THE CrossFit JOURNAL KIDS

Journal Club: Insulin and Exercise

Dr. Jonathan Gary offers an introduction to the cell biology behind increasing glucose uptake into cells.

By Dr. Jonathan Gary **March 2011**

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For readers who previously subscribed to *CrossFit Kids Magazine*, this article will be another in the series I've written trying to explain the basic scientific research that supports the benefits of exercise, both for the mind and body.

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I call this series "Journal Club" from my time in graduate school. The closest thing I can compare it to is a book report. Perhaps a dozen students would get together every week, and it was the responsibility of one of us to present a paper (or perhaps a couple linked by a common theme). We could present the paper as something imaginative or groundbreaking in order to let everyone else in on the cool science, but it could also be a paper that was a bit questionable, either in its experimental procedures or conclusions. In either case, it was a great learning tool for critically reading papers. It was also a great way to disseminate knowledge to the rest of the group. I have the same purpose here: to present papers that I think are pretty interesting in the hopes that it starts a dialogue, opens some eyes or even makes you do a bit of research for yourself. These papers are not presented as the end-all be-all on topics but a point from which further consideration and research can begin.

A Look at GLUT4

I think I can take it as a given with this audience that we are all aware of the obesity epidemic that is affecting many nations around the world. And we understand that it is not limited to adults but is also quite pervasive among younger generations. With obesity comes a whole host of health problems, not the least of which is insulin resistance. The primary result of insulin resistance is an inability to properly maintain optimal blood-glucose homeostasis. Elevated blood glucose means cells are not getting the energy they may require (because it's not being imported as effectively without the sensitivity to the insulin signal), and the continuous presence of excess glucose in the blood can also lead to many detrimental health effects.

There is essentially only one way to reduce the amount of glucose in the blood: import it into cells. Once out of the bloodstream, glucose can be converted directly to energy (via glycolysis and the Krebs cycle), stored for later use (as fats and glycogen), or utilized for the formation of other cellular building blocks (pentose phosphate pathway, nucleotide and amino acid synthesis). To date, at least 12 different proteins are responsible for the transport of glucose into cells. All these proteins belong to the same general family. They sit within the cellular membrane, creating a pore that makes the membrane specifically permeable to glucose. Without these transporters, the cellular membrane is a pretty good barrier to the exchange of soluble molecules between cells and their surrounding milieu. Our interest for this article is the transporter called GLUT4. GLUT4 is the primary glucose transporter present in skeletal muscle (and adipose tissue) and therefore the most relevant one for our discussions here.

Both insulin and exercise are capable of lowering blood-glucose levels, but surprisingly, at the level of cellular biology, they do it through two different modes. How do these signals affect the increase in glucose transport across GLUT4-containing membrane? It turns out that a significant reservoir of GLUT4 is actually stored in the membrane of the lipid vesicles that sit just inside the cellular membrane. Signals that result in an increase in glucose transport actually cause the fusion of these GLUT4-containing vesicles with the cellular membrane, thereby increasing the number of glucose transporters performing their function. As the signal diminishes, regions of the cellular membrane that contain GLUT4 invaginate and pinch off to reform the vesicles, readying them for the next round. The papers I'd like to go over in this article examine how the reservoir of vesicles that are insulin responsive are different from the set of vesicles that are induced to fuse with the cellular membrane in response to exercise.

Examining the Research

Douen et al. (1) confirm an observation they made a year earlier that there might be two distinct pools of GLUT4-containing vesicles, one responsive to insulin and one to exercise. Their work was done using skeletal muscle removed from rats. In one case, the rats were dosed with insulin; in a separate experiment, the animals ran on a treadmill for 45 minutes at a 15 percent grade; and a third set of rats were left untreated (controls)(1). A mixture of cellular and internal membranes were isolated from these muscle preparations and then subjected to separation on a density gradient (1). This particular procedure allows the separation of membranes based on the unique properties of what they contain (density). Resident proteins specific to each type of membrane were used to assess the separation (1). Cellular membranes migrated to the 25 percent layer, while the intracellular vesicles remained in the 35 percent layer (1). Once these membranes were separated and isolated from each other, assays were done on the samples to quantitate the amount of GLUT4 present compared to samples from untreated control animals. Their results show that upon insulin treatment, a significant amount (33 percent) of GLUT4-containing internal vesicles disappear

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and there is a concomitant increase (150 percent) in GLUT4 at the cellular membrane (1). Exercise treatment showed a 250 percent increase in GLUT4 at the cellular membrane, while only an 8 percent decrease in the vesicle fraction (1).

Incorporating the results from previous studies, Douen et al. conclude that insulin causes the fusion of some of the intracellular vesicles to the cellular membrane, thereby increasing the amount of active GLUT4 on the cell surface (1). Exercise shows an equally large increase in GLUT4 at the cellular membrane. However, this GLUT4 comes from a different source, presumably located in an unidentified portion of the density gradient (1). Brozinick et al. (2) subsequently confirmed the idea of separate insulin- and exercise-sensitive GLUT4 vesicles. They went a logical step further and showed that both treatments actually lead to an increased uptake of glucose by rat muscle cells, not just an increase in the protein itself (2). Using four different isolated muscle preparations, the average increase in the rate of glucose uptake after insulin treatment or electrically induced contractions was 9.75- and 8.47-fold, respectively (2).

At this point, the two populations of GLUT4 vesicles had only been differentiated by their densities. Lund et al. therefore conducted experiments to determine the nature of the signaling pathways controlling the trafficking of these two vesicle populations (3). Comparing insulin treatment and electrically stimulated contractions, the addition of the compound wortmannin only inhibited the glucose uptake following insulin treatment (3). Wortmannin is a fungal metabolite that primarily inhibits a subclass of signaling proteins called phosphoinositide 3-kinases (PI3Ks). One of the many roles PI3Ks fulfill is the control of intracellular vesicle trafficking; therefore, the specific effect of wortmannin on insulin-induced GLUT4 vesicle fusion is quite significant. It is not only consistent with the evidence that the two membrane vesicle populations are distinct, but also that they are controlled by separate mechanisms.

Indeed, the quantitative evidence supporting two pools of GLUT4 is significant, but there's nothing like qualitative verification—seeing is believing. Using isolated rat muscle, Ploug et al. (4) conducted several light and electron microscopy studies on tissue that was untreated (basal state)(4); the images are pretty amazing. Using immuoflourescence techniques to specifically visualize the location of GLUT4 in the preparations, it becomes apparent that the protein is arranged in very interesting patterns at the muscle cell surface, just below it and in the core of the fiber. At and near the surface, GLUT4 is arranged in either quite regularly spaced string-like structures parallel to the long fiber axis or in more amorphous arrangements depending on the point of contact of blood vessels (4). The parallel structures are maintained in the core as well, though perpendicular strings can also be seen with equivalent spacing as a sarcomere (the single actin/myosin muscle unit)(4). Upon higher magnification the GLUT4 can be described as being present on either large, intensely stained structures or finer, punctate and tubular elements (4). Using electron microscopy and immunogold labeling procedures, the localization of GLUT4 was further characterized. The larger structures correspond to the Golgi, while the other features appear to be endosomal membrane vesicles and tubulovesicular bodies (4).

Upon exposure to a mixture of insulin and glucose or electrically induced contractions, GLUT4 can be seen to accumulate at the cellular membrane and the tubules at the expense of material from both the large and small intracellular stores (Golgi and endosomal membranes) (4). Additional characterization of the small endosomal membrane vesicles by Ploug et al. revealed that they fall into two subclasses: those that have the transferrin receptor (TfR) and those that do not (4). The TfR is another protein that resides within membranes, and it is often observed to cycle between the endosomal and cellular membranes. For the purpose of this article, it is only important to use TfR as a molecular marker for a specific type of membrane vesicle rather than focus on what TfR is doing biologically. Double-labeling experiments further show that the endosomal membranes containing the TfR partially overlap with those containing GLUT4 in the basal state (4). Interestingly, the TfR only accumulates at the cellular membrane after muscle contractions, not insulin treatment (4). In fact, quite the opposite occurs after insulin treatment: the endosomal co-localization of GLUT4 and TfR increases (4).

This paper has a couple of significant conclusions. First, the confidence with which the intracellular compartments containing GLUT4 can be identified has moved from the realm of fractionation to actual visualization. Second, the two distinct intracellular pools of GLUT4 that fuse with the cellular membrane after different stimuli are molecularly defined as TfR positive or negative. Combined with the previous papers, we are coming to a greater understanding of what differentiates the two pathways and the membranes involved. However, how does a cell establish and keep these two populations separate?

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Within a cell there are numerous compartments, each separated by distinct lipid membranes. The compartments are defined for not only their contents, but also for the constituents of their respective membranes. The study of how these specific compartments arise and are maintained despite a significant shared flux between them is a field called "membrane trafficking." This is a vast area of study, with researchers around the globe doing experiments to answer these questions in even finer detail. For this article, suffice it to say that each compartment contains within its membrane protein tags that help to define it as well as target its movement and fusion to other compartments.

Randhawa et al. (5) looked at the role two such protein tags have on the insulin-dependent appearance of GLUT4 at the cellular membrane. Rather than using excised rat muscle as in the above studies, Randhawa et al. used rat muscle cells grown on tissue culture plates (5). These L6 myoblasts were used because of the simplicity with which they can be manipulated. Treating the L6 myoblast cells with tetanus toxin prevented insulin from triggering an increase in GLUT4 at the cellular membrane (5). Tetanus toxin is a neurotoxin that degrades the membrane-embedded protein tags. In order to figure out which tag is responsible for the insulin-dependent GLUT4 trafficking, genes for toxin-resistant tags were added back to the cells and the experiment was repeated (5). In those cells receiving the tag called vesicle-associated membrane protein 2 (VAMP2), the translocation of GLUT4 was rescued; the presence of a different tag, VAMP3, did not have the same result (5).

Acknowledging the results of Ploug et al. (4), Randhawa et al. hypothesize that the insulin-sensitive membrane vesicles are defined by the presence of VAMP2 and perhaps the absence of TfR, while the contraction-sensitive TfR membrane vesicles have a different resident tag, maybe VAMP3 (5). This hypothesis was not directly tested by Randhawa et al., and indeed VAMP3 appears not to be absolutely required for contraction-induced GLUT4 trafficking (6). A strain of mice was genetically altered to be lacking VAMP3, and in these animals, exercise-stimulated glucose uptake was not significantly different from normal mice (6). This type of experiment can be misleading, however; animals with genetic alterations often engage compensatory mechanisms that do not relate to what exists in normal animals to overcome the deficiency. Perhaps another VAMP family member takes over for the loss of VAMP3 in this extreme scenario. Final elucidation as to the differences between the vesicles will require more research.

The Human Angle

The papers I have chosen above used rat muscle (or cells) in their studies. Although the rat-to-human connection in many studies is taken for granted, I want to include at least one article demonstrating an overall similar phenomenon in human muscle cells. Thorell et al. (7) used human subjects in their experiments and showed exercise and insulin treatment caused an increase in GLUT4 transporters at the cellular membrane in humans as well (7). In their study, seven males and two females underwent exercise and/or an insulin and glucose infusion followed by experiments on muscle biopsies from the vastus lateralus (7). In humans as well, exercise and insulin treatment caused an increase in GLUT4 transporters at the cellular membrane (7). Subsequent research with human muscle biopsies or human-derived cell culture will hopefully someday match the detailed conclusions concerning GLUT4 trafficking that have been found in rats.

Although the elucidation of the intracellular trafficking of GLUT4 is basic research, knowing it can inform everyday decisions we make. For instance, from these results it becomes more apparent why post-WOD carbohydrate does not produce as large an insulin spike as you might expect and yet the sugar is still efficiently imported into cells. Similarly, it now may be clearer why self-management of diabetes prescribes regular aerobic and resistance exercises with a general goal of increasing lean muscle mass. Muscle contraction is an alternate way of getting glucose out of the bloodstream rather than relying solely on a prescription drug (like thiazolidinediones) that may have negative side effects.

References:

1. Douen AG, Ramlal T, Rastogi S, Bilan PJ, Cartee GD, Vranic M, Holloszy JO and Klip A. Exercise induces recruitment of the "insulin-responsive glucose transporter." Evidence for distinct intracellular insulin- and exercise-recruitable transporter pools in skeletal muscle. *J Biol Chem.* 265(23): 13427-30, 1990. PubMed PMID: 2199436.

2. Brozinick JT Jr., Etgen GJ Jr., Yaspelkis BB 3rd, and Ivy JL. The effects of muscle contraction and insulin on glucose-transporter translocation in rat skeletal muscle. *Biochem J.* 297(Pt. 3): 539-45, 1994. PubMed PMID: 8110191.

3. Lund S, Holman GD, Schmitz O and Pedersen O. Contraction stimulates translocation of glucose transporter GLUT4 in skeletal muscle through a mechanism distinct from that of insulin. *Proc Natl Acad Sci USA* 92(13): 5817-21, 1995. PubMed PMID: 7597034.

4. Ploug T, van Deurs B, Ai H, Cushman SW, and Ralston E. Analysis of GLUT4 distribution in whole skeletal muscle fibers: identification of distinct storage compartments that are recruited by insulin and muscle contractions. *J Cell Biol.* 142(6): 1429-46, 1998. PubMed PMID: 9744875.

5. Randhawa VK, Bilan PJ, Khayat ZA, Daneman N, Liu Z, Ramlal T, Volchuk A, Peng XR, Coppola T, Regazzi R, Trimble WS, and Klip A. VAMP2, but not VAMP3/cellubrevin, mediates insulin-dependent incorporation of GLUT4 into the plasma membrane of L6 myoblasts. *Mol Biol Cell* 11(7): 2403-17, 2000. PubMed PMID: 10888677.

6. Yang C, Mora S, Ryder JW, Coker KJ, Hansen P, Allen LA, and Pessin JE. VAMP3 null mice display normal constitutive, insulin- and exercise-regulated vesicle trafficking. *Mol Cell Biol* 21(5): 1573-80, 2001. PubMed PMID: 11238894

7. Thorell A, Hirshman MF, Nygren J, Jorfeldt L, Wojtaszewski JF, Dufresne SD, Horton ES, Ljungqvist O, and Goodyear LJ. Exercise and insulin cause GLUT4 translocation in human skeletal muscle. *Am J Physiol* 277 (4 Pt. 1): E733-41, 1999. PubMed PMID: 10516134.

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Image courtesy of Danell Marks

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Dr. Jonathan Gary is a CrossFit Level 1 and CrossFit Kids trainer and satellite BrandXer. He is also a member of the CrossFit Kids HQ staff and the CrossFit Kids Training Course presentation team. Jon received his B.A. in biology from Northwestern University and his Ph.D. from UCLA in molecular biology. He is a principal scientist at a biotech company in San Diego, where he lives with his wife and dog. He has been CrossFitting since late 2003 after being introduced to it by Jeff Martin.

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