Effect of Acute Exercise on Insulin Generation of Pyruvate Dehydrogenase Activator by Rat Liver and Adipocyte Plasma Membranes

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SUMMARY

Groups of young adult rats with body weights of 125–135 g (group A) or 300–400 g (group B) were subjected to one bout of prolonged exercise to exhaustion on a treadmill and were studied 2 h postexercise. Liver glycogen levels were markedly depleted in the exercised rats. Adipocytes from group A exercised rats showed a significantly greater increase in pyruvate dehydrogenase (PDH) activity in response to insulin than those from sedentary controls. Incubation with insulin of liver particulate fractions from exercised group A rats resulted in an increased production of a mitochondrial PDH activator compared with preparations from sedentary controls. The tissues of both exercised and sedentary group B rats were less responsive to insulin than those of the smaller rats. A significant effect of exercise on increased production of a PDH activator in response to insulin was found only in experiments in which adipocyte plasma membranes were coincubated with mitochondria and insulin. For group B rats exercise provided no significant enhancement of insulin activation of intact adipocyte PDH or stimulation of the production of a PDH activator by liver particulate preparations. Insulin binding to fat cells was not affected by exercise. Group A rats made insulin resistant by a high-fat diet did not respond to exercise by significantly increasing the insulin stimulation of PDH activator by liver membranes. The enhancing effect of a single bout of exercise on insulin response was not readily demonstrable in rats resistant to insulin either in association with age and weight or with a high-fat diet. In insulin-sensitive, young adult rats a single bout of exercise to exhaustion increases the effect of insulin on adipocyte PDH activity and on the generation of PDH-stimulating activity by liver and fat cell membranes. A postbinding site is probably responsible for the exercise effect. DIABETES 1986; 35:785–90.

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Our recent studies have shown that insulin resistance induced by fat feeding or dexamethasone administration results in decreased production of mitochondrial pyruvate dehydrogenase (PDH) activator by insulin-treated adipocyte and liver plasma membranes.[1–3] Our studies were designed to answer the question, Is the opposite situation, increased responsiveness to insulin, associated with increased insulin-stimulated production of PDH activator? One model for increased response to insulin is the exercised rat. Several studies have shown that both acute exercise and exercise training result in increased insulin sensitivity and increased responsiveness in both humans and rats.[4–9] Furthermore, an improvement in glucose tolerance in diabetic subjects in response to exercise has also been reported.[10–12]

Our study was designed to investigate *1*) whether acute prolonged exercise to exhaustion induces enhanced insulin-stimulated production of PDH activator and *2*) whether the well-known, fat-diet–induced insulin resistance could be reversed by acute exercise.

MATERIALS AND METHODS

ANIMALS AND TREATMENT

Male Sprague-Dawley rats (110–120 and 300–400 g, obtained from Taconic Farms, Germantown, NY) were adapted to treadmill running for 15 min every day for 3 days before initiation of the experiment. On the day of the experiment, they were randomly divided into a resting and an exercise group. The exercising rats were run to exhaustion (90 ± 6 min) on a motor-driven treadmill by alternating 10-min periods at speeds of 20 and 30 m/min for the first 90 min and then at a constant speed of 30 m/min thereafter; the rats were studied 2 h postexercise.

In experiments on fat-fed rats, young rats with an initial body weight of 70–80 g were fed a high-fat diet[13] for 7 days and subjected to acute exercise until exhaustion on the 7th day of fat feeding. The rats were adapted to treadmill running

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TABLE 1
Plasma glucose, insulin, and liver glycogen levels in 130- to 140-g rats 2 h after exercise

Group	Run time (min)	Plasma glucose (mg/dl)	Plasma insulin (μU/ml)	Liver glycogen (mg/g wet wt)
Sedentary	0	121 ± 12.3	28 ± 3.9	20.6 ± 2.03
Exercised	116 ± 24	130 ± 14.8*	18 ± 2.1*	7.2 ± 1.09†

Results are the means ± SE of 6 individual experiments performed in duplicate.
*P value for sedentary vs. exercised group is NS. †$P < .005$, sedentary vs. exercised group.

before the experiment as described above. On the day of the experiment, fat-fed rats were divided into a resting and an exercise group. The exercise group was run to exhaustion (116 ± 24 min) on a treadmill as described above and studied 2 h postexercise.

EXPERIMENTS ON FAT CELLS

Adipocytes from sedentary and exercised rats were prepared by the collagenase digestion of epididymal fat pads.[14] The cells were washed twice with Krebs-Ringer bicarbonate (KRB) buffer containing 4% bovine serum albumin (BSA) and resuspended 1:1 in the same buffer. Aliquots (1 ml) of fat cells were added to vials containing buffer alone (control) and increasing concentrations of insulin (25–1000 μU/ml) in a final volume of 2 ml. The vials were sealed, gassed with mixture of 95% O_2 and 5% CO_2 for 1 min, and incubated for 1 h at 37°C. The cells were transferred to plastic centrifuge tubes, diluted four times with KRB buffer containing 4% BSA, centrifuged for 5 s, and the infranatant was aspirated. The cells were resuspended 1:1 in homogenizing medium containing 10 mM potassium phosphate buffer, 1 mM EDTA, and 1 mM dithiothreitol, pH 7.2. The cells were homogenized and the homogenates were centrifuged for 30 s at 2500 rpm. The fat-free homogenates were assayed immediately for active PDH by the method of Taylor et al.[15] The assay mixture included final concentrations of 11 mM potassium phosphate (pH 7.4), 1.1 mM EDTA, 2.8 mM $MgCl_2$, 1.6% BSA, 1.2 mM dithiothreitol, 0.16 mM CoA, 1.6 mM NAD, .08 mM thiamine pyrophosphate, and 0.6 mM [1-^{14}C]pyruvate. After incubation at 37° for 2 min, the reaction was stopped by the addition of H_2SO_4 and the $^{14}CO_2$ collected on filter paper strips to which hyamine hydroxide was added.

EXPERIMENTS ON LIVER

Preparation of liver particulate fractions. Rats from the resting and exercised groups were anesthetized with ether, livers were removed, and a portion of liver was immediately frozen in liquid nitrogen, stored at −70°C, and used for the assay of liver glycogen by the method of Hassid and Abraham.[16] Another portion of liver (2–3 g) was rinsed in 10 mM potassium phosphate buffer containing 0.25 M sucrose, pH 7.4. Liver particulate fractions were prepared by the method of Saltiel et al.[17] Liver tissue was homogenized at low speed with a Teflon/glass homogenizer and the homogenate was centrifuged at 10,000 × g for 10 min at 4°C. The supernatant was centrifuged at 30,000 × g for 25 min. The pellet was resuspended in 10 mM potassium phosphate buffer, pH 7.4, at a final protein concentration of 15–20 mg/ml.

Liver mitochondria were prepared from sedentary rats by the method of Parsons et al.[18] A portion of liver tissue (2 g) was homogenized in 0.07 M sucrose, 0.21 M mannitol, 0.1 mM EDTA, and 10 mM potassium phosphate buffer, pH 7.2. The homogenate was centrifuged at 500 × g for 10 min. The supernatant was centrifuged at 9000 × g for 10 min. The pellet was resuspended in 5 ml of buffer and pelleted at 500 × g for 10 min. The pellet containing mitochondria was resuspended in 1 ml of 50 mM potassium phosphate buffer, pH 7.4, and was used on the same day. The mitochondria therefore were not intact.

Generation of insulin mediator. The mediator was generated by incubation of aliquots (2 ml) of liver particulate fractions in the absence and presence of various concentrations of insulin (25–300 μU/ml) for 15 min at 37°C. The membrane suspensions were then sedimented at 30,000 × g for 25 min. Aliquots of supernatants (equivalent to 2 mg original protein/ml liver particulate fraction) were tested on liver mitochondria of sedentary rats for their ability to activate PDH.

Pyruvate dehydrogenase activity was assayed as the release of 1-$^{14}CO_2$ from [1-^{14}C]pyruvate.[19] Mitochondria (2 mg protein/ml) and liver particulate fraction supernatants were added to tubes containing 50 mM potassium phosphate buffer, pH 7.4, 50 μM calcium chloride, 50 μM magnesium chloride, and 200 μM ATP (final volume 0.1 ml). The tubes were incubated for 15 min at 37°C. Pyruvate dehydrogenase assay was then initiated by the addition of 25 μl of the assay mixture (to achieve final concentrations of 50 mM potassium phosphate buffer, pH 8.0, 1 mM dithiothreitol, 0.1 mM CoA, 0.1 mM cocarboxylase, 0.25 mM [1-^{14}C]pyruvate, and 0.5 mM NAD) and was terminated after 10 min by the addition of 25 μl of 3 M H_2SO_4. Enzyme activity was expressed as nanomoles $^{14}CO_2$ produced per milligram of protein per 10 min.

EXPERIMENTS ON ADIPOCYTE CELL-FREE SYSTEM

The plasma membranes and mitochondria were prepared as described by Seals and Czech[20] from adipocytes of exer-

TABLE 2
Effect of acute exercise on insulin stimulation of adipocyte pyruvate dehydrogenase (PDH) in rats with body weight of 300–400 g

Insulin (μU/ml)	% PDH stimulation by insulin*	
	Sedentary	Exercised†
25	19 ± 5.3	42 ± 8.8‡
50	43 ± 9.6‡	73 ± 10.1‡
75	57 ± 10.8‡	81 ± 7.9‡
100	65 ± 23.4‡	92 ± 20.5‡

Adipocytes from 300- to 400-g exercised and sedentary rats were incubated with and without insulin (25–100 μU/ml) for 1 h at 37°C. The cells were washed, homogenized, and assayed immediately for active PDH. Results are the means ± SE of 5 individual experiments performed in triplicate. Basal values were 3.6 ± 0.73 nmol [^{14}C]-pyruvate oxidized · ml fat cell^{-1} · min^{-1} for the sedentary group, 3.3 ± 0.57 for the exercised rats.
*Overall insulin effect, $P < .001$; †exercise effect, $P < .06$; ‡significant insulin response, $P < .05$, Duncan's new multiple-range test.

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TABLE 3
Effect of acute exercise on insulin stimulation of adipocyte pyruvate dehydrogenase (PDH) in 130- to 140-g adult rats

Insulin (μU/ml)	% PDH stimulation by insulin*	
	Sedentary	Exercised†
25	23 ± 5.1	57 ± 8.9‡
50	46 ± 9.2‡	92 ± 15.1‡
75	58 ± 16.3‡	101 ± 21.2‡
100	88 ± 20.9‡	120 ± 16.2‡

Adipocytes from 130- to 140-g exercised and sedentary rats were incubated with and without insulin (25–100 μU/ml) for 1 h at 37°C. The cells were washed, homogenized, and assayed immediately for active PDH. Results are the means ± SE of 6 individual experiments performed in triplicate.
*Overall insulin effect, $P < .001$; †exercise effect, $P < .025$; ‡significant insulin response, $P < .05$, Duncan's new multiple-range test.

cised and sedentary rats. The two subcellular components were separated on a 32% sucrose gradient as described earlier.[1]

Pyruvate dehydrogenase assay. Pyruvate dehydrogenase was assayed by the method of Seals and Jarett.[19] Mitochondria from sedentary rats (250 μg protein/ml) were added to tubes containing plasma membranes (200 μg protein/ml), 50 mM potassium phosphate buffer, pH 7.4, with 50 μM $CaCl_2$, 50 μM $MgCl_2$, and 250 μM ATP in the absence and presence of increasing concentrations of insulin (25–100 μU/ml). Control tubes received 0.0005% BSA instead of insulin. The tubes were incubated for 5 min at 37°C, and the PDH assay was initiated by the addition of 25 μl assay buffer as described for the liver preparation and terminated after 5 min by the addition of 250 μl 3 M H_2SO_4. Enzyme activity was expressed as nanomoles $^{14}CO_2$ produced per milligram of protein per minute.

^{125}I-LABELED INSULIN BINDING

Radioiodination of insulin was carried out by the chloramine-T method and the radioactive insulin was purified by column chromatography on a cellulose column.[21] Specific activity of ^{125}I-labeled insulin was 120 mCi/mg. For measurement of insulin binding, adipocytes (~2–4 × 10^6/tube) were incubated at 24°C for 90 min in 1 ml Tris–5% albumin buffer containing bacitracin[22] and 1 ng ^{125}I-insulin in plastic culture tubes. Nonspecific binding was determined in the presence of high concentrations of unlabeled insulin (100 μg/ml) and was <10% of total binding. Specific binding was calculated by subtracting nonspecific binding from total binding. The incubation was stopped by the oil centrifugation method of Gammeltoft and Gliemann.[23] Aliquots (250 μl) of cell suspension were layered over 100 μl of silicone oil in 400-μl microfuge tubes, and the cells were separated from buffer by centrifugation at 10,000 × g for 30 s. The top portions of the tubes containing fat cells were cut off, and cell-associated radioactivity was determined. The degradation of insulin during each incubation was evaluated by measuring the trichloroacetic acid (TCA) precipitability of the label in the incubation medium. Protein contents were determined by the method of Lowry et al.[24]

Statistics. Two-factorial repeated-measures analysis of variance was used to evaluate the effect of insulin and exercise on the production of PDH activator. The unpaired t test was used to compare sedentary and exercised rats in Table 1. Statistically significant differences were accepted at $P < .05$. Values of $P > .05$ are indicated by NS.

Porcine insulin was a kind gift from Dr. Mary Root (Lilly, Indianapolis, IN). [1-^{14}C]pyruvate, [^{125}I]iodide, and hydroxide of hyamine were purchased from Amersham (Arlington Heights, IL). Omnifluor was from New England Nuclear (Boston, MA). Tris, sodium pyruvate, and the other cofactors for the assay of PDH were obtained from Sigma (St. Louis, MO). Collagenase was from Worthington Biochemical (Freehold, NJ). Bovine serum albumin was a product of Reheis (Phoenix, AZ).

RESULTS

The average run time to exhaustion in various experiments in older rats weighing 370–400 g was 80–90 min. Younger adult rats (body wt 115–130 g) ran a longer time than the older rats (110–125 min). Plasma insulin and glucose levels of the 130- to 140-g rats 2 h after exercise were not significantly different from those of sedentary controls. However,

TABLE 4
Effect of acute exercise on insulin generation of pyruvate dehydrogenase (PDH) activator by rat adipocyte plasma membranes

Insulin* (μU/ml)	PDH activity (nmol [^{14}C]pyruvate oxidized · mg protein^{-1} · min^{-1})					
	Sedentary			Exercised†		
	Basal activity	Plus plasma membranes	% Insulin effect	Basal activity	Plus plasma membranes	% Insulin effect +
0	1.6 ± 0.21	1.6 ± 0.25		1.8 ± 0.27	1.8 ± 0.29	
25		1.8 ± 0.31	13 ± 2.8		2.6 ± 0.50‡	44 ± 7.8
50		1.8 ± 0.31	16 ± 3.6		2.4 ± 0.37‡	37 ± 3.8
75		2.0 ± 0.34‡	22 ± 3.6		2.4 ± 0.42‡	36 ± 4.9
100		2.0 ± 0.35‡	25 ± 3.7		2.7 ± 0.44‡	50 ± 3.4

Plasma membranes and mitochondria were prepared from adipocytes from sedentary and exercised rats (body wt 380–400 g). Adipocyte mitochondria from sedentary rats (250 μg protein/ml) were preincubated with plasma membranes from sedentary controls or exercised rats (200 μg protein/ml) with and without insulin (25–100 μU/ml) for 4 min at 37°C in the presence of ATP (250 μM) in a final volume of 0.1 ml. PDH was assayed as described in the text. Results are the means ± SE of 5 individual experiments performed in triplicate.
*Overall insulin effect, $P < .001$; †exercise effect on % change, $P < .001$; ‡significant insulin response, $P < .05$, Duncan's new multiple-range test.

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TABLE 5
Effect of acute exercise on insulin generation of pyruvate dehydrogenase (PDH) activator by rat liver particulate fractions

	PDH activity (nmol [1-^{14}C]pyruvate oxidized · mg protein^{-1} · 10 min^{-1})			
	Sedentary		Exercised†	
Insulin* (μU/ml)	Control mitochondria + LPF	% Insulin effect	Control mitochondria + LPF	% Insulin effect +
0	2.9 ± 0.39		3.1 ± 0.36	
25	3.1 ± 0.39	6 ± 1.7	3.5 ± 0.39‡	13 ± 1.6
50	3.3 ± 0.33‡	15 ± 5.8	3.9 ± 0.49‡	24 ± 5.8
75	3.3 ± 0.39‡	14 ± 3.8	3.7 ± 0.47‡	17 ± 2.9
100	3.2 ± 0.39	11 ± 3.1	3.7 ± 0.49‡	17 ± 5.0

Liver particulate fractions (LPF) from sedentary controls and exercised rats (body wt 375–400 g) were incubated with and without insulin (25–200 μU/ml) for 15 min at 37°C. The membrane suspensions were centrifuged at 30,000 × *g* for 25 min. Aliquots of supernatants (equivalent to 2 mg original protein/ml) were tested for their ability to stimulate PDH from liver mitochrondria from sedentary control rats. Results are the means ± SE of 7.
*Overall insulin effect, $P < .001$; †exercise effect, $P < .2$; ‡significant insulin response, $P < .05$, Duncan's new multiple-range test.

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liver glycogen levels were markedly diminished in exercised rats (Table 1). The larger rats also showed a marked reduction in liver glycogen levels after prolonged exercise and 2-h recovery to 31% of the levels in sedentary controls.

Table 2 indicates the results of adipocyte experiments performed on sedentary and exercised rats weighing 370–400 g. Basal adipocyte PDH activities were not different between the exercised (3.3 ± 0.57 nmol [^{14}C]pyruvate · ml fat cell^{-1} · min^{-1}) and sedentary (3.7 ± 0.73) groups, whereas the response to various low concentrations of insulin was significantly increased in both groups ($P < .001$). Although the percent increases for the exercised-rat fat cells incubated with insulin were consistently higher than those for the sedentary group, the difference did not quite reach statistical significance ($P < .06$). However, as shown in Table 3, when a similar experiment was carried out with the more insulin-responsive, smaller rats, there was a highly significant ($P < .025$) exercise effect. The response to insulin, again, was clearly significant ($P < .001$).

Adipocyte cell-free preparations from the 300- to 400-g exercised rats showed a significant enhancement in insulin's effect (percent) over sedentary controls when plasma membranes of exercised rats were coincubated with mitochondria prepared from adipocytes from sedentary rats (Table 4). Maximal PDH stimulation was observed with 100 μU/ml insulin in the coincubation mixture in both sedentary and exercised groups. Insulin binding to fat cells was not changed by exercise (8.7 ± 2.5 ^{125}insulin bound/10^5 cells for the sedentary group, 8.8 ± 1.4 for the exercised group).

Table 5 indicates the effect of acute exercise in 375- to 400-g rats on the production of PDH activator by liver particulate fractions exposed to various concentrations of insulin. Preparations from the sedentary rats demonstrated 6–15% stimulation of liver mitochondrial PDH, whereas preparations from older exercised rats showed 13–24% stimulation of mitochondrial PDH, which was not a significant difference. The insulin effect, although small, was statistically significant ($P < .001$).

However, as shown in Table 6, when the experiment was repeated with smaller rats (115–130 g) fed chow, the insulin effect was greater than in the larger rats, and the increased hormone response in the exercised rats compared to the sedentary group was clearly significant ($P < .025$).

It has previously been observed that a lard diet decreases the insulin-induced formation of a PDH stimulator by liver particulate fractions.[2] To test the possibility that exercise might overcome this diet effect, the effect of exercise on this action of insulin was studied on a similar group of 115–130

TABLE 6
Influence of high-fat diet on the insulin generation of pyruvate dehydrogenase (PDH) activator by liver particulate fractions of rats (115–130 g) after acute exercise

	% Liver mitochondrial PDH stimulation			
	Chow diet		Lard diet	
Insulin* (μU/ml)	Sedentary	Exercised†	Sedentary	Exercised†
25	15 ± 3.2‡	19 ± 3.1‡	3 ± 2.0	6 ± 3.2
50	18 ± 3.1‡	26 ± 2.9‡	7 ± 1.7	13 ± 2.8
75	21 ± 4.8‡	42 ± 3.2‡	8 ± 1.9	12 ± 3.1
100	31 ± 2.0‡	31 ± 5.9‡	5 ± 2.3	12 ± 3.0

Liver particulate fractions were incubated with and without insulin (25–200 μU/ml) for 15 min at 37°C as described in Table 2. Membrane supernatants were used to test their ability to activate PDH from liver mitochondria from sedentary chow-fed rats. Results are the means ± SE of 5 individual experiments performed in triplicate. Basal values were 1.71 ± 0.47 nmol [1-^{14}C]pyruvate oxidized · mg protein^{-1} · 10 min^{-1} for liver mitochondria from chow-fed rats and 1.96 ± .132 for mitochondria from lard-fed rats.
*Overall insulin effect, $P < .001$ for chow group, $P > .05$ for lard group; †exercise effect, $P < .025$ for chow group, $P > .05$ for lard group; ‡significant insulin response, $P < .05$, Duncan's new multiple-range test.

rats that had been fed the lard diet for 7 days. The results given in Table 6 confirm the effect of the diet in reducing insulin responsiveness because the small insulin effect was not significant. Although there was a trend toward a somewhat greater stimulation of PDH by the insulin-exposed liver fractions from the exercised group compared to the sedentary lard-fed rats, the difference was not significant. More importantly, exercise did not return the lard-fed animals to the response found in chow-fed rats.

DISCUSSION

Our experiments demonstrate that young adult rats (130–140 g) that have been subjected to a single bout of exhaustive exercise followed by a 2-h recovery period develop an increased ability to respond to insulin by an enhanced in vitro activation of adipocyte PDH (Table 3). In addition, the insulin-induced production of mitochondrial PDH activator by liver particulate fractions (crude plasma membranes) was also greater in preparations from the exercised rats compared to those of sedentary controls (Table 6). This exercise effect seems to depend on adequate insulin responsiveness. For example, somewhat older rats (300–400 g) were less responsive to insulin, and a similar exercise-recovery regime did not result in a significant increase in the insulin effect on adipocytes or liver particulate fractions. However, when purified plasma membranes from the adipocytes of the larger exercised rats were incubated with insulin and mitochondria, the activation of PDH was enhanced in the exercised group.

When insulin resistance was produced in the smaller rats by feeding them a high-fat diet, it was again not possible to demonstrate an effect of exercise on the insulin-stimulated liver membrane production of PDH activator. Thus, the effect seems to become statistically significant only in groups of rats already relatively insulin sensitive.

Although the role of the PDH stimulator in the coordinate response of tissue to insulin is not yet clear, the observation that its production varies directly with the responsiveness of the tissue to other effects of the hormone suggests that it participates in the total response. Our animals were taken at a time postexercise when insulin is near normal and glycogen is resynthesized in the liver and muscle.[25] Recent studies by a number of investigators have indicated that acute exercise as well as exercise training results in elevations of glycogen synthase levels in muscle.[12,26,27] The activity of this enzyme is regulated in part by the putative mediator(s) of insulin action that also activates PDH.[28,29] It can be speculated that the observed increase in the activation of PDH by insulin-exposed membranes is related to the activation of liver and muscle glycogen synthase reported by others.[26,27] We believe that the observed elevations in the production of PDH activator after acute exercise are related to postbinding effects because insulin binding was not affected. Also, note that fat-diet-induced insulin resistance (which is accompanied by both binding and postbinding defects) cannot be reversed by a single bout of acute exercise (to that found in animals on a chow diet).

Studies by Craig et al.[30] showed that the rate of glucose oxidation at the maximally effective insulin concentration was sixfold greater in fat cells from young female rats (body wt 164 ± 7 g) compared with larger (328 ± 5 g), somewhat older female rats. However, exercise training of larger rats greatly increased the rate of glucose oxidation in response to insulin, returning it to values similar to those found in the smaller sedentary rats. The fat cells of the trained rats and the young rats were similar in size and were much smaller than those of sedentary controls. These investigators suggested that in the age range of the animals in their study, the decrease in the sensitivity and responsiveness of glucose oxidation to insulin that occurs as rats get older is primarily a fat-cell size phenomenon. In our studies, also, the fat cells from the 370- to 400-g rats were larger than those from the 110- to 125-g group. However, in both cases there was no difference in mean cell diameter between sedentary and exercised rats, so cell size was not involved in the acute-exercise-induced increase in insulin response (mean cell diameter, sedentary vs. exercised, 76 ± 1.1 vs. 77 ± 1.0 μm for the larger rats, and 54 ± 1.5 vs. 54 ± 1.1 μm for the younger rats).

In summary, we have shown that the enhanced insulin responsiveness of acute exercise is accompanied by changes in plasma membrane capacity to generate PDH activator without a change in insulin-binding capacity, a pattern that is consistent with a postbinding effect.

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