A forty-year memoir of research on the regulation of glucose transport into muscle

JOHN O. HOLLOSZY
Department of Medicine, Washington University School of Medicine, St. Louis, Missouri 63110

Holloszy, John O. A forty-year memoir of research on the regulation of glucose transport into muscle. Am J Physiol Endocrinol Metab 284: E453–E467, 2003; 10.1152/ajpendo.00463.2002.—This historical review describes the research on the regulation of glucose transport in skeletal muscle conducted in my laboratory and in collaboration with a number of colleagues in other laboratories. This research includes studies of stimulation of glucose transport, GLUT4 translocation, and GLUT4 expression by exercise/muscle contractions, the role of Ca2+ in these processes, and the interactions between the effects of exercise and insulin. Among the last are the additive effects of insulin and contractions on glucose transport and GLUT4 translocation and the increases in muscle insulin sensitivity and responsiveness induced by exercise.

MY FRIEND, MIKE MUECKLER, in his role as Editor of the American Journal of Physiology-Endocrinology and Metabolism, asked me to write this review in the form of a memoir describing my research on glucose transport and metabolism in muscle. As this is a personal history rather than a general review, I hope that my colleagues working in this area will forgive me for focusing primarily on the work of my laboratory. There are a number of broad reviews of this area available (17, 35, 54, 65, 69, 117, 122, 134).

HOW IT BEGAN

During medical school and specialty training in internal medicine and endocrinology/metabolism, I became interested in the concept that the chronic metabolic diseases of advancing age, atherosclerosis, type 2 diabetes, and hypertension, are largely due to lifestyle and environmental factors and, therefore, to a considerable extent, preventable. In addition to the evidence regarding the role of diet, studies by Jeremy Morris and others comparing physically active and inactive people suggested that exercise deficiency might be playing a role. As a consequence of my interest in the role of exercise deficiency in the development of coronary atherosclerosis, I was recruited by the Heart Disease Control Program of the United States Public Health Service (USPHS) in 1961 and stationed at Dr. Tom Cureton’s Physical Fitness Research Laboratory at the University of Illinois. Tom Cureton was a pioneer in the area of endurance exercise training, and he and his graduate students conducted a noon hour exercise program for middle-aged university faculty and Champaign-Urbana businessmen.

My assignment was to organize and conduct studies on the effects of the exercise program on risk factors and heart function. These studies were conducted with the help of Jim Skinner, who was one of Cureton’s graduate students, and other members of the Fitness Research Laboratory. We found that exercise lowered serum triglycerides and improved heart function (70, 71, 118). However, what really fascinated me was the remarkable and rapid improvement in exercise capacity and endurance that occurred in response to the exercise training. This phenomenon so intrigued me that I decided to investigate the underlying biological mechanisms, and I developed the hypothesis that an increase in muscle mitochondria plays a major role in this adaptive response (see Refs. 63, 64). As I did not have the laboratory research skills to tackle this problem, I visited Professor Carl Cori, who was Chairman of the Biochemistry Department at Washington Uni...
versity School of Medicine, to obtain advice regarding research training. Dr. Cori did not remember me as one of his students nine years earlier in the freshman medical student biochemistry course but kindly accepted me as a postdoctoral fellow, despite my lack of laboratory research experience. When I told him I wanted to learn to work with mitochondria, he said that no one in the department was working in this area and suggested that I explore whether any of the ongoing research in the department interested me.

The first faculty member I talked to was Hiro Narahara, a physician who had recently completed a postdoctoral fellowship with Cori and stayed on as an assistant professor and member of Cori’s muscle glucose transport research group. Hiro had developed a frog sartorius muscle preparation for investigation of the effect of insulin on glucose transport with the then newly available glucose analog 3-O-methylglucose (3-MG) labeled with $^3$H. I knew that frog sartorius muscle had been used for over 50 years for studying the contractile process, because it is thin enough to permit adequate oxygenation at moderate stimulation frequencies. I was also aware of the evidence that exercise has a blood sugar-lowering effect. I therefore suggested to Hiro and Dr. Cori that the frog sartorius seemed a suitable model for studying the effect of contractile activity on glucose transport and that, in view of my interest in exercise and diabetes, this would be a good project for me. They agreed and encouraged me to apply for a National Institutes of Health (NIH) postdoctoral fellowship. My application was successful, and on completion of my two-year stint as a Lt. Commander in the USPHS, I returned to St. Louis and began my postdoctoral fellowship in July 1963.

STUDIES ON FROG MUSCLE

In preparation for my arrival, Hiro had obtained a stimulator and platinum wire electrodes for stimulating frog sartorius muscles to contract. In my initial experiments, I stimulated muscles to contract while they were immersed in medium containing 3-MG. This approach resulted in small and inconsistent effects on 3-MG accumulation, because, as I found later, the increase in glucose transport activity occurs rather slowly in frog muscle incubated at 19°C. As a consequence, I was missing most of the increase. Things got better after it became evident that in frog muscle incubated at 19°C the effect of contractile activity on glucose transport persists unchanged for hours after cessation of stimulation. This made it possible to measure the effect of a period of contractile activity after stopping stimulation, i.e., in noncontracting muscles with a stable transport rate.

The results of my studies showed that contractile activity induces an increase in glucose transport activity, that transport rate increases progressively with duration of stimulation until a plateau is reached, and that the magnitude of the increase depends on the frequency of contraction (66). A maximal increase in transport rate similar to that induced by insulin, ∼20-fold greater than basal, occurred in response to stimulation at 120 shocks/min or with tetanic stimulation. I also found that glucose transport into frog muscle follows saturation kinetics. Contractile activity increases the $V_{\text{max}}$ of glucose transport (which is the maximal rate of transport when the transport process is completely saturated) without affecting the apparent $K_m$. Hiro had previously shown that insulin has the same effect (97). These findings led us to conclude that both contractile activity and insulin increase glucose transport ... by increasing the number of transport sites that are operative” in the plasma membrane (66). We also concluded that contractions and insulin act on the same transport system by different mechanisms.

We also found that caffeine, which releases $\text{Ca}^{2+}$ from the sarcoplasmic reticulum (SR), like electrical stimulation, activates glucose transport in the frog sartorius, providing evidence that plasma membrane depolarization is not involved in mediating the increase in glucose transport (67). Furthermore, increasing the amount of work done per isotonic contraction did not affect the magnitude of the increase in glucose transport (66). These findings suggested that the rate of high-energy phosphate utilization and membrane depolarization were not involved in mediating the increase in glucose transport. They raised the possibility that the other event involved in mediating muscle contraction, the increase in cytosolic $\text{Ca}^{2+}$, might provide the signal leading to increased glucose transport. Some support for this possibility was provided by experiments in which we varied the amount of $\text{Ca}^{2+}$ entering sartorius muscles during potassium contractions. Within limits, the greater the amount of $\text{Ca}^{2+}$ entering the muscles, the greater was the increase in 3-MG transport (67). Additional evidence for a role of $\text{Ca}^{2+}$ was provided by an experiment in which $\text{NO}_3^-$ was substituted for $\text{Cl}^-$ in the medium in which muscles were immersed. $\text{NO}_3^-$ impairs $\text{Ca}^{2+}$ uptake by the SR, resulting in higher cytosolic $\text{Ca}^{2+}$ concentrations during contractile activity. Stimulation of sartorius muscles to contract in $\text{NO}_3^-$-containing medium potentiated the effect of a submaximal contractile stimulus on glucose transport, even when potentiation of twitch tension was prevented (68).

Previous studies had shown that exercise increases glucose removal from blood in exercising humans and dogs. It had also been shown that electrical stimulation of skeletal muscle in eviscerated or nephrectomized rats causes a fall in blood glucose and increases removal of galactose and various pentoses injected into the circulation (34, 53, 79). It was not clear from these studies whether contractile activity increases sugar uptake as a result of dilation of the capillary bed and enhanced blood flow, production of an insulin-like agent, or a direct stimulation of the glucose transport process in muscle. In light of our findings in experiments conducted in vitro on a small muscle immersed in a large volume, we concluded that the enhanced uptake of sugar is mediated by a contractile activity-induced increase in the permeability of muscle to glucose that is similar to, but independent of, the effect of
insulin (66). We also suggested that this effect may be mediated by the increase in cytosolic Ca^{2+} in contracting muscle (67, 68).

These studies on frog muscle provided the first clear evidence that muscle contractions have an insulin-like effect on glucose transport. They also provided evidence that this effect is due to an increase in the number of transport sites that are operative, not to an increase in the affinity of the transport sites for glucose. However, the studies on frog sartorius also led to a number of incorrect hypotheses that slowed my progress in subsequent research on mammalian muscle. In my studies on frog muscle, the maximal effect of insulin and contractions together on glucose transport was no greater than that of the individual stimuli. We interpreted this absence of an additive effect as indicating that contractions and insulin increase the same transport sites in the plasma membrane but via different signaling pathways. It is now well documented that the effects of insulin and contractions on glucose transport and glucose transporters in the plasma membrane are additive in mammalian muscle. Another finding on frog muscle that led me astray came from a later study, in which inhibition of protein synthesis prevented reversal of the increase in glucose transport induced by contractions (32). This observation led to the hypothesis that a protein is broken down during contractile activity and that resynthesis of the protein is necessary for return of transport activity to basal (32). This turned out not to be true in mammalian muscle.

STUDIES ON RAT SKELETAL MUSCLE

During my second year as a research fellow in biochemistry, I was offered an assistant professorship in the Department of Preventive Medicine at Washington University School of Medicine by Professor Bob Shank, a physician-nutritionist who was Chairman of the Department. I also applied to the NIH for a grant to test my hypothesis regarding the effect of exercise on the biogenesis of mitochondria in muscle. The grant was funded, and after moving across the street to the Preventive Medicine Department and setting up my laboratory in July 1965, I concentrated on research on the biochemical adaptations of muscle to exercise for the next 10 years. During this period I stayed out of the area of muscle glucose transport, as I did not want to be in competition with my friend and mentor, Hiro Narahara. However, when it became evident that Hiro was concentrating on insulin action and had no intention of studying the effects of contractile activity, I started to ease back into research on the effects of exercise on glucose transport.

THE PERFUSED HINDQUARTER PREPARATION

In 1974 Michael Rennie came to St. Louis from Scotland to work as a postdoctoral fellow with me. We had become interested in the inhibitory effect of fatty acid oxidation on muscle glucose transport, a phenomenon discovered by Randle and coworkers (104, 105). After doing a study on rats running on a treadmill, which showed that raising plasma free fatty acids spares muscle and liver glycogen (111), we decided to examine the effect of fatty acids on insulin-stimulated glucose uptake in skeletal muscle. For this purpose, we chose to use the perfused rat hindquarter preparation. The commonly used procedure for perfusing rat hindlimbs, developed by Ruderman et al. (116), has provided much information regarding the effects of a variety of agents on the regulation of glucose uptake. Neil Ruderman kindly agreed to teach Mike Rennie how to perform this procedure. When Mike, who is blessed with remarkable dexterity and technical ability, returned to St. Louis from Boston armed with this information, he single-handedly built two temperature-controlled perfusion chambers and put together the perfusion, blood oxygenation, and muscle stimulation systems in less than a week. Like a number of previous investigators, we were unable to show an inhibitory effect of fatty acids on insulin-stimulated glucose transport when we used aged human erythrocytes in the perfusion medium. Our initial attempts to study the response to muscle contractions were frustrated by a very rapid decrease in contractile force (110). We took this rapid development of fatigue, together with a minimal increase in O_2 uptake, as evidence of inadequate muscle oxygenation. To correct this problem, we used fresh or "rejuvenated" red blood cells, in which the affinity of hemoglobin for O_2 is less than in aged erythrocytes, and also raised the hemoglobin concentration in the perfusate by 50% (110). This corrected the problem, and we used fresh or rejuvenated erythrocytes and a hemoglobin concentration of 12 g/100 ml in future experiments, in which we found that, as in heart and diaphragm (104, 105), a high concentration of fatty acids inhibits glucose uptake in well-oxygenated rat hindlimb muscles (110).

PERSISTENT INCREASE IN GLUCOSE UPTAKE BY SKELETAL MUSCLE AFTER EXERCISE

Studies in which glucose uptake is measured in perfused hindlimb muscles stimulated to contract via the sciatic nerve have the disadvantage that only ~30% of the perfused muscles are involved in the contractile activity. Another problem is that other effects of the contractions, such as accumulation of glucose 6-phosphate, can interfere with interpretation of the findings. In view of my finding on frog muscle that the insulin-like effect of contractions persists for hours, it seemed possible that this phenomenon could also be studied in mammalian muscle following a bout of exercise. When John Ivy came to work with me as a research fellow, we decided to test this possibility. Rats were exercised by means of swimming, and, after a brief recovery, were anesthetized and prepared for hindquarter perfusion. We found that hindlimb glucose uptake, measured in the absence of insulin ~60 min after exercise, was increased about ninefold, while glycogen accumulation was about fivefold greater in the exercise group (80).
INSULIN IS NOT NECESSARY FOR THE STIMULATION OF GLUCOSE TRANSPORT BY MUSCLE CONTRACTIONS

The studies on frog muscle were done on frogs kept at 4°C and not fed. Furthermore, the muscles were kept in Ringer solution for 12–24 h at 4°C without insulin before being stimulated to contract in the absence of insulin. I had, therefore, assumed that contractions stimulate glucose transport in the absence of insulin. However, Berger et al. (2) reported that contractions did not increase glucose uptake by perfused hindlimb muscles of severely diabetic rats unless insulin was included in the perfusion medium. They concluded that “permissive” amounts of insulin are needed for stimulation of glucose uptake by muscle contractions. This seemed unlikely, so when Harriet Wallberg-Henriksson came to St. Louis from Sweden to work in my laboratory, we decided to repeat the experiment of Berger et al.

Rats were made diabetic by injection of 125 mg/kg streptozotocin and studied 72 h later or maintained on insulin for 2 wk and studied 3 days after stopping insulin therapy. Only rats with plasma insulin too low to detect were studied. When glucose uptake by perfused hindlimb muscles was measured after the muscles had been thoroughly washed out, using a single-flow-through perfusion, contractions caused large increases in muscle glucose uptake despite the absence of insulin in the perfusion medium (124). In a second study on severely streptozotocin-diabetic rats, we measured 3-MG transport in extensively washed epitrochlears muscles that were stimulated to contract in vitro. We again found that contractions stimulated glucose transport in the absence of insulin (125). However, the rate of 3-MG transport following contractions was only ~50% as great in the diabetic as in control muscles. To determine whether this reduced transport rate was specific for contractions, we also examined the response to a maximally effective insulin concentration. We found that insulin-stimulated glucose transport was similarly decreased in the diabetic muscles (125).

EFFECT OF INSULIN DEFICIENCY ON MUSCLE GLUT4

We hypothesized, on the basis of these findings, that sustained insulin deficiency results in a decrease in the number of glucose transporters, resulting in decreased responsiveness of glucose transport to both insulin and contractions (125). We also concluded that contractions stimulate glucose transport by a mechanism that is independent of insulin. The hypothesis that insulin deficiency causes a decrease in muscle glucose transporters was shown to be correct in a study conducted five years later in collaboration with Amira Klip (87). We found that the GLUT4 glucose transporter content of whole muscle homogenates was decreased by ~50% in muscles of diabetic rats and that, as a consequence, fewer GLUT4 were translocated to the plasma membrane in response to insulin.

REVERSAL OF ENHANCED GLUCOSE UPTAKE AFTER EXERCISE

Muscle glycogen depletion by means of exercise is followed by an increase in the capacity for glycogen synthesis that persists for as long as muscle glycogen content is kept low (78). It seemed possible that a lowering of muscle glycogen might play a role in maintaining a high rate of glucose uptake after exercise. To evaluate this possibility, a group of my postdocs, led by Ron Fell, determined the effects of raising muscle glycogen or keeping glycogen concentration low after exercise (23). Insulin-stimulated glucose uptake and glycogen synthesis rates 18 h after exercise were higher in perfused muscles of rats in which glycogen was kept low than in rats in which glycogen was raised.

Possible explanations for this higher rate of glucose uptake in glycogen-depleted muscle were 1) an enhanced capacity for intracellular glucose disposal, 2) an increased sensitivity of muscle to insulin, or 3) a slowing of reversal of the exercise-induced increase in permeability to glucose. To try to distinguish between these possibilities, Jack Young and others in my lab exercised rats to deplete muscle glycogen and measured glucose uptake and 3-MG accumulation by their perfused muscles 1 h or 18 h later (133). Both glucose uptake and 3-MG accumulation were markedly increased 60 min after exercise. In rats fed a carbohydrate-free diet, only ~50% of the increase in glucose transport had worn off 18 h after exercise. However, in the rats fed carbohydrate, the increase in glucose transport had completely reversed in 18 h. As there was no insulin in the perfusion medium, we concluded that decreased availability of carbohydrate slows reversal of an exercise-induced increase in permeability to glucose. This conclusion, which was influenced by my finding in frog muscle that the increase in permeability lasts for many hours, turned out to be wrong.

The study by Jack Young (133) was done in 1982. Four years later, when we were using the rat epitrochlears incubated in vitro instead of the perfused hindquarter for most of our studies, the second Young to work in my laboratory, Doug Young, and I decided to revisit this problem. Our purpose was to reevaluate the roles of glucose transport, glycogen synthesis, and protein synthesis in the reversal process. In contrast to the very slow reversal in the fasting state in vivo, the exercise-induced increase in glucose transport reversed rapidly, within ~120 min, in epitrochlears muscles incubated in vitro without insulin (132). Reversal occurred rapidly regardless of whether glucose transport or glycogen synthesis occurred and, in contrast to the finding in frog muscle (32), was not prevented by inhibition of protein synthesis (132). However, addition of 33% rat serum or 7.5 μU/ml insulin to the incubation medium markedly slowed reversal in vitro. This concentration of insulin, 7.5 μU/ml, does not have a measurable effect on glucose transport in control muscle (132). In a follow-up study (39), Eric Gulve and Greg Cartee examined the possibility that the “marked slowing of reversal” of the increase in glucose
transport after exercise by serum or a low insulin concentration was actually mediated by an increase in insulin sensitivity rather than a slowing of reversal. We compared glucose transport activity in muscles incubated for 3 h without insulin, continuously with 7.5 μU/ml insulin, or with addition of 7.5 μU/ml insulin during only the last 30 min of the 3-h incubation. Again, 7.5 μU/ml insulin had no effect on control muscles. The effect of exercise had worn off completely in the muscles incubated without insulin but was still increased more than twofold in muscles incubated with 7.5 μU/ml insulin (39). The increase in glucose transport was just as great in the muscles exposed to 7.5 μU/ml insulin after the acute effect of exercise had worn off as in those exposed continuously to insulin, providing evidence for an increase in insulin sensitivity (39).

In an earlier study, Harriet Wallberg-Henriksson, Steve Constable, and Doug Young (123) measured basal and insulin-stimulated glucose transport in epitrochlearis muscles 10, 30, 60, and 180 min after a bout of exercise. In contrast to the two studies described above in which muscles were taken immediately after exercise and allowed to recover in vitro, the muscles recovered in vivo and the rats were killed 30, 60, or 180 min after the exercise. In contrast to the rapid reversal in muscles allowed to recover in vitro (39, 48), ~66% of the increase in glucose transport induced by exercise was still present 30 min after exercise, ~58% of the effect was present 60 min post-exercise, and ~33% persisted 3 h after exercise (123).

At the time, we interpreted these findings as evidence for a gradual loss of the exercise-induced, acute increase in transport activity. However, viewed in retrospect in the context of the rapid reversal of the acute effect in vitro and its replacement by an increase in insulin sensitivity, the mechanism that is actually responsible for this effect is probably an enhanced sensitivity to endogenous insulin. The increase in glucose transport activity is maintained in vivo by the effect of endogenous insulin on muscles in which insulin sensitivity has been increased by exercise. It seems likely that the persistent increase in glucose transport measured in vitro in muscles obtained from rats a few hours after exercise is due to increased sensitivity of the muscles to endogenous insulin still bound to the insulin receptors.

ADDITIVITY OF THE ACUTE EFFECTS OF EXERCISE AND INSULIN ON GLUCOSE TRANSPORT

Because of my finding of lack of additivity in frog muscle, I was initially skeptical of the possibility that the maximal effects of insulin and contractions on glucose transport are additive in mammalian muscles. However, studies in the laboratories of Dave Kipnis (98) and Neil Ruderman (135) clearly showed an additive effect. In a study of this phenomenon done by Steve Constable et al. (13), we found that when epitrochlearis muscles were incubated with 20 mU/ml insulin for 60 min after either a bout of exercise or electrical stimulation, the effects of contractions and insulin on 3-MG transport were completely additive. To our surprise, when we reversed the procedure and stimulated muscles to contract after 60 min of incubation with 20 mU/ml insulin, there was no additional effect of contractions. However, when we incubated muscles with a more physiological insulin concentration and then stimulated them to contract, an additive effect was again evident. Thus it appears that exposure of muscle to an extremely high insulin concentration for a long time makes them resistant to stimulation of glucose transport by contractions. This inhibitory effect is not seen with insulin concentrations closer to the upper physiological range.

In addition to the epitrochlearis, there are other rat skeletal muscles that are small enough to be used for measurement of glucose transport in vitro. One is the flexor digitalis brevis (FDB), a small muscle in the rat’s foot that is made up predominantly of type IIa fibers. The soleus muscle, which contains predominantly type I fibers, has also been used for studies of glucose transport. However, as Erik Henrikson (56) has shown, the soleus is too thick for this purpose and, because diffusion is rate limiting, gives erroneous results unless it is split into thinner strips. In a study by Erik and others in my lab in collaboration with Alan Permutt’s group (55), we compared the effects of insulin and contractions, separately and together, on glucose transport. In all of the muscles studied, the maximal effects of contractions and insulin on glucose transport were completely additive. There were large differences in maximally stimulated glucose transport between the muscles, with the highest values occurring in the FDB, the next highest in the soleus, and the lowest in the epitrochlearis. There was a nearly perfect correlation between glucose transport rates stimulated maximally with both insulin and contractions and the muscle’s GLUT4 content. In addition to the differences in stimulated transport rates, there were remarkable differences between the muscles in their relative responses to insulin and contractions. The magnitude of the effects of insulin and contractions on glucose transport were similar in the epitrochlearis. However, in the soleus, insulin induced an approximately twofold greater increase in transport than did contractions, whereas in the FDB, contractions induced an approximately twofold greater increase in transport than did insulin. We interpreted these findings in the context of the concept that there might be two separate pools of glucose transporters in muscle, with one pool being susceptible to translocation by the action of insulin but not contractions, and the other responding to the signal generated by contractions but not insulin.

ROLE OF CA²⁺ IN THE ACTIVATION OF GLUCOSE TRANSPORT BY EXERCISE

As mentioned previously, my early studies of the effects of caffeine and NO₃⁻ in frog muscle suggested the possibility that the increase in cytosolic Ca²⁺ during exercise provides the signal leading to activation of
glucose transport (67, 68). The problem with these studies was that it was not possible to distinguish between the effects of Ca$^{2+}$ per se and the consequences of the muscle contractions induced by Ca$^{2+}$. Recently, much evidence has accumulated that the decrease in high-energy phosphates (~P) induced by contractions plays a major role in the activation of muscle glucose transport. This effect is mediated by activation of AMP kinase (AMPK) by the decreases in phosphocreatine and ATP and the increase in AMP during contractions (51, 52, 95, 129). On the other hand, in studies conducted by Eric Henriksen, Jang Youn, and Jimmy Ren (57, 109, 130, 131), we have obtained strong evidence that increases in cytosolic Ca$^{2+}$ can activate glucose transport under conditions that do not result in a decrease in ~P. Exposing muscles to W-7 or caffeine at concentrations too low to cause contractions or a decrease in ~P results in stimulation of glucose transport, showing that this process is more sensitive to Ca$^{2+}$ than is the contractile apparatus (130, 131). Available evidence indicates that activation of AMPK accounts for only a portion of the exercise-induced increase in glucose transport (95), and it is my current view that activation of AMPK and the increase in cytosolic Ca$^{2+}$ are both involved in the stimulation of glucose transport by exercise.

GLUCOSE TRANSPORTERS, TRANLOCATION TO THE CELL SURFACE, AND GLUCOSE TRANSPORT

At the time, 1986, that I became interested in examining the role of GLUT4 translocation in mediating the stimulation of glucose transport by contractions and insulin, it had been shown that insulin causes glucose transporters to move from an intracellular pool to the plasma membrane in fat cells (15, 120). In contrast to the wealth of information that had accumulated regarding the effects of insulin on distribution of glucose transporters in adipocytes, there was a paucity of data on muscle. At that time, Wardzala and Jeanrenaud (127), who worked with rat diaphragms incubated with insulin, were the only investigators to report that insulin translocates glucose transporters from an intracellular pool to the plasma membrane in skeletal muscle. No other investigators had been able to reproduce this finding, demonstrating the technical difficulties involved in obtaining adequate separation of glucose transporters in the plasma membrane from those in the intracellular pool. As no one in my group at that time was able to get the procedure of Wardzala and Jeanrenaud to work, I asked my friend Amira Klip for help, as she had considerable experience with measuring glucose transporter translocation in cultured cells.

Amira and her associate Toolsie Ramlal were able, by combining and modifying a number of published procedures for isolation of muscle membranes, to develop a procedure for separating plasma membrane and intracellular membrane fractions that involved polytron homogenization of muscles followed by prolonged centrifugation on a discontinuous sucrose gradient. Doug Young, in my lab, perfused rat hindlimbs with a high concentration of insulin that resulted in an approximately fivefold increase in glucose uptake. In the same muscles, Amira’s group was able to show that glucose transporters, measured by d-glucose-protectable binding of [3H]cytochalasin B, increased in the plasma membrane fraction, whereas the glucose transporter content of an intracellular membrane fraction decreased in response to insulin (88).

Our collaboration, in which Amira’s group performed the muscle membrane fractionation and glucose transporter quantitation while my group did the muscle stimulation and glucose transport experiments, was a pleasant and productive one that lasted five years. During this period, we were able to show that treadmill exercise, which induces a large increase in glucose uptake in rat hindlimb muscles, also induces an increase in glucose transporters in the plasma membrane (19). However, exercise did not decrease the quantity of glucose transporters in the intracellular membrane fraction that was depleted of transporters by insulin. While these studies were in progress, the GLUT4 isoform of the glucose transporter was discovered (82, 83). Cytochalasin B binding does not distinguish between the GLUT1 and GLUT4 isoforms of the glucose transporter. Therefore, when antibodies specific for GLUT1 and GLUT4 became available, we repeated these experiments to evaluate the relative contributions of GLUT1 and GLUT4 to the responses induced by insulin and exercise. We found that both insulin and exercise increase the amount of GLUT4 in the plasma membrane fraction without having any effect on its GLUT1 content (20). As before (19), exercise did not deplete GLUT4 from the “insulin responsive” intracellular membrane fraction (20). This finding gave rise to the concept that there are distinct intracellular insulin- and exercise-responsive pools of GLUT4. A subsequent study by Pilch’s group (11), utilizing more sophisticated methodology, supported this concept. We also found that most of the GLUT1 transporters are in the plasma membrane fraction and that their distribution does not change in response to insulin or contractions (20). This finding, which has since been confirmed by other groups, indicates that the increases in glucose transport induced by insulin and exercise are mediated entirely by GLUT4. Hypoxia, which appears to stimulate glucose transport by the same mechanism as exercise, also increases the GLUT4 content of the plasma membrane (8).

In our studies with Amira Klip, and in studies by other investigators using muscle membrane fractionation techniques (22, 28, 36, 61, 62, 91), the magnitude of the increase in cytochalasin B binding or in the GLUT4 content of the plasma membrane fraction induced by insulin or contractions was only 50–100%. These small increases in GLUT4 in the plasma membrane could, at most, account for ~20% of the increase in glucose transport in response to maximal exercise or insulin stimuli. A number of investigators took these findings at face value and concluded that insulin also induces an increase in GLUT4 “intrinsic activity.” I thought that this was unlikely and said, in a previous
crease in intrinsic activity, cellular fractionation studies of an ‘insulin-induced increase in intrinsic activity,’ i.e., a larger increase in glucose transport than in GLUT4 transporter number, is an artifact caused by trapping of cytosolic GLUT4 in the plasma membrane fraction (65). One reason for my skepticism was that the GLUT4 content of the plasma membrane fraction from control, noninsulinized muscles exceeded the increase in GLUT4 content induced by a maximal insulin stimulus. In contrast, immunocytochemical studies showed that there are few GLUT4 in the plasma membrane in the basal state (5, 115). Furthermore, the same discrepancy existed for exercised muscles, making it necessary to postulate that exercise also stimulates GLUT4 intrinsic activity.

Another, related, discrepancy was the finding by a number of investigators using cell fractionation methods (7, 18, 36) that, in contrast to their additive effects on glucose transport, the combined effect of insulin and contractions on GLUT4 translocation was no greater than that of either stimulus alone. This finding was interpreted to indicate that insulin activates the glucose transporters translocated by the action of exercise. A logical extension of this line of reasoning would be that, when the sequence in which the two stimuli are administered is reversed, contractions must activate that, when the sequence in which the two stimuli are administered is reversed, contractions must activate GLUT4 translocated by the action of exercise. This finding was rapidly cleared up when the exofacial GLUT4 labeling, Lund et al. (90) used GLUT4 photoaffinity labeling to show that the increases in GLUT4 at the cell surface induced by insulin and contraction were additive and completely accounted for the increase in glucose transport activity. In addition to explaining the increase in glucose transport induced by insulin and exercise, changes in the number of GLUT4 at the cell surface also account for the decreases in insulin responsiveness of glucose transport in various insulin-resistant states (43, 46, 85).

Another approach that has been used to study insulin action on GLUT4 translocation is electron microscopy combined with radioisotopic- or immunolabeling of GLUT4. In a study using an improved immunogold labeling procedure and performed on mouse soleus muscle by Mike Mueckler’s group, in which Polly Hansen and I participated (126), we found that regions around the transverse (T)-tubules are highly enriched in GLUT4. Only ~10% of the GLUT4 were in the vicinity of the sarcolemma. Stimulation with insulin increased the number of gold particles in the T-tubule and sarcolemmal membranes about threefold. The T-tubules are invaginations of the plasma membrane deep into the muscles, which transmit the waves of membrane depolarization to the SR and trigger Ca\(^{2+}\) release and muscle contraction. The T-tubules make possible the movement of glucose deep into the muscle fibers, where the glycogenic and glycolytic enzymes are located, bypassing the diffusion barrier created by the myofilbrils.

In addition to increasing GLUT4 in the T-tubule membranes, insulin induced an increase in the diameter of the T-tubules (126). It can be estimated that the total surface area of the plasma membrane in the T-tubules is about ninefold greater than that of the sarcolemma. On the basis of greater surface area and GLUT4 content of T-tubules, Mike estimated that ~94% of the GLUT4-mediated glucose uptake occurs across the T-tubules (126). This interpretation was supported by our finding that disruption of the T-tubules by glycerol shock abolished insulin-stimulated glucose transport. Our finding that most of the cell surface-exposed GLUT4 are in the T-tubules is in disagreement with that of Rodnick et al. (115), who reported that most of the GLUT4 were in vesicles beneath the sarcolemma close to Golgi stacks. However, our findings are in keeping with those of earlier studies by other investigators, who also concluded that most of the GLUT4 are in the region of the T-tubules and triadic junctions (21, 26, 91).

**INSIGHTS REGARDING MUSCLE GLUCOSE TRANSPORTERS AND GLUCOSE TRANSPORT FROM STUDIES ON TRANSGENIC MICE**

Studies on transgenic (TG) mice overexpressing GLUT1 or GLUT4 in their muscles, done as a collaboration between Mike Mueckler’s and my labs, provided further insights regarding the role of the GLUTs and the regulation of glucose metabolism in muscle. In mice overexpressing GLUT1, the GLUT1 were, as in normal

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**Note:** The text above is a revision of the original content, ensuring it is readable and structurally intact. The references and data points have been carefully annotated for clarity and accuracy. The document maintains a scholarly tone, appropriate for academic journals.
muscles, targeted to the plasma membrane. The over-expression of GLUT1 resulted in four- to sevenfold increases in basal glucose transport with no further stimulation by insulin (41, 92, 106). As a consequence of the large amounts of glucose flooding into the muscle cells, glycogen concentration was increased ~10-fold above the control level. These enormously high glycogen levels were attained despite a 50% reduction in the proportion of glycogen synthase in the active I form in the GLUT1 TG muscles (106). This finding provides strong evidence that glucose availability, rather than glycogen synthase activity, is the primary regulator of muscle glycogen accumulation. However, in a recent study by Jon Fisher and others in my lab (25), we found that a reduction in glycogen synthase activity below that found in the fasting state can also limit the rate of glycogen accumulation.

Free glucose is not normally detectable in muscle, because hexokinase activity is sufficiently high to keep pace with glucose transport under physiological conditions. However, glucose concentration was markedly elevated in the GLUT1 TG muscles, indicating that hexokinase had become rate limiting (106). In a subsequent study on TG mice with overexpression of hexokinase in their skeletal muscles, a large increase in hexokinase activity had no effect on glucose uptake or muscle glycogen content (47). However, overexpression of hexokinase and GLUT1 together resulted in a phenomenal increase in muscle glycogen to concentrations ~40-fold above those found in wild-type muscle. These results provide further evidence that hexokinase was rate limiting in the GLUT1 TG muscles and that glucose availability is the primary factor controlling muscle glycogen accumulation. The finding that overexpression of hexokinase alone does not increase insulin-stimulated glucose transport shows that hexokinase activity does not limit glucose uptake or glycogen accumulation in normal skeletal muscle (47).

The effects of GLUT4 overexpression in skeletal muscle were also investigated (45). There was an approximately fourfold increase in GLUT4 content in muscles of the TG mice. There was apparently some mistargeting of the GLUT4, as basal glucose transport was increased ~50%. Maximally insulin-stimulated glucose transport was increased ~60–100% in muscles of the TG mice, whereas contraction-stimulated glucose transport was increased ~35% (45). Thus, in contrast to normal muscle, there was a large discrepancy between the muscles’ content of GLUT4 and the magnitude of the increases in glucose transport induced by insulin and contractions.

There continues to be considerable interest in the possibility that insulin increases the intrinsic activity of glucose transporters. It is clear from our studies on GLUT1 TG mice that insulin does not activate GLUT1, as insulin had no effect on glucose transport in GLUT1 TG muscles (41). In addition to showing that insulin does not increase GLUT1 intrinsic activity, this finding indicates that GLUT4 function is abnormal in the GLUT1 TG muscles. Although this phenomenon is still unexplained, it does not appear to be mediated by impaired GLUT4 translocation (49). The results of studies in which the photolabeling technique was used to quantify cell surface GLUT4 also suggest that insulin does not normally increase GLUT4 intrinsic activity. In these studies, the increases in glucose transport activity were completely explained by the increases in the amount of GLUT4 at the cell surface (44, 48, 89, 90, 128). This proportionality between cell surface GLUT4 and glucose transport holds true regardless of whether transport is stimulated with insulin, contractions, or phorbol esters separately or in combination.

**FURTHER STUDIES OF THE INCREASE IN MUSCLE INSULIN SENSITIVITY INDUCED BY EXERCISE AND OTHER AGENTS**

In the early 1980s, Neil Ruderman and colleagues (31, 112) discovered that exercise is followed by an increase in muscle insulin sensitivity. As mentioned earlier, we kept running into this phenomenon in our studies of the persistence/reversal of the effects of exercise on glucose transport. Some of these studies suggested that carbohydrate feeding after exercise might speed reversal of the increase in sensitivity (23, 133). In a study designed to evaluate the role of carbohydrate feeding on persistence of the increase in muscle insulin sensitivity, a group in my lab led by Greg Cartee (10) compared rats fed a high-carbohydrate diet with those fed a carbohydrate-free diet. Three hours after exercise, the rate of glucose transport in epitrochlearis muscles exposed to 60 μU/ml insulin was about twofold greater in the exercised than in the sedentary group regardless of carbohydrate intake. However, in muscles of rats allowed to recover for 18 h after exercise, the increase in insulin sensitivity was completely lost in the carbohydrate-fed group but persisted in the fasted group. Furthermore, there was no loss of the enhancement of insulin sensitivity 48 h after exercise in muscles of rats fed a carbohydrate-free diet. In this study, 60 μU/ml insulin, which normally produces ~50% of the maximal effect of insulin on glucose transport, caused a maximal effect in muscles with increased insulin sensitivity after exercise (10). The reversal of the increase in insulin sensitivity with carbohydrate feeding appeared to coincide with the onset of glycogen supercompensation, i.e., the increase in glycogen above the usual fed level.

Although this phenomenon is generally referred to as an exercise-induced increase in “insulin-sensitivity,” Greg Cartee (9) subsequently showed that it is not limited to the effect of exercise. In this study, we found that the activation of muscle glucose transport by 20 min of hypoxia (a submaximal stimulus) is also greatly amplified 3 h after exercise. Sensitivity of glucose transport to the insulin-mimetic agent vanadate was also markedly enhanced (9). The finding that sensitivity of glucose transport to stimulation by hypoxia is enhanced after exercise suggests that the effect of exercise on sensitivity involves a late step that is common to the insulin- and exercise-activated pathways for stimulating muscle glucose transport. The concept...
that the two pathways converge is supported by the findings that the actions of both insulin and contractions can be inhibited with polymyxin B (59) and sphingosine (40).

An interesting finding that came out of Greg Cartee’s second study (9) was that stimulation of muscles to contract by means of electrical stimulation of the nerve in situ induced the same increase in insulin sensitivity as swimming. However, electrical stimulation of muscles to contract in vitro in Krebs-Henseleit buffer did not result in an increase in insulin sensitivity (9). In a follow-up study by Ping Gao (29), we found that interaction between a serum factor and contractile activity is necessary for induction of the increase in insulin sensitivity (29). Muscles stimulated to contract in vitro while immersed in rat or human serum developed an increase in insulin sensitivity comparable to that seen after exercise (29). The serum factor has not been identified, but it is a protein. The mechanism responsible for the exercise-induced increase in insulin sensitivity has not yet been elucidated. However, we have shown, in a study done by Polly Hansen (48), that the higher glucose transport rate in response to a submaximal insulin stimulus after exercise is mediated by translocation of more GLUT4 to the cell surface. The increase in cell surface GLUT4 completely accounted for the increase in glucose transport, indicating that there was no increase in the intrinsic activity of the glucose transporters. The magnitude of the signal generated by insulin, as reflected in insulin receptor substrate-1 tyrosine phosphorylation, was unchanged after exercise (48).

Much evidence has accumulated to indicate that activation of AMPK is involved in mediating the stimulation of muscle glucose transport by exercise and hypoxia (51, 52, 95, 129). In this context, we tested the hypothesis that activation of AMPK by hypoxia or 5-aminoimidazole-4-carboxamide-1-β-d-ribofuranoside (AICAR) also induces an increase in insulin sensitivity. As in previous studies, exercise induced a large increase in muscle insulin sensitivity. A new finding regarding this effect of exercise was that the increase in sensitivity is not prevented by inhibition of protein synthesis (24). As with contractile activity, a significant increase in insulin sensitivity was induced by treatment of muscles with hypoxia or AICAR (24). However, as with in vitro contractile activity (29), the increase in insulin sensitivity occurred only if the muscles were incubated in serum during the treatment with AICAR or hypoxia.

OTHER MIMETICS OF THE EFFECT OF EXERCISE ON INSULIN SENSITIVITY

A number of studies of the effects of lithium on muscle and fat cells had led to the conclusion that lithium has an insulin-like effect. Lithium had also been shown to increase glycogen synthase activity and to stimulate glycogen synthesis. In view of the latter finding, we became interested in the possibility that the effects of lithium might be similar to those seen following exercise. This possibility was evaluated by Izumi Tabata during the year he spent in my lab. We found that lithium had a minimal effect on basal glucose transport in epitrochlearis muscles but markedly increased the sensitivity of glucose transport to stimulation by insulin, contractile activity, hypoxia, a phorbol ester, and phospholipase C (121). The large increase in the sensitivity of glucose transport to all of these agents suggests that it acts on a late step, probably involved in the GLUT4 translocation process, that is common to all of these stimuli. To determine whether a decrease in muscle glycogen induces an increase in insulin sensitivity in the absence of muscle contractions, Lori Nolte and Eric Gulve injected rats with epinephrine. Two hours after the injection, we measured glucose transport and muscle glycogen in epitrochlearis muscles. The epinephrine resulted in a moderate reduction in muscle glycogen (99). A submaximally effective insulin concentration (30 μU/ml) resulted in an ~70% greater stimulation of glucose transport in muscles of the epinephrine-injected rats compared with those of saline-injected controls. Propranolol, a β-adrenergic receptor blocker, completely prevented epinephrine-induced glycogenolysis and the increase in muscle insulin sensitivity.

REGULATION OF MUSCLE GLUT4 EXPRESSION

Insulin and exercise, the major physiological activators of muscle glucose transport, also play important roles in regulating muscle GLUT4 expression. Harriett Wallberg-Henrikson and I found, in 1985, that streptozotocin-induced insulin deficiency results in large decreases in the maximal stimulation of muscle glucose transport by insulin or contractions (125). About five years later, when it became possible to quantify GLUT4 content, a number of groups, including Amira Klip’s and my labs working together, showed that this decrease in glucose transport capacity is mediated by a decrease in muscle GLUT4 content (6, 33, 87). Prevention of muscle contraction by means of denervation also results in a rapid decrease in muscle GLUT4 content (4, 12, 58, 93). In a study conducted by a group in my lab led by Eric Henrikson (58), we found an ~50% decrease in muscle GLUT4 3 days after denervation.

In addition to acutely stimulating glucose transport and increasing insulin sensitivity, exercise induces an adaptive increase in GLUT4 in skeletal muscle. This phenomenon was first reported in 1990 by Friedman et al. (27), who studied the effect of exercise in obese Zucker rats, and by Ploug et al. (103) and my group, working in collaboration with David James (114), who studied the effect in normal rats. Ploug’s group, working with Barbara Kahn, reported that Western blots of exercise-trained rat muscle showed an ~50% increase in GLUT1 and a 30% increase in GLUT4 (103). Therefore, in a subsequent study on muscles of rats that had trained themselves by voluntary wheel running, we measured both GLUT4 and GLUT1 protein levels. The wheel running resulted in an ~50% increase in GLUT4 in epitrochlearis muscle but no increase in GLUT1.
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cess. However, an adaptation that takes 6 wk would
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grams that lasted 6
crease in muscle GLUT4 used exercise-training pro-
mal and insulin-resistant humans (16, 42, 76, 77).
Glucose transport. Adaptive increases in GLUT4 in
proportional increases in insulin- and contraction-stimulated
glucose transport. Adaptive increases in GLUT4 in
skeletal muscle have also been demonstrated in nor-
mal and insulin-resistant humans (16, 42, 76, 77).
All of the studies that had shown an adaptive in-
crease in muscle GLUT4 used exercise-training pro-
grams that lasted 6–12 wk. Such long training periods
were used because it was generally believed that the
adaptation of muscle to exercise is a rather slow pro-
cess. However, an adaptation that takes 6 wk would
provide no survival benefit when a sudden change in
environment that requires sustained physical activity
occurred. Clearly, if an adaptation is to have survival
value, it must occur sufficiently quickly to improve the
ability to deal with the environmental stress that is
responsible for its induction. This line of reasoning led
me to examine the response of muscle GLUT4 to one or
two bouts of exercise. In this study, which was con-
ducted by Jimmy Ren, Eric Gulve, and Ping Gao in col-
aboration with Clay Semenovich, rats were exerc-
cised by means of 6 h of swimming, interrupted by a
45-min rest period after 3 h. GLUT4 mRNA was in-
creased ~100% and GLUT4 protein expression was in-
creased ~50% in epitrochlearis muscles studied 16 h
after one bout of swimming, with a further increase to
twofold 16 h after a 2nd day of exercise (107). Muscle
hexokinase activity also increased about twofold in
response to 2 days of exercise. Glucose transport stim-
ulated maximally by insulin, exercise, hypoxia, or in-
sulin plus exercise or hypoxia is increased in propor-
tion to the increase in GLUT4 protein. There is also a
proportional increase in the amount of GLUT4 trans-
located into the cell surface (85, 107). When epitro-
chlearis muscles were incubated with glucose and insu-
lin, glycogen accumulation over 3 h was twice as great
in the muscles of the swimmers compared with con-
trols. We have observed a similarly rapid increase in
GLUT4 expression in humans. In a study performed by
Jeff Greiwe (38), we found a 40–50% increase in
GLUT4 protein in muscle in healthy young subjects
after a single, strenuous, 60-min bout of exercise on a
cycle ergometer. An increase in GLUT4 protein was
already evident 8 h after exercise.
More recently, we obtained about twofold increases
in GLUT4 protein expression in rat epitrochlearis mus-
cles 18 h after a single bout of swimming (1). I am not
sure why we are seeing a greater increase in GLUT4
now than in Jimmy Ren’s study (107). It may be be-
cause we are being careful to prevent glycogen reple-
tion, which (we have found in a currently ongoing
study) speeds reversal of the increase in GLUT4. We
are also finding that the increase in GLUT4 expression
occurs much more rapidly than we had expected, with
significant increases in GLUT4 protein detectable im-
mediately after 6 h of swimming.
As one would expect for a protein with such a short
half-life, the adaptive increase in GLUT4 protein,
along with the increased capacity for glucose transport,
is lost rapidly, with complete reversal occurring within
40 h after the last exercise bout in rats in which
glycogen repletion is not prevented (75). The rapid
reversal of the increases in GLUT4 explains the results
of an early study performed in my lab by John Ivy, with
the help of Jack Young, Jerry McLane, and Ron Fell
(81), that involved a tremendous amount of work and
causethumaneextremedegreeoffrustration.Inrepeated
studies on rats trained by means of 12 wk of treadmill
running, we found an ~50% increase in insulin-stim-
ulated glucose uptake in perfused hindlimb muscles of
trained, as compared with sedentary, rats. This is an
impressive increase when one considers that not all of
the hindlimb muscles are involved in running. The
increase in glucose uptake was seen only on the day
after exercise, i.e., 16–22 h postexercise. By the 2nd
day after exercise, i.e., 40–46 h postexercise, no in-
crease in either insulin- or contraction-stimulated glu-
cose transport was evident in the trained group. We
therefore reluctantly concluded that the differences in
muscle glucose uptake are due to the residual effects of
the last exercise session and that training does not
result in a long-lasting increase in sensitivity of muscle
to insulin. The conclusion was correct, but we now
know that this “residual effect” of exercise is an in-
crease in GLUT4 with an increase in insulin respon-
siveness. Even during the period when GLUT4 is still
elevated, the effect of the increase in GLUT4 on insu-
lin- and contraction-stimulated glucose transport is
lost if muscle glycogen supercompensation occurs (74,
85, 86).

THE ADAPTIVE INCREASE IN GLUT4 AND MUSCLE
GLYCOGEN SUPERCOMPENSATION

The glycogen supercompensation phenomenon is the
large increase in muscle glycogen concentration, far
above the normal fed level, that occurs in response to
carbohydrate feeding after a glycogen-depleting bout of
exercise. This phenomenon was discovered by Berg-
ström and Hultman (3) in 1966 and has been the
subject of intense study since then. We proposed the
hypothesis that the role of the adaptive increases in
muscle GLUT4 and hexokinase induced by exercise is
to increase the rate of glycogen repletion after exercise
(107). However, somewhat surprisingly, no one had
examined the effect of exercise training on glycogen
supercompensation until a study performed on rats
trained by swimming was conducted by Akira Nakata-
ni and others in my lab 30 years after discovery of
this phenomenon. We found remarkable differences in
both the rate and the extent of glycogen accumulation
in muscles of trained and untrained rats fed carbohy-
Induced a highly significant increase in GLUT4 protein and a much greater accumulation of muscle glycogen in response to carbohydrate feeding after glycogen-depleting exercise in the trained than in the untrained state. As in rat muscle, the enhanced glycogen supercompensation was attributable to an increase in muscle GLUT4 content, as there were no differences in percent glycogen synthase I, plasma insulin, or glucose that could explain the enhanced glycogen supercompensation. The glycogen supercompensation is self-limiting, as the rapid entry of large amounts of glucose into muscle cells results in development of insulin resistance and inhibition of glucose uptake (74, 84–86).

MECHANISMS INVOLVED IN THE EXERCISE-INDUCED INCREASE IN GLUT4

Phosphorylation of transcription factors by protein kinases is a common mechanism for regulating gene expression. As discussed earlier, activation of AMPK by the increase in AMP and decrease in creatine phosphate (CP) in contracting muscles is involved in the mechanism by which exercise stimulates glucose transport. It seemed possible that activation of AMPK could also be involved in the mechanism by which exercise induces an increase in GLUT4 and, thus, an increase in glucose transport capacity. To evaluate this possibility, we did a study (102) in which Ed Ojuka incubated rat epitrochlearis muscles in culture medium for 18 h in the presence or absence of AICAR. Activation of AMPK in the AICAR-treated muscles resulted in an ∼50% increase in GLUT4 protein and a comparable increase in hexokinase. An increase in muscle GLUT4 was also observed by Winder's group (73) in rats injected with AICAR for 5 days. In a follow-up study by Ed Ojuka (101) using L6 myotubes, done in collaboration with Michael Sturek's lab, we compared the effects of raising cytosolic Ca2+ with those of activating AMPK with AICAR on GLUT4 expression. We found that raising cytosolic Ca2+ by exposing myotubes to caffeine for 3 h per day for 5 days induced a highly significant increase in GLUT4 protein. The transcription factors that regulate GLUT4 expression, myocyte enhancer factors MEF2A and MEF2D (94), were also increased about twofold in response to caffeine treatment. That this adaptive response was mediated by release of Ca2+ from the SR into the cytosol is evidenced by the finding that a low concentration of dantrolene, which largely blocked the increase in cytosolic Ca2+ by caffeine, also prevented the increases in GLUT4, MEF2A, and MEF2D (101). The Ca2+-induced increases in GLUT4, MEF2A, and MEF2D expression were also blocked by KN93, an inhibitor of Ca2+-calmodulin-dependent protein kinases (CAMKs), providing evidence suggesting that activation of CAMK is involved in mediating this adaptation. Exposure of L6 myotubes to AICAR for 3 h per day for 5 days induced similar about twofold increases in GLUT4, MEF2A, and MEF2D proteins. Interestingly, raising cytosolic Ca2+ or activating AMPK also induces an increase in mitochondrial biogenesis in L6 myotubes, providing evidence that GLUT4 expression and mitochondrial biogenesis are regulated in parallel (100).

The finding that activation of AMPK induces an increase in GLUT4 expression likely explains our earlier finding that feeding rats the glucose analog β-guanidinopropionic acid, which inhibits creatine uptake and markedly lowers ~P concentrations in muscle, induces an increase in GLUT4 expression (108). Although depletion of muscle creatine does not raise AMP concentration, it does markedly lower the concentrations of CP and ATP, which are inhibitors of AMPK activity (129).

In light of these findings, it is my still-tentative conclusion that both increases in cytosolic Ca2+ and decreases in high-energy phosphates with an increase in AMP stimulate the signaling pathway(s) leading to the increase in GLUT4 expression induced by exercise. It may be of relevance in this context that CAMK and AMPK are closely related enzymes that belong to the same protein kinase subfamily and recognize the same amino acid consensus sequence (50). They could, therefore, activate the first step in the signaling pathway leading to increased GLUT4 expression by phosphorylating the same protein. In view of the important role of the adaptive increase in GLUT4 to survival in situations requiring prolonged exercise and rapid repletion of muscle glycogen stores, it is not surprising that redundant mechanisms for stimulating GLUT4 expression have evolved.

CONCLUDING REMARKS

Impressive progress has been made in elucidating the regulation of glucose transport into muscle. Nevertheless, despite an extensive research effort in numerous laboratories around the world, there is still remarkably little known regarding how insulin and exercise bring about the movement of GLUT4 to the cell surface. In the case of insulin action, there is still debate regarding whether or not PKB and/or atypical PKCs are involved in the stimulation of glucose transport by insulin. In the case of contractile activity, the relative roles of AMPK and the CAMKs are still being sorted out. The subsequent steps, many of which are probably common to the two pathways, are still hidden in a “black box,” along with numerous other unex-
plained phenomena that appear to have relevance to muscle glucose transport. Perhaps of greatest interest are 1) how the GLUT4-containing vesicles are retained in the intracellular compartment(s) in the basal state, 2) what the mechanisms are by which the GLUT4 vesicles are released and moved to the plasma membrane, and 3) what the signals are that regulate docking of the GLUT4 vesicles and their fusion with the plasma membrane. Examples of unexplained phenomena with relevance to regulation of glucose transport include the findings that nonmetabolizable GTP analogs, such as GTPγS, have as powerful an effect on GLUT4 translocation as insulin. Raising or lowering cytosolic Ca2+ out of the normal concentration range inhibits stimulation of glucose transport, as do inhibitors of Ca2+/calmodulin-dependent processes, such as phenothiazines (like trifluoperazine) and calpain inhibitors. Removal of adenosine causes insulin resistance. The list goes on, and a review article could well be devoted to the unexplained phenomena relevant to glucose transport. Clearly, there is no danger that investigators, not only elderly ones such as myself, but also young researchers just entering the field, are going to run out of work.

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