. While chronic physical activity provokes numerous well-documented physiological adaptations, little is known of the effects of repeated exercise on the biotransformation capacity of the liver and intestine [1, 2].

Limited past work hints that chronic exercise alters xenobiotic biotransformation. Frenkl *et al.* [3, 4] reported a decreased hexobarbital sleeping time in treadmill-run and swim-trained rats when compared to controls. In addition, increased hepatic concentrations of cytochrome P450 and cytochrome b_5, as well as higher activities of several phase I enzymes were found [3, 4]. In contrast, Day and Weiner [5] noted in treadmill-run rats decreased hepatic cytochrome P450 concentrations as well as decreased cytochrome P450 enzyme activities which may have provided protection from carbon tetrachloride toxicity in hepatocytes of the exercised versus the sedentary rats, since carbon tetrachloride must first be metabolized to exert its toxic effect [5]. Given these conflicting results and that forced exercise is more stressful than voluntary exercise [6], this study has evaluated the effects of a voluntary exercise model on hepatic and intestinal biotransformation reactions. Whereas no phase II pathways were examined in previous reports, the present study has included model substrates for the major conjugation reactions—acetylation, sulfation, glucuronidation and glutathione conjugation—in addition to two phase I biotransformations. Because voluntary exercise is associated with an increase in food intake [7, 8], while forced exercise is not [9–11], food intake was monitored and controlled to match body weight.

MATERIALS AND METHODS

Materials. Acetyl coenzyme A, 2-naphthylamine, 2-aminofluorene, *p*-aminobenzoic acid, *N*-1-(naphthyl)ethylenediamine dihydrochloride, *N*-ethylmaleimide, glutathione, ethacrynic acid, sulfobromophthalein, 1-chloro-2,4-dinitrobenzene, NAD^+, glucose-6-phosphate, semicarbazide hydrochloride, glucose-6-phosphate dehydrogenase, Tris, 2-naphthol, 2-mercaptoethanol, adenosine 3′-phosphate 5′-phosphosulfate, methylene blue, trichloroacetic acid, UDP-glucuronic acid, polyoxyethylene 20 cetyl ether (Brij 58†), glycine, sucrose, 1-naphthol, estrone, *p*-nitrophenol, *cis*-oxaloacetate, and hexobarbital were obtained from the Sigma Chemical Co. (St. Louis, MO); styrene oxide was purchased from the Aldrich Chemical Co. (Milwaukee, WI); benzphetamine HCl was provided by the Upjohn Co. (Kalamazoo, MI); morphine

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† Abbreviations: Brij 58 (polyoxyethylene 20 cetyl ether); and GSH (glutathione).

alkaloid was a gift from the Penick Corp. (Lyndhurst, NJ); [2,4,6,7-^{3}H]estrone (103 Ci/mmol), [*n-methyl*-^{3}H]morphine (58.5 Ci/mmol), [1-^{14}C]1-naphthol (57 mCi/mmol), [^{3}H]acetyl coenzyme A (6.48 Ci/mmol), and [7(n)-^{3}H]styrene oxide (150 mCi/mmol) were obtained from Amersham–Searle (Arlington Heights, IL). Scintillation counting fluid (Budget Solve) was obtained from Research Products International (Elk Grove, IL) and halothane from Halocarbon Laboratories (Augusta, SC). All other chemicals were the highest quality available. Deionized water was used throughout.

Animal care. Male Sprague–Dawley rats (150 g, Harlan, Indianapolis, IN) were randomly assigned to either an active or inactive group. The inactive rats were housed in individual stainless steel cages measuring 7 × 7 × 9.5 inches in which they had free mobility. The rats allowed more activity were housed in individual galvanized steel cages measuring 5 × 10 × 6 inches which were attached to voluntary running wheels, 44 inches in circumference. The freely accessible exercise wheels were fitted with revolution counters. The rats were housed at 21–24°, exposed to 13 hr of light per day, allowed water *ad lib.*, and fed standard ground rodent chow (Purina No. 5012). The active group ate rat chow *ad lib.*, while the inactive group had food intake adjusted to match body weight to the active group. For both groups of rats, the body weights increased from an average of 150 to 300 g within the 6-week experimental period, which is the normal growth rate for male Sprague–Dawley rats [12]. The rats' body weight, food intake and distance run were measured daily during the experimental period of 6 weeks.

Hexobarbital sleeping time. At the end of the 6-week exercise or sedentary period, hexobarbital sleeping time was determined in one group of rats. The active rats were allowed to run the previous night, and the experiment was performed in the mid-morning about 1 hr after the rats were removed from their cages. Hexobarbital sleeping time was defined as the time between the loss and return of the righting reflex [13]. A solution of 40 mg/mL of hexobarbital free acid in deioinized water, prepared fresh, was injected i.p. at a dose of 125 mg hexobarbital/kg body weight. A blinded observer determined sleeping times for all animals. The sleeping environment was free from draft or loud noises, and core temperature was maintained constant by heat lamps controlled by rectal thermistors.

Tissue preparation. At the end of the 6-week exercise or sedentary period, activities of several biotransformation enzymes were determined in livers and intestines from rats anesthetized with 1.5% halothane, a dose which does not affect any of the studied enzyme reactions. The entire liver and entire small intestine were removed and immediately placed in ice-cold 1.15% potassium chloride. The red soleus muscle was removed, immediately frozen in liquid nitrogen, and stored at −70°. After being cleaned, blotted and weighed, the liver and intestine were either frozen at −70° or immediately processed for the *N*-acetyltransferase assays.

All tissues were homogenized with a Brinkmann polytron at a setting of 5.5. Red soleus muscle was homogenized in 175 mM potassium chloride, 10 mM glutathione (GSH) and 2 mM EDTA, pH 7.4, and assayed for citrate synthase activity. For the glucuronosyltransferase assays, 10% liver and intestinal homogenates were prepared in 0.25 M sucrose containing 5.0 mM Tris–HCl at pH 7.4. Cytosolic and microsomal subcellular fractions from both intestine and liver were prepared by first homogenizing the tissue in 1.15% potassium chloride and then centrifuging the homogenate at 10,000 *g* for 20 min to remove the mitochondrial fraction and nuclear debris. The supernatant was then recentrifuged at 100,000 *g* for 65 min. The resultant microsomal pellet was resuspended in 0.1 M potassium phosphate buffer, pH 7.4, and used for the benzphetamine *N*-demethylase and styrene oxide hydrolase assays. The cytosolic fraction (supernatant) was used to determine *N*-acetyltransferase and glutathione *S*-transferase activities. The cytosol for the sulfotransferase assay was prepared by homogenizing tissue in 0.25 M sucrose containing 10 mM Tris–HCl and 3 mM 2-mercaptoethanol, pH 7.4, and then centrifuging the homogenate at 105,000 *g* for 65 min.

Protein determination. Protein concentration in the whole tissue homogenates and in the microsomal and cytosolic subcellular fractions was measured by the method of Lowry *et al.* [14].

Enzyme assays. N-Acetyltransfrerase activity toward 0.04 mM *p*-aminobenzoic acid was determined colorimetrically [15], whereas activities toward 0.125 mM 2-naphthylamine and 0.125 mM 2-aminofluorene were determined radiometrically [16]. Glutathione *S*-transferase activity toward 0.2 mM ethacrynic acid, 0.12 mM sulfobromophthalein and 1.0 mM 1-chloro-2,4-dinitrobenzene was determined spectrophotometrically [17]. *N*-Demethylase activity toward 1 mM benzphetamine was determined using colorimetric determination of formaldehyde [18, 19]. Epoxide hydrolase activity toward 1.5 mM styrene oxide was measured radiometrically [20]. Aryl sulfotransferase activity toward 0.25 mM 2-naphthol was determined according to the method of Sekura and Jakoby [21]. Glucuronosyltransferase activity toward 1.5 mM morphine and 0.5 mM 1-naphthol was measured radiometrically [22]; activity toward 0.1 mM estrone was also determined radiometrically [23] and activity toward 0.5 mM *p*-nitrophenol was quantitated colorimetrically [24]. Citrate synthase activity was determined by an enzymatic method [25].

All assays were performed in duplicate along with the appropriate blanks and were proportional to protein concentrations and incubation time.

Statistics. All results are given as means ± SEM. At the end of the exercise period, the activity and inactive rats were paired by weight, the heaviest active rat being paired with the heaviest inactive rat, and so on. All of the data were then analyzed by comparing the difference between the active and inactive rats by paired *t*-tests, with significance defined as $P < 0.05$.

RESULTS

Distance run, food intake, body weight, liver and

Table 1. Distance run, food intake, body weight, liver weight, intestinal weight, citrate synthase activity and hexobarbital sleeping time in inactive and active rats

	Inactive	Active
Distance run (miles/day) N = 22	0	2.83 ± 0.61*
Range (miles/day)		0.7 to 7.1
Food intake (g/day) N = 22	18.2 ± 0.15	22.2 ± 0.34*
Body weight (g) N = 22	300 ± 1.8	304 ± 2.3
Liver weight (g) N = 22	9.8 ± 0.2	11.2 ± 0.2*
Intestinal weight (g) N = 12	8.0 ± 0.27	8.2 ± 0.18
Citrate synthase activity in red soleus muscle (nmol/min/g) N = 9	15.0 ± 0.82	17.2 ± 1.6
Hexobarbital sleeping time (min) N = 9	31.2 ± 10	36.0 ± 13

Values are means ± SEM.
* Significantly different from inactive group ($P < 0.05$).

intestinal weights, citrate synthase activity and hexobarbital sleeping time are all listed in Table 1. Within the first 2 weeks, the rats with access to wheels voluntarily increased their running to an average of 2.8 miles/day (range 0.7 to 7.1 miles/day). Similar mean daily milage was maintained for the next 4 weeks. The weight-matched exercised rats ate 22% more food than the sedentary rats. Although there was no difference in body weight or intestinal weight, there was a significantly greater liver weight in the active rats. Citrate synthase activity in the red soleus muscle and hexobarbital sleeping time were not significantly different between the active and inactive rats. Although miles run/day was positively correlated with food intake, it was not correlated with liver weight, specific enzyme activity, or protein content.

Whole liver homogenate protein concentration was higher in inactive rats when compared with active rats (278 ± 9.8 vs 301 ± 11 mg protein/g; $P < 0.05$). The microsomal and cytosolic subcellular protein concentrations were similar in the two groups (23 ± 1.5 and 146 ± 9.7 mg protein/g liver, respectively). However, when the protein content was calculated for the whole liver, homogenate, microsomal and cytosolic protein contents were greater in active rats (Fig. 1) than in the sedentary controls. There was no comparable difference in either relative (126 ± 4.1, 8.0 ± 1.1 and 58.3 ± 3.1 mg protein/g intestine for homogenate, microsomal, and cytosolic fractions, respectively) or absolute intestinal protein contents (i.e. mg/g tissue or mg/total tissue weight) between the two groups of rats.

The relative hepatic and intestinal biotransformation enzyme activities expressed in nmol/min/mg protein were not significantly different between inactive and active rats (Tables 2 and 3). When the liver biotransformation enzyme activities were expressed per whole liver (nmol/min/liver), there was an increase in total activity of styrene oxide hydrolase (74%), benzphetamine *N*-demethylase (61%), ethacrynic acid glutathione *S*-transferase (26%), morphine UDP-glucuronosyltransferase (25%) and 2-naphthol sulfotransferase (14%) in active rats (Fig. 2). All other biotransformation enzyme activities expressed per whole liver were identical in the two groups; all intestinal enzyme activities per whole organ were similarly unaffected by animal activity.

DISCUSSION

In this study, voluntarily active rats weight-matched to relatively inactive controls had increased liver weights and increased whole liver homogenate, microsomal, and cytosolic protein contents. These changes resulted in increased total activity of several hepatic biotransformation enzymes. There was no difference, however, in the relative hepatic enzyme activity, or in intestinal weight, intestinal protein content or intestinal biotransformation enzyme activity between the two groups of rats. There was also no difference in hexobarbital sleeping time, an indirect measure of one component of hepatic biotransformation enzyme activity [26].

Voluntary wheel running was chosen for this study because it is analogous to most human exercise and is less stressful for the rat than forced swimming or treadmill running [6]. The low stress of voluntary wheel running apparently results both from the activity being spontaneous and from maintenance of a constant environment in active and inactive situations [6]. Although there was clearly an increase in energy expenditure in the active rats (as evidenced by increased food intake compared to weight-matched controls), a training effect upon red soleus citrate synthase activity was not found. The rats ran an average of 2.8 miles/day, but the intensity of the exercise apparently was not sufficient to elevate citrate synthase activity. Citrate synthase activity has been shown to increase as a result of forced treadmill running [11], which is typically more intense than voluntary wheel running. The 22% increase in food intake of the voluntarily active rats, which closely agrees with past studies [7, 8], is typically not seen in forced exercise [9–11].

The association of increased relative liver weight in rats with increased food intake has also been found in other studies of voluntarily active animals [8]; in contrast, exercise that fails to provoke hyperphagia can decrease liver weight [27]. Similarly, lactating rats eating twice as much food as non-lactating rats have a 50% increase in hepatic weight [28]. While the increase in food intake per unit body weight clearly correlates with liver growth [28], the mechanism for this growth is unclear. Indeed, while the specific protooncogenes and growth factors that

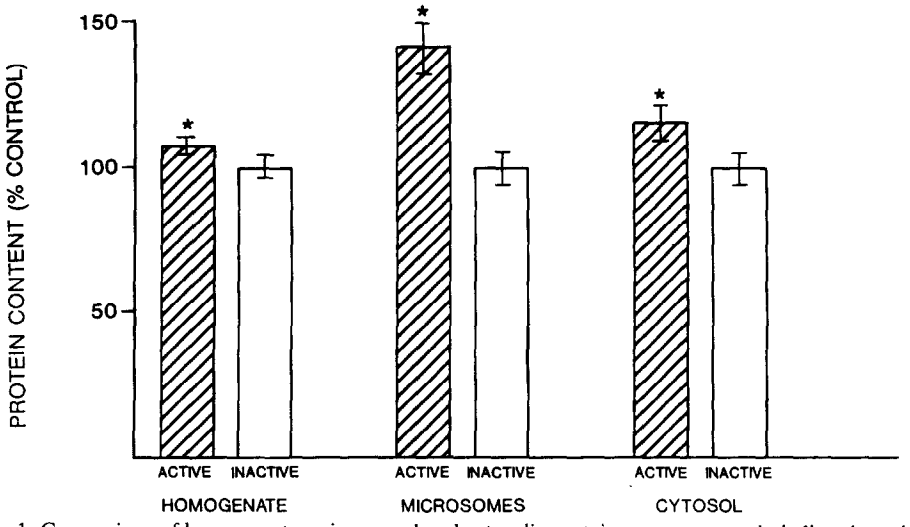

Fig. 1. Comparison of homogenate, microsomal and cytosolic protein contents per whole liver in active versus inactive rats. Control values (100%) were: homogenate, 2,920 ± 100, microsomes, 212 ± 14, and cytosol, 1,460 ± 92 mg protein/total liver. Values are means ± SEM (N = 11). Key: (*) Significantly different from inactive at $P < 0.05$.

Table 2. Relative liver enzyme activities in inactive and active rats

	Inactive	Active
2-Naphthol sulfotransferase	0.501 ± 0.034	0.442 ± 0.028
Styrene oxide hydrolase	1.19 ± 0.11	1.25 ± 0.17
Benzphetamine *N*-demethylase	2.39 ± 0.29	2.89 ± 0.27
Glutathione *S*-transferase toward:		
Ethacrynic acid	22.3 ± 1.9	23.3 ± 2.2
1-Chloro-2,4-dinitrobenzene	1640 ± 258	1480 ± 248
Sulfobromophthalein	2.08 ± 0.21	2.00 ± 0.22
UDP-glucuronosyltransferase toward:		
Morphine	1.34 ± 0.09	1.53 ± 0.09
4-Nitrophenol	34.1 ± 2.8	28.9 ± 3.1
Estrone	0.002 ± 0.0004	0.003 ± 0.001
1-Naphthol	7.90 ± 0.79	7.23 ± 0.70
N-Acetyltransferase toward:		
p-Aminobenzoic acid	0.750 ± 0.081	0.755 ± 0.081
2-Naphthylamine	0.254 ± 0.031	0.254 ± 0.018
2-Aminofluorene	0.240 ± 0.036	0.232 ± 0.018

N-acetyltransferase, glutathione *S*-transferase and sulfotransferase activities were determined with cytosol, UDP-glucuronosyltransferase with homogenate, and *N*-demethylase and epoxide hydrolase with microsomes. Values are means ± SEM for 8 rats and are expressed in nmol/min/mg protein; P = NS for all enzyme activities.

regulate hepatic compensatory growth at the cellular and subcellular level are fairly well defined, the precise signals that ultimately link liver size to the metabolic activity of the body are unknown [29].

Whatever its mechanism, liver hypertrophy resulted in increased total hepatic protein in active rats, leading to increased absolute activity (nmol/min/liver) of several biotransformation enzymes. This change occurred despite decreased relative hepatic protein/unit liver, most likely due to enhanced hepatic glycogen storage in active animals [30]. We found no changes in relative enzyme activity (enzyme induction) by physical activity. This last result contrasts with earlier work, using treadmill-run or swim-trained rats, that shows either an increase [3, 4] or a decrease [5] in the relative hepatic activity and concentration of several biotransformation enzymes. It is not surprising that increased metabolic activity leads to neither induction nor inhibition of the enzymes involved in xenobiotic metabolism, since both induction and inhibition are substrate and, therefore, isozyme-specific. If, however, a specific inducer or inhibitor (e.g. Brassica vegetables) were present at constant concentration

Table 3. Relative intestinal activities in inactive and active rats

	Inactive	Active
2-Naphthol sulfotransferase	ND*	ND
Styrene oxide hydrolase	ND	ND
Benzphetamine *N*-demethylase	ND	ND
Glutathione S-transferase toward:		
Ethacrynic acid	15.1 ± 1.0	14.8 ± 1.2
1-Chloro-2,4-dinitrobenzene	358 ± 35	349 ± 34
Sulfobromophthalein	ND	ND
UDP-glucuronosyltransferase toward:		
Morphine	ND	ND
4-Nitrophenol	ND	ND
Estrone	ND	ND
1-Naphthol	0.93 ± 0.13	1.55 ± 0.38
N-Acetyltransferase toward:		
p-Aminobenzoic acid	0.810 ± 0.113	0.922 ± 0.097
2-Naphthylamine	0.168 ± 0.038	0.189 ± 0.048
2-Aminofluorene	0.079 ± 0.009	0.110 ± 0.009

N-Acetyltransferase, glutathione *S*-transferase and sulfotransferase activities were determined with cytosol, UDP-glucuronosyltransferase with homogenate, and *N*-demethylase and epoxide hydrolase with microsomes. Values are means ± SEM for 8 rats and are expressed in nmol/min/mg protein; P = NS for all enzyme activities.
* ND = not detectable.

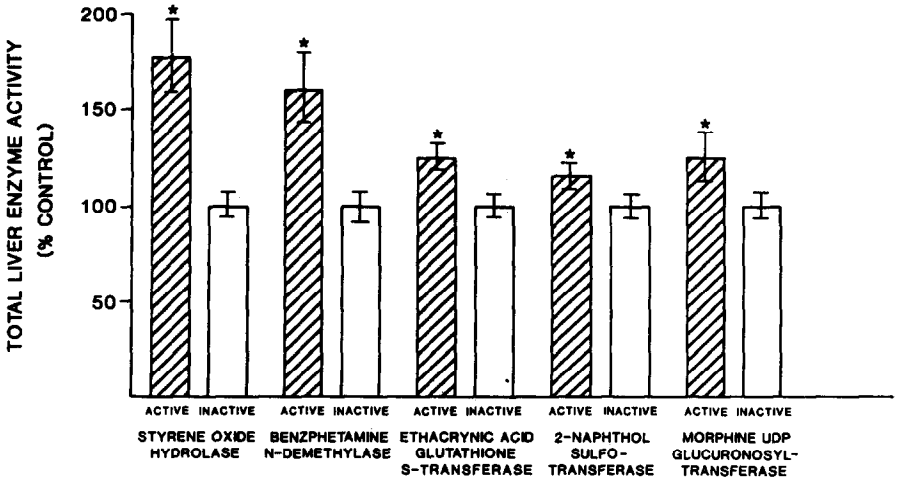

Fig. 2. Comparison of liver enzyme activities, adjusted per whole liver, that were greater in active rats versus inactive rats. The control values (100% activity) for the substrates were: styrene oxide hydrolase, 214 ± 14, benzphetamine *N*-demethylase, 488 ± 39, ethacrynic acid glutathione *S*-transferase, 28,900 ± 1,600, 2-naphthol sulfotransferase, 706 ± 44, and morphine UDP-glucuronosyltransferase, 3,240 ± 220 nmol/min/liver. Values are means ± SEM (N = 8). Key: (*) Significantly different from inactive at $P < 0.05$.

in the diet, hyperphagia could be expected to alter substrate availability and thereby provoke changes in relative enzyme activity [31]. It is unclear why forced exercise leads to an increased or decreased relative enzyme activity, while voluntary exercise does not. Treadmill-running or swim-training is more intense, usually a maximum of 1 hr per day, while voluntary exercise is not as intense and the rats run for several hours per day [6]. Acute bouts of exercise are associated with an increased body temperautre [32], increased free radical formation [33], altered hepatic blood flow [34], and changes in the hormonal milieu [35]. It is possible that the more intense forced exercise results in an increased response to those changes, all of which may affect substrate availability and therefore lead to substrate specific differences in biotransformation enzyme activity. In contrast, voluntary exercise is not as intense and does not elicit substrate specific changes. Instead, it is associated with an increased food intake which may

be responsible for the increase liver weight and total protein content resulting in an increased total biotransformation enzyme activity.

Despite finding enhanced total activity of several biotransformation enzymes, we found no difference in hexobarbital sleeping time between the active and inactive rats. In contrast, Frenkl *et al.* [3, 4] noted a decreased hexobarbital sleeping time in swim-trained and treadmill-run rats, which he attributed to the increased concentration and the activity of various components of the microsomal monooxygenase system. Of the enzyme activities that we measured, only benzphetamine *N*-demethylase is part of the monooxygenase system, and does not participate in the metabolism of hexobarbital. Instead, hexobarbital is metabolized by hexobarbital hydroxylase to hydroxy-hexobarbital and keto-hexobarbital [36]. A constant hexobarbital sleeping time suggests that its rate of hepatic biotransformation is unchanged despite activity-induced hyperphagia and hepatic hypertrophy.

There was no significant difference in intestinal weight or intestinal biotransformation enzyme activity between the inactive and active rats. Although several reports have noted an association of intestinal hypertrophy with hyperphagia due to cold exposure or lactation [28, 37], we found none. Rather than reflecting a qualitative difference between chronic activity and these other causes of hyperphagia, this result likely is due to the relatively small degree of hyperphagia induced by exercise (22%). In contrast, lactation-induced gut hypertrophy was detected as food intake increased 100% [28, 37].

In conclusion, voluntary wheel running for 6 weeks resulted in increased liver weights and increased total hepatic activity of several biotransformation enzymes in rats weight-matched to relatively inactive controls. At the same time, a functional index of a specific enzyme activity, hexobarbital sleeping time, was unchanged. Since the liver is the primary organ for drug metabolism, these results suggest that individuals with physical activity-induced increases in energy expenditure resulting in hyperphagia could metabolize certain drugs or toxins at different rates than the sedentary counterparts. Further studies in animals and humans with different energy expenditure will determine if chemicals that undergo hepatic metabolism require different dosages for similar effects.

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