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Genetic selection of mice for high voluntary wheel running: effect on skeletal muscle glucose uptake

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Dumke, C. L., J. S. Rhodes, T. Garland Jr, E. Maslowski, J. G. Swallow, A. C. Wetter, and G. D. Cartee. Genetic selection of mice for high voluntary wheel-running: effect on skeletal muscle glucose uptake. *J Appl Physiol* 91: 1289–1297, 2001.—Effects of genetic selection for high wheel-running activity (17th generation) and access to running wheels on skeletal muscle glucose uptake were studied in mice with the following treatments for 8 wk: *1*) access to unlocked wheels; *2*) same as *1*, but wheels locked 48 h before glucose uptake measurement; or *3*) wheels always locked. Selected mice ran more than random-bred (nonselected) mice (8-wk mean ± SE = 8,243 ± 711 vs. 3,719 ± 233 revolutions/day). Body weight was 5–13% lower for selected vs. nonselected groups. Fat pad/body weight was ~40% lower for selected vs. nonselected and unlocked vs. locked groups. Insulin-stimulated glucose uptake and fat pad/body weight were inversely correlated for isolated soleus ($r = -0.333$; $P < 0.005$) but not extensor digitorum longus (EDL) or epitrochlearis muscles. Insulin-stimulated glucose uptake was higher in EDL ($P < 0.02$) for selected vs. nonselected mice. Glucose uptake did not differ by wheel group, and amount of running did not correlate with glucose uptake for any muscle. Wheel running by mice did not enhance subsequent glucose uptake by isolated muscles.

glucose transport; exercise; artificial selection; insulin sensitivity

EXERCISE AND INSULIN are the most important physiological stimuli for increasing skeletal muscle glucose uptake. A variety of in vivo exercise models have been used with rats, the most common being forced exercise by running on motorized treadmills (8, 32) and swimming (4, 5, 21, 27, 44). Substantial effects on insulin-stimulated glucose uptake can occur after a single bout of exercise in rats, and these effects can persist for several hours up to a day or more (5). In addition to increases in insulin action following a single exercise session, chronically performed exercise (swimming, running on a motorized treadmill, or voluntary running) by rats can induce adaptations that increase the capacity of muscle for insulin-stimulated glucose uptake (11, 20, 21, 26, 33, 35).

Few studies have used the mouse to study exercise-related changes in muscle glucose uptake. Bonen and colleagues published several important studies (1, 2, 43) that used mice to evaluate the effects of exercise (motorized treadmill running) on muscle glucose uptake and metabolism. Glucose uptake (both insulin independent and insulin dependent) was enhanced following a single bout of treadmill running (1, 2). Recently, with the advent of transgenic technology, there has been a resurgence of publications describing effects of exercise (swimming or treadmill running) or electrically stimulated (in situ) contractile activity on muscle glucose uptake using mice (12, 13, 19, 36, 45).

None of these studies has assessed voluntary exercise in mice. Therefore, this study aimed to fill this gap in knowledge using a novel mouse model: animals that had been genetically selected (17 generations) for high levels of wheel running (39). In brief, eight closed lines were established from an original outbred base population. Each generation, mice were given access to voluntary wheels for a period of 6 days, and selective breeding was based on the mean number of revolutions run on *days 5* and *6*. The male and female with the greatest number of revolutions per day propagated the four replicate "selected lines," and randomly bred control mice propagated the four replicate "nonselected lines" (39). After 17 generations of selection, selected mice ran an average of >2.5-fold more (revolutions/day) than did nonselected mice (31). Knowledge about phenotypic differences between the nonselected and selected groups is valuable for beginning to elucidate the genetic basis of differences at the phenotypic level. In addition, because mice from the selected lines exhibit substantially elevated levels of wheel running, they may be more likely to exhibit a training response compared with ordinary laboratory mice. Hence, power to detect effects of voluntary exercise should be increased by use of these lines of mice.

In this study, we evaluated glucose uptake by isolated muscles from nonselected and selected mice, with and without access to functional running wheels. Male mice (nonselected and selected; 17th generation of se-

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lection) were randomly assigned to one of three treatments for a period of 8 wk: *1*) continuous access to an unlocked (free to turn) running wheel; *2*) same as *treatment 1*, but wheel locked 48 h before measurement of glucose uptake (to identify persistent effects of wheel running independent of responses to relatively recent activity); and *3*) wheel locked throughout the experiment. We hypothesized that mice given unlimited access to unlocked running wheels would have enhanced glucose uptake in skeletal muscle treated with insulin compared with mice without access to functional wheels, and we expected the effect to be diminished when wheels were locked 48 h before measurement of glucose uptake. Evidence from prior generations that genetic selection was accompanied by several metabolic differences, even when mice did not have access to unlocked wheels (22, 40), suggested there also might be a genetic effect on glucose uptake in locked-wheel groups.

METHODS

Breeding design. As previously described in detail (39, 40, 42), outbred, genetically variable laboratory house mice of the Hsd:ICR strain were purchased from Harlan Sprague Dawley. After two generations of random mating, mice were randomly paired and assigned to eight closed lines (10 pairs in each). In each subsequent generation, when the offspring of these pairs were 6 to 8 wk old, they were housed individually with access to a running wheel for 6 days, and wheel revolutions were determined in 1-min intervals. In the four selected lines, the highest-running male and female from each family were selected as breeders to propagate the lines to the next generation (i.e., within-family selection). In the four nonselected lines, a male and a female were randomly chosen from each family. Within all lines, the chosen breeders were randomly paired except that sibling matings were not allowed. The lines were propagated this way for 17 generations.

Experimental design. In this study, we used animals from the 17th generation that were not among those chosen as breeders to propagate lines to the 18th generation. Because exclusion of the top runners would have caused our samples from the selected lines to be biased downward with respect to wheel running, we also excluded the lowest-running animals in selected-line families. These mice are a subset of *generation 17* mice used in a previous study (31). Male ($n = 72$) mice from the four replicate selected lines and the four nonselected control lines were randomly assigned to three groups of varying wheel treatment (initially 12 per treatment group): *1*) continuous access to an unlocked running wheel (unlocked), *2*) same as *treatment 1*, but wheels locked 48 h before experiment (48 h), and *3*) wheel continuously locked (locked).

Mice (mean age ± SD = 70 ± 3 days) were placed in standard clear plastic cages (27 × 17 × 12.5 cm) attached to Wahman-type activity wheels (1.12-m circumference, 10-cm-wide running surface of 10-mm wire mesh bounded by clear Plexiglas walls; model 86041 with modifications, Lafayette Instruments, Lafayette, IN) and provided food [Harlan Teklad laboratory rodent diet (W)-8604] and water ad libitum. Twenty-four wheels were secured with wire ties to prevent them from rotating (locked groups) and 48 were left free to rotate (unlocked and 48-h groups). Mice were left undisturbed in their cages for 8 wk. Approximately 48 h before the muscle incubation experiment, 24 of the freely rotating wheels were locked with wire (48-h groups). On the day of the muscle incubation experiment, all animals were removed from wheel cages 4–8 h before death. They were provided with water, but not food, after being removed from wheel cages.

Voluntary wheel-running behavior. Voluntary wheel running was measured as previously described (39, 40, 42). We attached a photocell counter to each wheel, which interfaced with an IBM-compatible personal computer. Customized software from San Diego Instruments (San Diego, CA) measured the number of clockwise and counterclockwise revolutions during every 1-min interval for each wheel. Data were downloaded every 24 h.

Tissue dissection. Animals were anesthetized (intraperitoneal injection of pentobarbital sodium, 50 mg/kg). The order of anesthetization among groups was alternated so that effects of the time of tissue sampling on glucose uptake was minimized. Mice were weighed, and blood was drawn from the periorbital vascular bed with heparin-lined capillary tubes. Tubes were centrifuged, and hematocrit was determined in quadruplicate; means of these four measurements were then analyzed. The remaining plasma was used to determine plasma glucose and insulin concentrations. The soleus and extensor digitorum longus (EDL) from the hindlimb and epitrochlearis from the forelimb were rapidly dissected for the glucose uptake assay. These muscles, because of their small size in mice, have been frequently used for measurement of in vitro glucose uptake (1, 2, 9, 12, 19, 36, 43, 45). The left retroperitoneal fat pad was removed and weighed. The liver was rapidly frozen and weighed, and a portion was stored at −80°C.

Glucose uptake. Erlenmeyer flasks (25 ml) were gently agitated in a shaking water bath and continuously gassed with 95% O_2-5% CO_2. Contralateral soleus, EDL, and epitrochlearis muscles from each mouse were initially placed in flasks containing 3 ml of Krebs-Henseleit buffer including 0.1% bovine serum albumin, 2 mM sodium pyruvate, and 36 mM mannitol. One muscle from each pair was incubated without insulin (basal), and the contralateral muscle was exposed to 0.6 nM insulin (Humulin R, Lilly, Indianapolis, IN) (30 min at 37°C).

Muscles were then transferred to a second flask containing Krebs Henseleit buffer supplemented with 1 mM ^{3}H-labeled 2-deoxyglucose (2 mCi/mmol; ARC, St. Louis, MO), 39 mM mannitol, [^{14}C]mannitol (0.022 mCi/mmol; ARC), and the same insulin concentration as the preceding preincubation step (20 min at 37°C). Muscles were then rapidly blotted on filter paper, trimmed of connective tissue, and quick frozen between aluminum clamps cooled to the temperature of liquid nitrogen. Muscles were stored at −80°C until weighed and then homogenized in 0.3 M perchloric acid at 4°C. The homogenate was centrifuged (10,000 *g* for 10 min), and aliquots of supernatant were quantified for ^{3}H and ^{14}C using a liquid scintillation counter. Glucose uptake activity was determined as previously described (3, 15).

Glycogen concentration. Glycogen concentration in the soleus and EDL was determined using aliquots of the perchloric acid homogenate by the amyloglucosidase method (28). Liver samples (~25 mg) were hydrolyzed in 2 M HCl at 100°C for 2 h. The hydrolyzed samples were neutralized with 0.67 M NaOH and vortexed. Liver glycogen concentration was determined as in muscle (28).

Plasma glucose and insulin concentration. Plasma glucose was determined using a spectrophotometer by the glucose oxidase method (Sigma Diagnostics, St. Louis, MO). Plasma insulin concentrations were quantified in duplicate using a

Table 1. *Wheel-running activity*

Genetic Group	Week 1	2	3	4	5	6	7	8
Selected	9,402 ± 696	7,963 ± 691	8,530 ± 1,028	7,871 ± 526	7,728 ± 648	7,140 ± 726*	4,307 ± 333*	5,756 ± 379*
Nonselected	3,943 ± 403	4,142 ± 595	4,337 ± 902	3,752 ± 484	3,884 ± 600	3,302 ± 688	2,915 ± 322†	3,375 ± 354
Selected vs. nonselected for each week	$P = 0.002$	$P = 0.006$	$P = 0.022$	$P = 0.001$	$P = 0.005$	$P = 0.009$	$P = 0.024$	$P = 0.004$

Values are means ± SE (mean revolutions/day). Sample size (including values for continuously unlocked group combined with group unlocked during 48 h before measurement of 2-deoxyglucose uptake) was 22 for selected and 24 for nonselected mice. Some mice had missing data for 1 or more days, in which case values for that animal were not included in the mean for the corresponding week. In some weeks, values for up to 3 animals have been deleted because they were statistical outliers, based on inspection of residuals. Each value is the mean of 7 days except for *week 8,* which included only 6 days for the continuously unlocked groups and 4 days for the groups unlocked until 48 h before 2-deoxyglucose measurement. *Within the selected group, running activity on *weeks 6* through *8* was significantly ($P < 0.05$) different from *weeks 1* through *5* based on post hoc analysis. †Within the nonselected group, running activity on *week 7* was significantly ($P < 0.05$) different from *weeks 2* through *5* based on post hoc analysis.

radioimmunoassay with rat insulin as the standard (Linco, St. Louis, MO).

Statistical analysis. All dependent variables were analyzed using a nested two-way ANOVA (general linear models procedure SAS, Cary, NC). Genetic background (selected vs. nonselected) and wheel treatment (unlocked, 48 h, and locked) were the grouping factors; replicate line was nested within genetic background. Main effects for genetic selection, wheel treatment, and the interaction of genetic selection × wheel treatment are reported. Wheel running was assessed each week, and repeated-measures, nested two-way ANOVA was used to analyze the data. When a significant interaction ($P < 0.05$) was found, post hoc analysis was completed to determine the source of significant variance by a Bonferroni correction of a matrix of simple *t*-test comparisons between each group. Data were considered outliers and removed if they exceeded three studentized residuals; two data points were the most removed from any one analysis. Pearson product-moment correlations were completed between running activity (during the last 2 days and final week of wheel access) and insulin-stimulated glucose uptake in wheel treatment groups, fat pad/body weight and insulin-stimulated glucose uptake in all groups, and muscle glycogen concentration and insulin-stimulated glucose uptake in all groups. A P value of < 0.05 was considered statistically significant.

RESULTS

During the experiment, one mouse died by an unknown cause (selected-unlocked group), and another mouse (selected-unlocked group) reduced its running for an unknown reason (possibly a wheel malfunction) to <5% during the final 3 wk of the experiment. Data from these two mice were not used in any of the statistical analyses.

Wheel running. During each of the 8 wk of wheel treatment, selected mice ran significantly more than nonselected mice (Table 1). Repeated-measures, nested two-way ANOVA indicated significant main effects of selection, week, and a genetic selection × week interaction. In selected lines, values for *weeks 1* through *5* were significantly greater than the final 3 wk. In nonselected lines, running activity in *week 7* was lower than *weeks 2* through *5*. The difference between the genetic groups was 48–58% in the first 5 wk and 32–41% during the final 3 wk. The difference was significant during every week. A similar reduction in wheel running in later weeks has been observed previously in males from these lines (24, 40) and may be related to inherent ontogenetic changes, seasonal effects, or fluctuations in temperature and humidity in the housing rooms (unpublished data).

Body and tissue weight. Selected mice weighed significantly less (~5–13%) than identically housed, nonselected mice (Table 2). There was not a significant main effect of wheel treatment on body weight. However, body composition was altered by wheel treatment as expected: retroperitoneal fat pad weight relative to body weight was lower (36–57%) for mice with some

Table 2. *Body weight and relative tissue weights*

	Nonselected Locked	Nonselected 48 h	Nonselected Unlocked	Selected Locked	Selected 48 h	Selected Unlocked	Genetic Selection Effect	Wheel Treatment Effect	Selection × Treatment Interaction
Body weight, g	36.0 ± 1.20	34.5 ± 0.97	34.1 ± 0.66	31.2 ± 0.85	32.4 ± 1.00	32.3 ± 0.58	$P = 0.05$	$P = 0.87$	$P = 0.16$
Relative tissue weight, mg/g body wt									
Fat pad	3.60 ± 0.43	1.56 ± 0.22	2.14 ± 0.22	1.80 ± 0.29	1.14 ± 0.24	1.15 ± 0.21	$P = 0.01$	$P = 0.01$	$P = 0.26$
Liver	0.052 ± 0.001	0.056 ± 0.001	0.055 ± 0.001	0.061 ± 0.002	0.064 ± 0.002	0.057 ± 0.001	$P = 0.01$	$P = 0.08$	$P = 0.12$
EDL	0.312 ± 0.011	0.347 ± 0.011	0.323 ± 0.009	0.264 ± 0.015	0.301 ± 0.009	0.326 ± 0.008	$P = 0.06$	$P = 0.06$	$P = 0.22$
Soleus	0.265 ± 0.012	0.269 ± 0.008	0.276 ± 0.011	0.318 ± 0.020	0.291 ± 0.011	0.301 ± 0.009	$P = 0.16$	$P = 0.73$	$P = 0.53$
Epitrochlearis	0.133 ± 0.006	0.153 ± 0.006*	0.130 ± 0.010	0.132 ± 0.011	0.148 ± 0.008	0.151 ± 0.008	$P = 0.53$	$P = 0.002$	$P = 0.02$

Values are means ± SE for 10–12 mice per group. Relative weight for each tissue was determined by dividing tissue weight by body weight for each animal. The mean value of both muscles (right and left) from each rat was used to calculate relative muscle weight. P values for main effects and interaction are provided. EDL, extensor digitorum longus muscle. *$P < 0.05$, nonselected-48-h vs. nonselected-unlocked and selected-locked groups.

Table 3. *Plasma glucose, plasma insulin, and hematocrit*

Measure	Nonselected Locked	Nonselected 48 h	Nonselected Unlocked	Selected Locked	Selected 48 h	Selected Unlocked	Genetic Selection Effect	Wheel Treatment Effect	Selection × Treatment Interaction
Plasma glucose, mM	7.54 ± 0.30	7.90 ± 0.16	7.93 ± 0.39	7.24 ± 0.44	7.91 ± 0.39	7.95 ± 0.48	$P = 0.87$	$P = 0.24$	$P = 0.87$
Plasma insulin, pM	127.5 ± 16.5	91.5 ± 10.5	123.0 ± 12.0	126.0 ± 27.0	102.0 ± 16.5	115.5 ± 22.5	$P = 0.98$	$P = 0.19$	$P = 0.87$
Hematocrit, %	46.9 ± 0.5	50.8 ± 0.5	50.6 ± 0.5	49.6 ± 0.7	51.8 ± 0.5	51.3 ± 0.5	$P = 0.14$	$P = 0.09$	$P = 0.76$

Values are means ± SE for 10–12 mice per group.

access to unlocked wheels (both unlocked and 48-h groups) compared with mice from locked groups of the same genetic background. Relative fat pad weight was also significantly lower (27–50%) in selected compared with nonselected mice with matched wheel treatment. Relative liver weight was slightly (~12%) but significantly greater in selected mice compared with nonselected controls. Relative liver weight was not significantly altered by wheel treatment.

Relative epitrochlearis weight differed significantly among wheel-treatment groups, and a significant interaction (genetic selection × wheel treatment) was found (Table 2). Post hoc analysis revealed that relative epitrochlearis weight for the nonselected-48-h group was significantly greater than nonselected-unlocked and selected-locked groups. No selection effect was apparent for the relative weights of the epitrochlearis. In the selected-unlocked group, four mice apparently had only one soleus muscle each, and this muscle was used for insulin-stimulated glucose uptake. Significant effects of genetic selection or wheel treatment were not detected for relative weights of EDL or soleus. Tissue weights were also analyzed using body weight as a covariate (data not shown), and this analysis did not change the significance of any main effects.

Glucose, insulin, and hematocrit. Hematocrit, plasma glucose, and insulin did not vary significantly with wheel treatment or genetic selection (Table 3).

Glycogen. Liver glycogen did not vary in relation to either wheel treatment or genetic selection (Table 4). Muscle glycogen was assessed in the absence (basal) and presence of insulin for both soleus and EDL. Wheel treatment had a significant effect on glycogen concentration in insulin-treated muscles, although the patterns of treatment effect were somewhat different for the soleus and EDL. A significant interaction occurred in the insulin-treated soleus: post hoc analysis revealed that the selected-48 h group was significantly greater than the selected-locked, and nonselected-48 h and nonselected-locked groups. Wheel treatment had no effect on glycogen in muscles without insulin. Genetic selection did not influence glycogen concentration with or without insulin.

Glucose uptake. Basal glucose uptake (no insulin) in isolated EDL muscles was not different between the selected and nonselected mice (Fig. 1). There was no significant wheel treatment effect on basal glucose uptake in the EDL. In the soleus (Fig. 2), there was a significant main effect of wheel treatment on basal glucose uptake: the locked-wheel groups appeared to have higher values than the other wheel-treatment groups. There was also a significant wheel-treatment effect on basal glucose uptake in the epitrochlearis muscle (Fig. 3): glucose uptake was highest in the continuous wheel treatment group for the nonselected mice and the locked group for the selected mice. A significant interaction (genetic selection × wheel treatment) was found for basal glucose uptake of the epitrochlearis, and post hoc analysis indicated that the selected-locked was higher than the nonselected-48 h and nonselected-locked epitrochlearis muscle.

In EDL treated with insulin, glucose uptake was significantly greater for selected compared with nonselected mice (Fig. 1). There was no significant effect of genetic selection in the soleus muscles (Fig. 2) or epitrochlearis muscles (Fig. 3) incubated with insulin. Delta glucose uptake (calculated as insulin-treated minus basal values for paired muscles) showed a significant main effect of genetic selection in the EDL but not in the soleus or epitrochlearis (data not shown).

Table 4. *Liver and muscle glycogen concentration*

Tissue	Nonselected Locked	Nonselected 48 h	Nonselected Unlocked	Selected Locked	Selected 48 h	Selected Unlocked	Genetic Selection Effect	Wheel Treatment Effect	Selection × Treatment Interaction
Liver, μmol/g	142.8 ± 29.4	142.0 ± 19.5	157.3 ± 24.9	143.5 ± 29.0	102.4 ± 18.4	144.0 ± 17.8	$P = 0.33$	$P = 0.63$	$P = 0.80$
Soleus, μmol/g									
Basal	1.90 ± 0.29	2.55 ± 0.30	2.65 ± 0.39	2.29 ± 0.34	3.23 ± 0.37	3.05 ± 0.58	$P = 0.38$	$P = 0.05$	$P = 0.88$
Insulin	2.25 ± 0.25	2.73 ± 0.29	3.19 ± 0.97	2.68 ± 0.57	4.46 ± 0.43*	2.97 ± 0.48	$P = 0.22$	$P = 0.007$	$P = 0.02$
EDL, μmol/g									
Basal	3.09 ± 0.30	2.88 ± 0.41	3.79 ± 0.36	5.96 ± 1.30	3.19 ± 0.48	2.98 ± 0.35	$P = 0.33$	$P = 0.42$	$P = 0.30$
Insulin	4.66 ± 0.53	3.04 ± 0.54	3.78 ± 0.53	6.21 ± 0.99	4.14 ± 0.39	3.68 ± 0.63	$P = 0.12$	$P = 0.003$	$P = 0.25$

Values are means ± SE for $n = 10–12$ mice per group. *$P < 0.002$ selected-48-h vs. selected-locked, nonselected-48-h, and nonselected-locked groups.

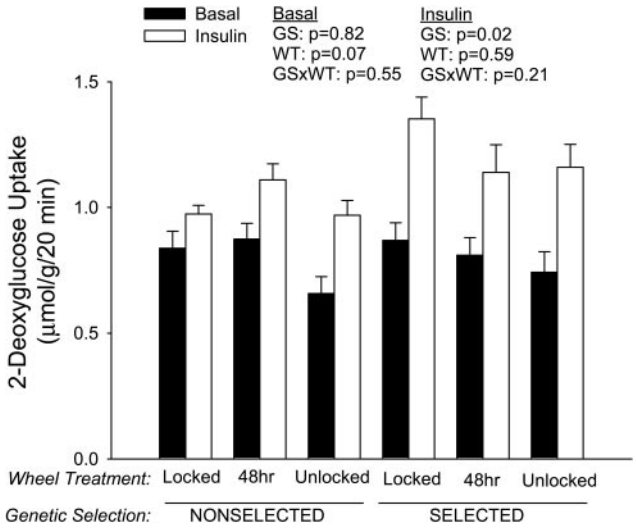

Fig. 1. Rate of 2-deoxyglucose uptake without insulin and with 100 μU/ml insulin in isolated extensor digitorum longus muscles of mice genetically selected for high voluntary wheel-running activity (selected) and of random-bred mice (nonselected). Treatments for 8 wk before measurement of 2-deoxyglucose uptake were as follows: access to locked (unable to turn) running wheel (locked), access to unlocked running wheel until wheel was locked 48 h before 2-deoxyglucose uptake measurement (48 h), and continuous access to unlocked running wheel (unlocked). Values are means ± SE for 9–12 mice per group. Data were analyzed using nested (by family lines) two-way ANOVA. GS, main effect of genetic selection; WT, main effect of wheel treatment; GS×WT, interaction between main effects.

Surprisingly, there was no evidence of a wheel treatment effect on insulin-stimulated glucose uptake in any of the muscles studied. Pearson product-moment correlations between the amount of wheel running during the final 2 days, or last week, and insulin-stimulated glucose uptake were not significant for any

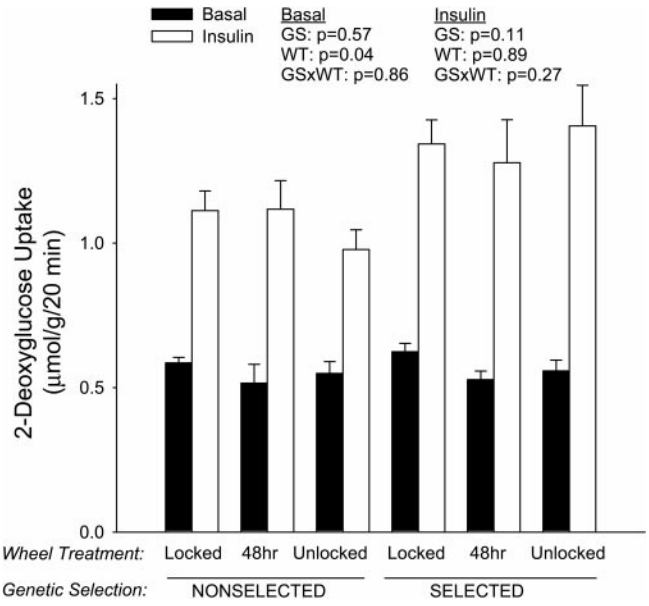

Fig. 2. Rate of 2-deoxyglucose uptake without insulin and with 100 μU/ml insulin in isolated soleus muscles. Values are means ± SE for 6–12 mice per group.

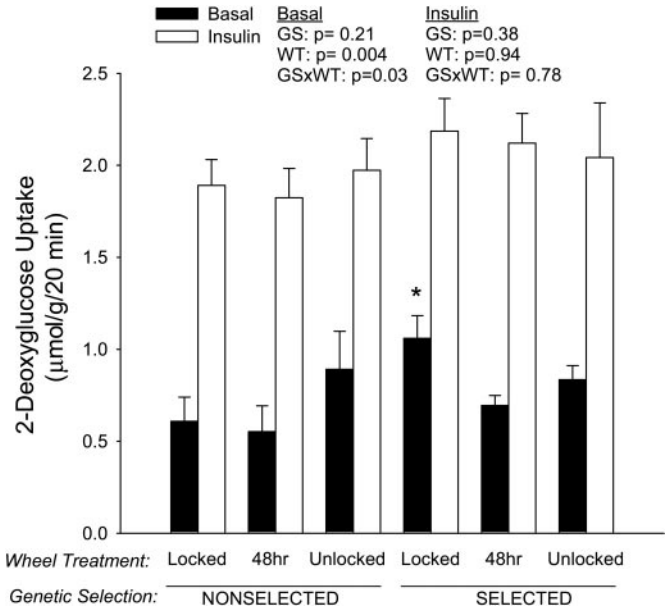

Fig. 3. Rate of 2-deoxyglucose uptake without insulin and with 100 μU/ml insulin in isolated epitrochlearis muscles. Values are means ± SE for 10–12 mice per group. *Post hoc analysis indicated that basal glucose uptake in the locked-selected group was different from locked-nonselected ($P < 0.002$) and 48 h-nonselected ($P < 0.0005$) groups.

of the muscles studied ($r < 0.20$ for each of the correlations; $P > 0.40$; data not shown).

The relationship between glucose uptake and fat pad/body weight was also assessed by Pearson product-moment correlations. The only statistically significant correlation found was for insulin-stimulated soleus (Fig. 4; $r = -0.333$; $P < 0.005$). No significant association was evident for EDL ($r = -0.082$; $P = 0.52$) or epitrochlearis ($r = -0.004$; $P = 0.98$).

Because previous research had indicated that muscle glycogen levels may influence the effect of exercise on insulin-stimulated glucose uptake (18), a Pearson

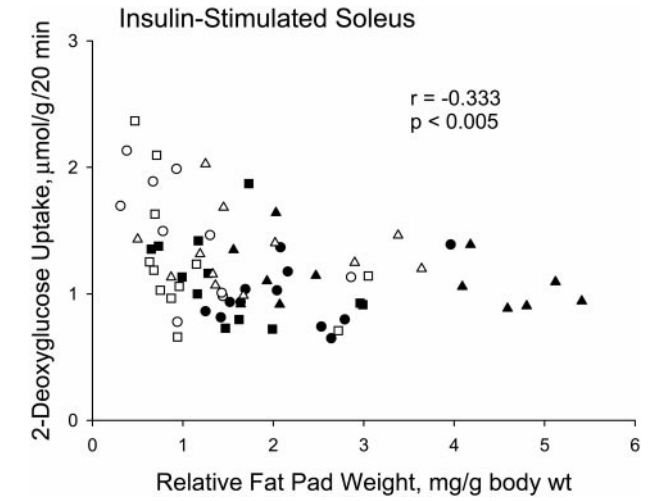

Fig. 4. Correlation of 2-deoxyglucose uptake in soleus and relative fat pad weight. ●, Unlocked-nonselected; ■, locked-nonselected; ▲, 48 h-nonselected; ○, unlocked-selected; □, locked-selected; △, 48 h-selected.

product-moment correlation was performed for glycogen concentration and insulin-stimulated glucose uptake. No significant association was found for soleus ($r = -0.057$; $P = 0.65$) or EDL ($r = -0.067$; $P = 0.59$).

DISCUSSION

Relatively few previous studies have used mice to evaluate exercise effects on muscle glucose uptake. Most of these have focused on effects immediately after a single bout of forced exercise (1, 2, 13, 19, 36, 45). The influence of forced exercise training on muscle glucose uptake in mice has also been described (43), but the effect of voluntary exercise on muscle glucose uptake in mice has not been previously reported. Mice have become a particularly useful research model because they can readily be used to generate transgenic lines, they have been extensively used for traditional breeding experiments to develop natural variants, and their genome will soon be mapped. Therefore, our major goal was to characterize the influence, in mice, of 8 wk of voluntary wheel running on glucose uptake by several isolated skeletal muscles.

By evaluating mice that had been selected for high wheel-running activity, our study included animals performing extremely high amounts of exercise, thus enhancing the likelihood for detecting effects of wheel running. Based on studies of voluntary exercise using rats and forced exercise using mice, we hypothesized that insulin-stimulated glucose uptake would be elevated in muscles from animals performing large amounts of wheel running. Perhaps the most valuable new information from this study was an unexpected result: despite a remarkable amount of wheel activity sufficient to reduce relative fat pad mass by ~40%, voluntary wheel running had no persistent effect on glucose uptake in any of the isolated skeletal muscles studied with insulin.

One possible explanation for the lack of a wheel treatment effect on insulin-stimulated glucose uptake would be if the mice performed little physical activity, but that was clearly not the case. The average number of revolutions per day performed by the nonselected (3,719 ± 233) and selected mice (8,243 ± 711) represents a daily distance of 4.2 and 9.2 km, respectively. The amount of voluntary wheel running by the mice in this study was consistent with data for mice from preceding generations of selected and nonselected mice (22, 24, 31, 39, 40, 42, 46). The groups with unlocked wheels (free to rotate) also had substantially lower relative fat pad weight compared with nonselected controls with access to locked wheels, indicating a classic training adaptation.

Although insulin-stimulated muscle glucose uptake was unaltered by wheel treatment, results from previous generations (10th and 14th), in which mice performed amounts of wheel activity similar to mice in this study, have demonstrated that wheel treatment leads to enhanced activity of various enzymes (succinate dehydrogenase, carnitine palmitoyl transferase, citrate synthase, pyruvate dehydrogenase, and hexokinase) in skeletal muscle (22, 46). The relative increases in activity of mitochondrial enzymes induced by wheel access in mice (i.e., ~20–40%) were roughly comparable to those reported for rats (~20–50%) undergoing voluntary wheel running (20, 33, 34). There does not appear to be some general inability of mouse muscle to adapt in response to wheel running.

To our knowledge, this is the first investigation that examined the influence of voluntary wheel-running activity on muscle glucose uptake in mice, but several studies using rats have indicated that, after 1–5 wk of voluntary wheel running, insulin-stimulated glucose uptake is increased in isolated epitrochlearis muscles compared with sedentary controls (20, 33). The timing between the last exercise bout and measurement of glucose uptake can influence the effect of exercise. The diurnal pattern of voluntary wheel running by mice is similar to that of the rat (38), and, in previous studies with enhanced glucose uptake in rats, animals were removed from running wheels ~8 h before measurement of glucose uptake (20, 33), which compares with ~4–8 h in our experiment. It is possible that insulin-dependent glucose uptake was enhanced in muscles less than 4 h after removal of mice from running wheels.

Henriksen and Halseth (18) reported enhanced insulin-stimulated glucose uptake in the soleus of rats after 1 wk but not after 2 or 4 wk of voluntary wheel running. They attributed reversal of the exercise effect at 2 and 4 wk to the coincident increase in soleus glycogen levels. Glycogen concentration in insulin-treated muscles was not significantly higher for mice in the unlocked- compared with locked-wheel groups. Furthermore, we found no significant correlation between glycogen concentration and insulin-stimulated glucose uptake in either muscle. The lack of an effect of wheel treatment on insulin-stimulated glucose uptake is not attributable to differences in glycogen concentration.

Goodyear et al. (11) found enhanced insulin-stimulated glucose uptake in plasma membrane vesicles from the forelimb muscles of voluntary wheel-running rats compared with sedentary controls. In another study, the same group found no influence of voluntary wheel running on glucose uptake (with or without insulin) in perfused rat hindlimb muscle (10). There are clearly some differences among individual muscles from rats in their response to wheel running, but the consistent finding of enhanced glucose uptake in insulin-stimulated, forelimb muscle from rats performing voluntary wheel running (20, 33) together with our data suggests that rats and mice may differ with regard to the postexercise effect of chronic, voluntary wheel running on insulin-stimulated glucose uptake in isolated muscle.

Our findings should be interpreted in the context of the experimental design. We studied male mice, as have previous researchers evaluating exercise and glucose uptake (1, 2, 12, 13, 19, 36, 43, 45). It would also be important to evaluate female mice, but it is notable that, at least in rats, both genders have robust, exer-

cise-induced increases in muscle glucose uptake (6, 23, 33, 35). In the only other published study with exercise training and muscle glucose uptake in mice, the age of animals was not provided (43); however, rats of ages across the life span respond to exercise with increased insulin-stimulated glucose uptake (7, 14, 20). The duration of training in this experiment (8 wk) is similar to that used in previous studies in mice (43) and rats (6, 23, 33, 35).

Although there was no influence of running-wheel treatment on glucose uptake in this study, it is possible that, with modifications, the voluntary wheel-running model can be used with mice to study the effects of exercise on muscle-glucose uptake. For example, rather than unlimited wheel access, which allows great variability among animals in timing of running activity, wheel access could be limited to only 2 h/day, and glucose uptake could be evaluated <4 h after wheel access. Peak running activity occurred during the first 3 wk of wheel access, so limiting the duration of the training period might optimize its effects. Also, preventing animals from eating after the final bout of exercise before measurement of glucose uptake might increase the likelihood of detecting an exercise effect. It is important to note that several studies with rats have found enhanced insulin sensitivity with voluntary wheel running even though they used experimental protocols (continuous wheel access with ad libitum food until the morning of glucose uptake measurement) quite similar to the procedure we used (7, 18, 20, 33).

The second key finding of this study was the genetic effect on insulin-stimulated glucose uptake by isolated skeletal muscle. Insulin sensitivity is strongly influenced by genetics (29, 30, 37). Compared with nonselected mice, selected mice had enhanced insulin-stimulated glucose uptake in the EDL. Results from previous generations of these lines have also revealed differences between genotypes (selected greater than nonselected) for maximal oxygen consumption and a trend for enhanced oxidative enzyme activities in skeletal muscle (22, 40). It seems reasonable to suspect that these differences between selected and nonselected mice are the consequence of greater amounts of spontaneous activity by the selected mice, independent of wheel access. However, based on systematic observations of *generation 13* mice housed with locked-wheel cages identical to the present study, genetic selection did not increase the frequency of locomotor or nonlocomotor activities (24). It is possible that subtle differences in recruitment of a particular muscle, especially one as infrequently recruited as the EDL (17), might escape detection based on observation of movement behaviors. Arguing against differences in physical activity accounting for the difference in EDL glucose uptake between selected and nonselected groups, there was no evidence that substantial amounts of wheel-running activity by nonselected or selected mice with unlocked wheels altered insulin-stimulated glucose uptake in any of the muscles studied. Taken together, these findings suggest that enhanced insulin action in selected mice may not be the direct consequence of greater physical activity.

The molecular mechanisms accounting for higher glucose uptake in the EDL of selected compared with nonselected lines is uncertain, but, in isolated muscles from rats, insulin-stimulated glucose uptake is closely associated with the amount of GLUT-4 glucose transporter in the cell surface membranes (16, 25). It would be reasonable to hypothesize that such a mechanism may underlie the genetic effect on insulin action in the EDL.

Consistent with previous reports of earlier generations (42), body mass was slightly (5–13%) but significantly less in selected vs. nonselected mice. Main effects on relative fat weight for genetic selection (nonselected > selected) and wheel treatment (locked-wheel groups > unlocked-wheel groups) were evident in the current study in a pattern similar to that reported previously for total body fat (41). The differences in relative fat weight among groups indicate that energy balance was altered both by genetic and treatment effects. In soleus, but not EDL or epitrochlearis, there was a modest, inverse correlation between insulin-stimulated glucose uptake and fat pad/body weight. The underlying reasons for the inverse association in the soleus, and for its absence in EDL and epitrochlearis, are unclear.

In conclusion, despite a high amount of documented physical activity sufficient to reduce relative fat pad mass by ~40%, there was no difference among wheel-treatment groups for glucose uptake and no correlation between wheel-running activity and glucose uptake by isolated skeletal muscle. These data do not support our main hypothesis: that voluntary wheel running would result in enhanced insulin-stimulated glucose uptake by isolated skeletal muscles. On a practical level, they indicate that voluntary running protocols similar to those previously used in rats may be ineffective for enhancing muscle glucose uptake in mice. A second novel finding was that, compared with nonselected controls, selected mice were characterized by enhanced insulin-stimulated glucose uptake in the EDL muscle. Consistent with this finding, research with earlier generations of these lines has indicated that, even without access to unlocked wheels, selected compared with nonselected lines exhibit characteristics that are reminiscent of classic endurance exercise training adaptations. The results indicate that these selected lines are potentially useful for identifying genes that affect the propensity for chronic voluntary exercise and its physiological consequences and the phenotype commonly found with endurance training.

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