

# Short-term exercise enhances insulin-stimulated GLUT-4 translocation and glucose transport in adipose cells

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**Ferrara, Cynthia M., Thomas H. Reynolds, Mary Jane Zarnowski, Joseph T. Brozinick, Jr., and Samuel W. Cushman.** Short-term exercise enhances insulin-stimulated GLUT-4 translocation and glucose transport in adipose cells. *J. Appl. Physiol.* 85(6): 2106–2111, 1998.—This investigation examined the effects of short-term exercise training on insulin-stimulated GLUT-4 glucose transporter translocation and glucose transport activity in rat adipose cells. Male Wistar rats were randomly assigned to a sedentary (Sed) or swim training group (Sw, 4 days; final 3 days:  $2 \times 3$  h/day). Adipose cell size decreased significantly but minimally (~20%), whereas total GLUT-4 increased by 30% in Sw vs. Sed rats. Basal 3-*O*-methyl-D-[<sup>14</sup>C]glucose transport was reduced by 62%, whereas maximally insulin-stimulated (MIS) glucose transport was increased by 36% in Sw vs. Sed rats. MIS cell surface GLUT-4 photolabeling was 44% higher in the Sw vs. Sed animals, similar to the increases observed in MIS glucose transport activity and total GLUT-4. These results suggest that increases in total GLUT-4 and GLUT-4 translocation to the cell surface contribute to the increase in MIS glucose transport with short-term exercise training. In addition, the results suggest that the exercise training-induced adaptations in glucose transport occur more rapidly than previously thought and with minimal changes in adipose cell size.

adipose cells; exercise; glucose metabolism

IT IS WELL DOCUMENTED that long-term exercise training profoundly affects the metabolic characteristics of rat skeletal muscle and adipose tissue (7, 8, 21, 24, 25). The latter effects are most clearly reflected by marked reductions in adipose cell size (7, 21). Metabolic adaptations in adipose cells, reported to be associated with long-term exercise training, include an increase in the intracellular pool of GLUT-4, a resulting increase in the amount of GLUT-4 translocating to the cell surface in response to insulin stimulation, and a corresponding increase in insulin-stimulated glucose transport (7).

These previous investigations involved up to 12 wk of running or swim training because it was believed that the adaptations in the regulation of glucose transport only occurred over an extended period of time. However, the marked decrease in adipose cell size so characteristic of long-term training confounds the interpretation of the metabolic adaptations with regard to identifying the responsible variables. In one previous investigation, various groups of control animals were

included in an attempt to separate the effects of changes in adipose cell size from those of the exercise training itself. In addition to the exercising group, an age-matched group, with larger adipose cells and body weight than the exercising group, and a group matched with the exercising group for adipose cell size (younger animals) were included in the investigation (7). Other investigators have included animals of similar age to the exercise-trained animals but these were given a food-restricted diet to match body weights to the exercising animals (24). It is well known that age, food intake, and adipose cell size itself individually have significant effects on adipose cell metabolism, thus confounding the interpretation of the results of these previous investigations (9, 12).

Recent investigations in skeletal muscle suggest that 4 days of vigorous swim training are sufficient to induce up to a 100% increase in skeletal muscle GLUT-4 content and insulin-stimulated glucose transport (17, 18). These investigations suggest that the adaptations in glucose transport in skeletal muscle as a result of exercise training occur within a very short period of time. The effects of a similar exercise program on adipose cell metabolism have not been investigated. A potential benefit of the short-term exercise protocol is that the changes in adipose cell size, if any, might be small enough so as to permit a direct evaluation of the effects of exercise training on adipose cell metabolism.

The present investigation was thus undertaken to determine whether 4 days of swim training are sufficient to induce significant adaptations in glucose transport in adipose cells, similar to those observed in skeletal muscle, and to evaluate the relationship between these adaptations and adipose cell size. In characterizing the effects of exercise training on glucose transport, we used the relatively new 2-*N*-4-*N*[1-azido-2,2,2-trifluoroethyl]benzoyl-1,3-bis-[D-mannos-4-yloxy]-2-propylamine (ATB-BMPA) photoaffinity labeling technique to determine how exercise training affects the relationship between insulin-stimulated glucose transport and cell surface GLUT-4 (10, 20). We also examined the effect of short-term exercise training on syntaxin 4, a target membrane-soluble *N*-ethylmaleimide-sensitive fusion protein receptor, recently proposed to play a role in targeting intracellular GLUT-4-containing vesicles to the plasma membrane (22). We anticipated that changes in GLUT-4 levels and translocation might be paralleled by changes in one or more of the proteins thought to be involved in vesicle docking and fusion, such as syntaxin 4. The answers to these questions will provide new and important information

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on the regulation of insulin-stimulated glucose transport in adipose cells.

## METHODS

**Materials.** Fraction V BSA was from Intergen (Purchase, NY); type I crude collagenase was from Worthington Biochemical (Freehold, NJ). Rabbit polyclonal anti-GLUT-4 and anti-GLUT-1 antisera, prepared against 20-amino acid peptides corresponding to the COOH-terminal sequences, were kindly supplied by Hoffmann LaRoche (Nutley, NJ). A rabbit polyclonal antiserum for syntaxin 4, directed against the unique N-terminal region of syntaxin 4, was kindly supplied by W. Trimble (Hospital for Sick Children, Toronto, Canada).  $^{125}\text{I}$ -labeled protein A, 3-*O*-methyl-D- $^{14}\text{C}$ ]glucose, and L- $^3\text{H}$ ]glucose were from Dupont-New England Nuclear (Boston, MA). ATB- $^{125}\text{I}$ ]BMPA (10 Ci/mmol specific activity) was kindly supplied by G. D. Holman (University of Bath, Bath, UK).

**Animal care and exercise program.** Specific-pathogen-free male Wistar rats weighing 45–60 g were obtained from Charles River Laboratories (Boston, MA). On arrival, rats were housed four per cage in a temperature-controlled animal room maintained on a 12:12-h light-dark cycle. The rats were fed ad libitum National Institutes of Health standard chow and water. Rats were randomly assigned to a swim training (Sw) or sedentary control (Sed) group. The rats assigned to the Sw group were acclimated to swimming for 10 min/day for 2 days, and then were exercise trained by using a previously published protocol (17, 18). Briefly, the animals performed two bouts of exercise in water maintained at 35°C, separated by a 1-h rest period. On the first day of swimming, rats swam for 1 h, followed by a 2-h bout. During the rest period, animals were given food and water. The duration of swimming was increased to two 3-h bouts of exercise on the second day of swimming and continued at that level for another 2 days.

**Preparation of adipose cells.** Sed and Sw rats were killed in the postprandial state 18–22 h after the last bout of swimming. The rats were anesthetized with 5 mg/100 g body wt of pentobarbital sodium. The epididymal fat pads were removed and weighed, and isolated adipose cells were prepared by the collagenase digestion technique described by Rodbell (19), as modified by Weber et al. (26). All buffers contained 200 nM adenosine and either 1 or 5% BSA. The isolated cells were incubated in the presence of 5% BSA.

**Measurement of 3-*O*-methyl-D- $^{14}\text{C}$ ]glucose transport.** The rates of 3-*O*-methyl-D- $^{14}\text{C}$ ]glucose transport were measured at a substrate concentration of 100  $\mu\text{M}$ , by using a double-isotope (3-*O*-methyl-D- $^{14}\text{C}$ ]glucose, and L- $^3\text{H}$ ]glucose) technique, as described by Karnieli et al. (13).

**Measurement of adipose cell size.** Adipose cell size, expressed as lipid weight/cell, was determined by the osmic acid fixation Coulter electronic counter method described by Hirsch and Gallian (6), as modified for isolated cell suspensions by Cushman and Salans (2).

**Preparation of total membranes.** After incubation, the adipose cells were washed with a buffer containing 20 mM Tris-HCl, 1 mM EDTA, and 255 mM sucrose (TES), pH 7.4, at 18°C. Total cell membranes were prepared as described by Vannucci et al. (23). Briefly, cells were homogenized in TES and centrifuged at 500 *g* for 10 min to remove large particulate matter. The supernatants were further centrifuged at 400,000 *g* for 60 min to pellet the cell membranes. The pellets were resuspended in a small volume of TES buffer. Protein concentrations were determined by the bicinchoninic acid assay (Pierce, Rockville, IL) by using crystalline BSA as the standard.

**Quantitation of GLUT-4, GLUT-1, and syntaxin 4.** GLUT-4, GLUT-1, and syntaxin 4 levels were assessed in total cell

membranes by SDS-PAGE and Western blotting. Samples from both groups were run on the same gel. Western blotting was performed by using anti-GLUT-4 and anti-GLUT-1 COOH-terminal peptide antisera and an anti-syntaxin 4 antiserum directed against the N-terminal region, and  $^{125}\text{I}$ -labeled protein A (16). The results were quantitated by using a Molecular Dynamics Phosphorimager (Sunnyvale, CA) and normalized to the amounts of protein loaded per well.

**Cell surface GLUT-4 and GLUT-1 photolabeling.** Diluted adipose cells were incubated without (basal) or with 670 nM of insulin for 20 min. After incubation, 1 ml of cells was photolabeled with 125  $\mu\text{Ci}$  of ATB- $^{125}\text{I}$ ]BMPA by using a Rayonet photochemical reactor (Southern New England Ultraviolet, Branford, CT) with 300-nm lamps ( $3 \times 1$  min). Photolabeled cells were then washed with TES buffer (18°C), and GLUT-4 was immunoprecipitated via the following procedure. The cells were solubilized for 20–30 min at room temperature in 2% Thesit (Boehringer-Mannheim, Indianapolis, IN) in PBS (pH 7.4). The cell lysate was centrifuged at 150,000 *g* for 10 min. The supernatant of each sample was then incubated overnight at 4°C with an immunocomplex consisting of anti-GLUT-4 antiserum and protein-A-sepharose (Pharmacia Biotech). The efficiency of immunoprecipitation of GLUT-4 is ~95%. The immunocomplexes were briefly centrifuged at high speed, and the supernatants were carefully removed and saved for the immunoprecipitation of GLUT-1. The complexes were washed and solubilized in electrophoresis sample buffer, and the immunoprecipitated GLUT-4 was subjected to SDS-PAGE, followed by gel slicing and counting, as previously described (20). Radioactivity was expressed as counts per minute per  $10^6$  cells.

GLUT-1 was immunoprecipitated twice in sequence from the supernatants of the GLUT-4-immune complexes, and photolabeled GLUT-1 was analyzed as described for GLUT-4. The efficiency of immunoprecipitation of GLUT-1 is ~75%.

**Statistical analysis.** Data were analyzed by using two-way analysis of variance and Student's *t*-test, when appropriate. Differences were accepted as significant at the level of  $P \leq 0.05$ . Results are expressed as means  $\pm$  SE.

## RESULTS

**Characteristics of experimental animals and adipose cells.** Rats in the Sw group weigh significantly less and have significantly smaller epididymal fat pads than those in the Sed group (Table 1). Adipose cell size is also reduced significantly by 20% in the Sw compared with the Sed group. Adipose cell water space, estimated as the 3-*O*-methyl-D- $^{14}\text{C}$ ]glucose equilibrium space, is significantly greater in the Sw compared with the Sed group, despite a smaller cell size (Table 1). Total cell membrane protein recovery in the Sw group is not changed compared with the Sed group. Four days of swimming result in a significant 30% increase in GLUT-4 content per milligram of recovered total cell membrane protein in the Sw compared with the Sed group, whereas GLUT-1 and syntaxin 4 levels are unchanged (Table 1).

**3-*O*-methyl-D- $^{14}\text{C}$ ]glucose transport and cell surface GLUT-4 and GLUT-1.** Under basal conditions, adipose cells from the Sw group exhibit a significant decrease in glucose transport activity, compared with cells from the Sed group (Fig. 1), but no significant changes in the cell surface photolabeling of either GLUT-4 (Fig. 2) or GLUT-1 (Fig. 3). In contrast, under conditions of maxi-

Table 1. *Body and fat pad weights; adipose cell sizes and water spaces; total membrane protein recoveries; and GLUT-4, GLUT-1, and syntaxin 4 levels in sedentary and swim-trained rats*

	Sedentary	Swim-Trained
Body weight, g	120 ± 1 (59)	104 ± 1* (60)
Fat pad weight, g	0.45 ± 0.02 (20)	0.35 ± 0.01* (24)
Cell size, µg lipid/cell	0.060 ± 0.002 (5)	0.050 ± 0.003* (4)
Protein, mg	0.41 ± 0.03 (5)	0.35 ± 0.06 (5)
Cell water space, pl/cell	1.69 ± 0.09 (5)	1.97 ± 0.07* (5)
GLUT-4, absorbance/mg protein	60 ± 5 (4)	80 ± 7* (4)
GLUT-1, absorbance/mg protein	10 ± 2 (6)	10 ± 1 (6)
Syntaxin 4, absorbance/mg protein	5 ± 1 (6)	3 ± 1 (6)

Values are means ± SE; nos. in parentheses are no. of samples/group. GLUT-4, GLUT-1, and syntaxin 4 values are expressed relative to a protein standard that is processed on all gels. \*Significant difference between sedentary and swim-trained rats,  $P \leq 0.05$ .

mal insulin stimulation, cells from the Sw group exhibit a 36% higher glucose transport activity (Fig. 1) and a 44% increase in cell surface GLUT-4 photolabeling (Fig. 2), compared with cells from the Sed group. Insulin-stimulated cell surface GLUT-1 photolabeling is increased compared with basal levels in both experimental groups, but no differences between the values are observed for the two groups. When the stimulatory effects of insulin are expressed as multiples of responses relative to the basal activities, swim training enhances the glucose transport activity markedly because of both decreased basal and increased insulin-stimulated glucose transport activity. Swim training

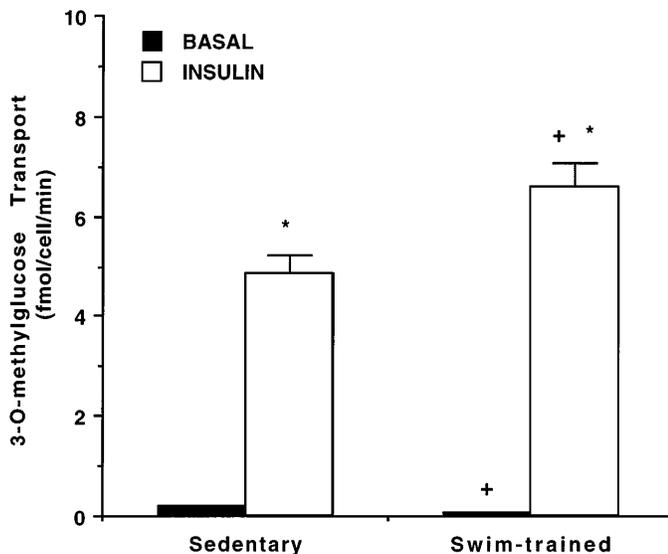


Fig. 1. 3-O-methyl-D-[ $^{14}\text{C}$ ]glucose transport in isolated adipose cells from sedentary and swim-trained rats. Values are means ± SE obtained from triplicate samples in 4–5 independent experiments. +Significant difference between sedentary and swim-trained rats,  $P \leq 0.05$ ; \*significant difference between basal and insulin-stimulated rats,  $P \leq 0.05$ .

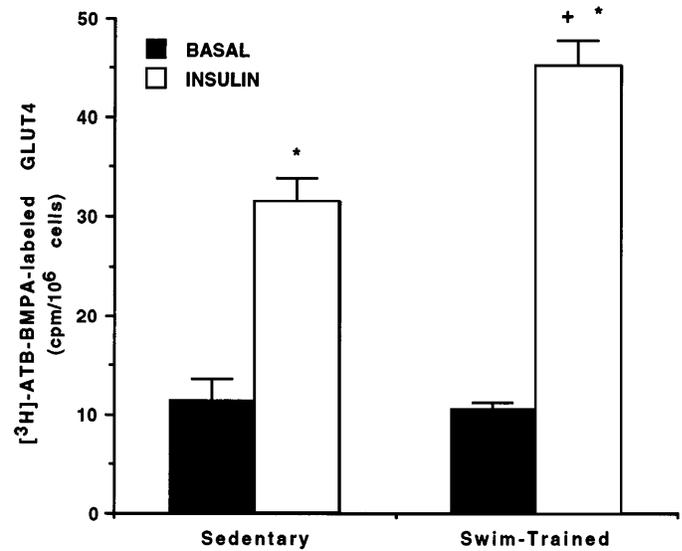


Fig. 2. Cell surface photolabeling of GLUT-4 in isolated adipose cells from sedentary and swim-trained rats. ATB-[ $^3\text{H}$ ]BMPA labeling of GLUT-4 is expressed relative to  $10^6$  cells. Cell no. was calculated from total lipid ( $\mu\text{g}$ ) per sample and average cell size ( $\mu\text{g}$  lipid/cell) for each respective group. Values are means ± SE from 3 independent experiments; cpm, counts per minute. +Significant difference between sedentary and swim-trained,  $P \leq 0.05$ ; \*significant difference between basal and insulin-stimulated,  $P \leq 0.05$ .

also enhances the cell surface GLUT-4 response but only because of increased insulin-stimulated activity.

## DISCUSSION

The present study clearly demonstrates that 4 days of swim training are sufficient to produce marked effects on adipose cell function, with minimal, albeit

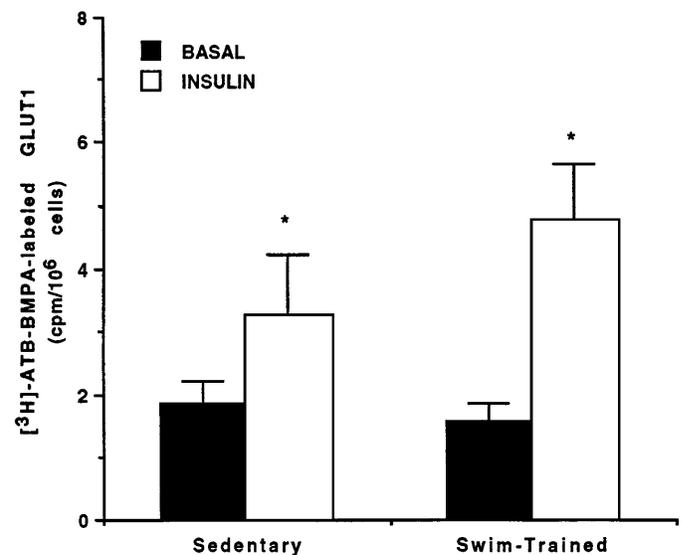


Fig. 3. Cell surface photolabeling of GLUT-1 in isolated adipose cells from sedentary and swim-trained rats. ATB-[ $^3\text{H}$ ]BMPA labeling of GLUT-1 is expressed relative to  $10^6$  cells. Cell no. was calculated from total lipid ( $\mu\text{g}$ ) per sample and average cell size ( $\mu\text{g}$  lipid/cell) for each respective group. Values are means ± SE from 5–6 independent experiments. \*Significant difference between basal and insulin-stimulated,  $P \leq 0.05$ .

statistically significant, changes in adipose cell size. Under these conditions, insulin-stimulated glucose transport activity, insulin-stimulated GLUT-4 translocation to the cell surface, and the total GLUT-4 content increase significantly and to a similar degree, but no effects are observed in total GLUT-1 or syntaxin 4. These effects of exercise training occur much more rapidly than previously believed and appear to be independent of large changes in adipose cell size.

In the present investigation, 4 days of swim training result in significant reductions in body weight and adipose cell size, although these reductions are much smaller than those observed with longer training regimens (1, 7, 21, 24). Previous investigations included various control groups to account for the changes in adipose cell size, including age-matched animals (with a larger cell size) and younger and food-restricted animals matched for either cell size or body weight. The small absolute reductions in adipose cell size observed in the present investigation allow more direct comparisons of glucose transport between the exercise-trained and sedentary controls than did previous investigations, since the problems of appropriate control groups with similar cell sizes are minimized.

Total membrane GLUT-4 levels increase by 30% as a result of 4 days of swim training. Thus significant increases in GLUT-4 levels with exercise training occur very quickly and appear to be independent of large changes in adipose cell size. In addition to the increase in total GLUT-4, a significant increase is also observed in cell water space, indicating an increase in cytosolic volume. This result suggests that exercise training not only affects GLUT-4 but may also result in increases in other cytoplasmic proteins that may be involved in carbohydrate metabolism. Syntaxin 4 and GLUT-1, on the other hand, are not affected by 4 days of swim training. We have also observed that syntaxin 4 does not change even after 6 wk of swim training (unpublished observations). Thus changes in syntaxin 4 do not appear to be required for the adaptations in the glucose transport response to insulin with exercise training, nor do changes in GLUT-1 appear to play a role in altered glucose transport activity.

Insulin-stimulated glucose transport activity is 36% higher in adipose cells from swim-trained compared with sedentary animals and is paralleled by a 44% increase in insulin-stimulated cell surface GLUT-4 photolabeling. This suggests that the increase in insulin-stimulated glucose transport in the swim-trained rats is a result of an increase in total cellular GLUT-4, which results in an increase in the number of GLUT-4 appearing on the cell surface in response to insulin. This conclusion is similar to that observed in long-term exercise-training studies (7), which utilized membrane fractionation of adipose cells to determine intracellular and plasma membrane-associated GLUT-4 content under basal and insulin-stimulated conditions.

Previous investigations have reported similar increases in glucose transport and cell surface GLUT-4 measured via ATB-BMPA photoaffinity labeling in adipose cells with insulin stimulation (10, 20), suggesting

no change in GLUT-4 intrinsic activity or the amount of glucose transported per cell surface GLUT-4. In this investigation, insulin stimulation results in >25-fold increases in glucose transport activity but in only 2.5-fold increases in cell surface GLUT-4 in cells from both the Sed and Sw groups. Because the reduction in the ATB-BMPA degree of response to insulin is observed in cells from both the Sed and Sw groups, it cannot be related to exercise training. Elevated cell surface GLUT-4 photolabeling in the basal state appears to be responsible for the lower degree of increases in cell surface GLUT-4 photolabeling in both groups.

The explanation for higher basal cell surface GLUT-4 levels is unknown but may be related to a number of factors. The present investigation used young Wistar rats (100–120 g), whereas previous investigations used older Sprague-Dawley rats (150–200 g). Adipose cells from Wistar rats have often been noted to exhibit higher basal glucose transport activity and higher basal cell surface GLUT-4 photolabeling than those from age-matched Sprague-Dawley rats (unpublished observations). Thus the disparity between the present and previous observations may reflect differences in the strains of rats studied.

Another possible explanation for higher basal cell surface GLUT-4 is differences in the function of the GLUT-4 on the cell surface. Kinetic analysis of the time course to maximal insulin stimulation for photolabeling and glucose transport suggests that during exocytosis GLUT-4 passes through a transitional state where the GLUT-4-containing vesicles are fused with the plasma membrane, exposing GLUT-4 on the cell surface so that they can be photolabeled with ATB-BMPA, but these exposed GLUT-4 are not yet able to transport glucose into the cell (10, 20, 23). It is possible that in adipose cells from Wistar rats more GLUT-4 may reside in this plasma membrane-associated transitional condition in the basal state, thus reducing the degree of increase in ATB-BMPA-detectable GLUT-4 on the cell surface with insulin stimulation.

Alternatively, the discrepancy between the increases in insulin-stimulated glucose transport and cell surface GLUT-4 may also suggest a potential increase in the activity of GLUT-4 with insulin stimulation in the adipose cells from young Wistar rats. Nevertheless, increased photolabeling in the insulin-stimulated state with training directly parallels increased glucose transport, suggesting that increased GLUT-4 number explains the effects of exercise training.

The rapid increase in insulin-stimulated glucose transport and total GLUT-4 in adipose cells after 4 days of swim training potentially contributes to the overall increase in metabolism and ability to utilize energy sources such as glucose in exercising vs. sedentary animals. The reason for the rapid increase in the amount of GLUT-4 and insulin-stimulated glucose transport in the adipose cells of exercise-trained rats is not known. Recent research by Dela et al. (3) suggests that one of the possible mechanisms by which exercise increases GLUT-4 expression in skeletal muscle is a direct result of muscle fiber contraction. The mecha-

nisms contributing to the exercise training-induced increase in insulin-stimulated glucose transport and GLUT-4 levels in adipose cells must certainly differ from those responsible for the adaptations in exercise-trained skeletal muscle.

Hormonal influences may contribute to the elevation in GLUT-4 levels in adipose tissue after exercise training. Elevated insulin concentrations are known to result in increased expression of adipose cell GLUT-4 (5, 11). In the present investigation, 4 days of swim training result in lower fasting insulin concentrations compared with the sedentary group (unpublished observations). Swimming itself may result in elevations in stress-related hormones such as thyroid hormone, cortisol, and catecholamines (4). Hyperthyroidism in rats increases insulin-stimulated glucose transport activity and plasma membrane GLUT-4, as well as total GLUT-4 content in isolated adipose cells (14). In contrast, elevated levels of epinephrine and glucocorticoids reduce insulin-stimulated glucose transport activity and, in the case of epinephrine, may also downregulate GLUT-4 expression (15). Thus changes in circulating insulin, epinephrine, and/or cortisol are not likely to explain the increases in insulin-stimulated glucose transport and total GLUT-4 observed in adipose cells from exercise-trained animals. Further research is needed to elucidate the mechanisms of the exercise-induced increases in insulin-stimulated glucose transport and total GLUT-4 content in adipose cells.

In conclusion, 4 days of swim training are sufficient to induce the marked changes in adipose cell metabolism normally observed after long-term exercise programs. In addition, the changes in insulin-stimulated glucose transport and cell surface GLUT-4 induced by the present short-term exercise program are larger than the simultaneous changes in adipose cell size. Thus the exercise-induced changes in adipose cell function are not likely to be the consequence of changes in adipose cell size but rather a functional adaptation to one or more exercise-generated, but still unknown, factors.

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