Topical Review

Glucose, exercise and insulin: emerging concepts

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(Received 30 May 2001; accepted after revision 13 July 2001)

Physical exercise induces a rapid increase in the rate of glucose uptake in the contracting skeletal muscles. The enhanced membrane glucose transport capacity is caused by a recruitment of glucose transporters (GLUT4) to the sarcolemma and t-tubules. This review summarises the recent progress in the understanding of signals that trigger GLUT4 translocation in contracting muscle. The possible involvement of calcium, protein kinase C (PKC), nitric oxide (NO), glycogen and AMP-activated protein kinase (AMPK) are discussed. Furthermore, the possible mechanisms behind the well-described improvement of insulin action on glucose uptake and glycogen synthase activity in the post-exercise period is discussed. It is concluded that both during and following muscle contractions, glycogen emerges as an important modulator of signalling events in glucose metabolism.

Glucose uptake in muscle is a function of different regulatory steps such as delivery of glucose from the blood to the interstitial space, transmembrane transport from the interstitial space to the inside of the muscle cell and intracellular metabolism of the glucose. Each step may be rate limiting under specific circumstances. In most cases transmembrane glucose transport is considered to be the limiting step. In reality it is often difficult to discern which step is limiting because mostly more than one step is altered by any given intervention (Halseth *et al.* 1998). For instance, exercise increases muscle membrane glucose transport capacity but at the same time increases glucose delivery by increasing muscle blood flow and also increases enzymatic activity related to glucose metabolism. In the present review we will discuss new insights into the mechanisms regulating glucose uptake in skeletal muscle during exercise and in the recovery period after exercise.

Contraction signalling of muscle glucose uptake

Stimulation of glucose transport in contracting muscles involves a plasma membrane- and t-tubule-directed mobilisation of GLUT4-containing vesicles from exercise-sensitive intracellular storage sites (Douen *et al.* 1989; Marette *et al.* 1992; Coderre *et al.* 1995; Ploug *et al.* 1998; Dohm & Dudek, 1998). Other glucose transporter isoforms such as GLUT1 and 5 are expressed at much lower levels than GLUT4 in skeletal muscle. Their role in contraction-induced glucose uptake is negligible as judged from the virtual absence of contraction-induced glucose transport in muscles from mice in which GLUT4 is not expressed (Ryder *et al.* 1999; Zisman *et al.* 2000).

Proximal signalling leading to contraction-induced muscle glucose uptake is at the moment under intense investigation by many research groups. Several candidates are emerging, from the oldest candidate, calcium, to several newer ones including protein kinase C (PKC), glycogen, adenosine, nitric oxide (NO) and 5′-AMP-activated protein kinase (AMPK). It is probably naive to believe that contraction-induced muscle glucose transport is regulated only through the action of one signalling pathway. Rather, glucose transport is the result of the interaction of several signalling pathways that are activated to different extents according to the prevailing metabolic needs of the muscle. As regards distal elements in the signalling cascade leading to mobilisation of GLUT4 from large storage membrane compartments, recent evidence supports the involvement of phospholipase D in this process (Kristiansen *et al.* 2001), although it is not clear whether it is involved in contraction signalling.

A role for glycogen? It has repeatedly been shown that the level of glycogen in skeletal muscle exerts a regulatory effect on glucose uptake during muscle contractions. *In vitro* this was first shown by Richter & Galbo (1986) and Hespel & Richter (1990), and the effect of glycogen was found at the glucose transport step (Hespel & Richter, 1990). Subsequent studies showed that surface membrane GLUT4 protein content after

contractions (Derave *et al.* 1999) was negatively associated with initial muscle glycogen levels, indicating that contraction-induced translocation of GLUT4 to the surface membrane was influenced by muscle glycogen levels (see Fig. 1). However, the effect of glycogen was restricted to the fast-twitch fibres and was not demonstrable in the slow-twitch soleus muscle (Derave *et al.* 1999). Also in humans, evidence for regulation of muscle glucose uptake during exercise by glycogen is available (Gollnick *et al.* 1981) although other studies demonstrated less convincing effects (Hargreaves *et al.* 1995). Recent studies in our laboratory have, however, clearly shown that muscle glucose uptake during exercise is markedly higher in the glycogen-depleted state compared to when muscles are glycogen loaded (Richter *et al.* 2001). Thus, leg glucose clearance during 1 h of bicycling at 70% of maximum oxygen consumption rate ($\dot{V}_{O_2,max}$) was twice as high when subjects commenced exercise with an average muscle glycogen content of 185 μmol (g dry wt)$^{-1}$ than when glycogen content was 800 μmol (g dry wt)$^{-1}$.

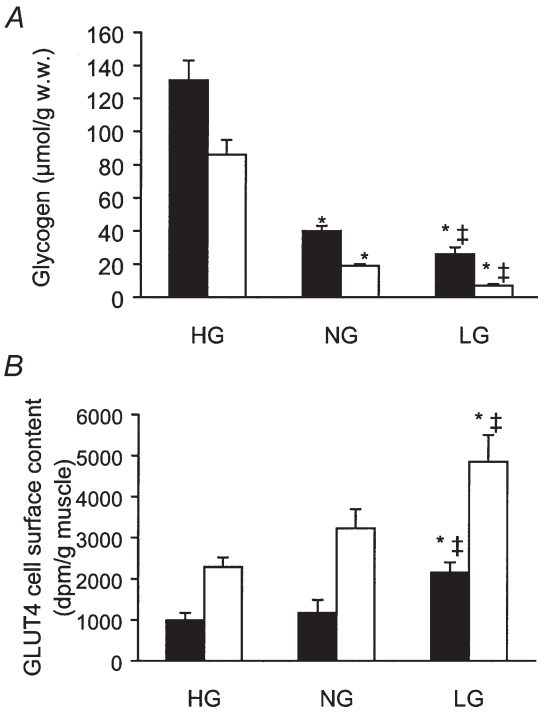

Figure 1. Glycogen content (*A*) and GLUT4 cell surface content (*B*) in plantaris muscles at rest (■) and after contractions (□)

Rats were pre-treated by swimming and diet to obtain muscles with high (HG), normal (NG) or low (LG) glycogen content. Hindlimbs were perfused and the calf muscles of one leg were electrically stimulated (100 ms trains with 2 s intervals) for 10 min. Plantaris muscles were dissected out of the rested and electrically stimulated leg and were incubated in 2-*N*-4-(1-azi-2,2,2,-trifluoroethyl)benzoyl-1,3-bis(D-mannose-4-yloxy)-2-propylamide (ATB-BMPA) to label cell surface GLUT4. Data are presented as means ± S.E.M. ($n = 5$–8). * Different from HG ($P < 0.05$). ‡ Different from NG ($P < 0.05$). Figure is adapted from Derave *et al.* (1999) with permission.

The fact that contraction-induced as well as insulin-stimulated (see below) glucose transport and GLUT4 translocation are inhibited by high muscle glycogen levels raises the possibility that glycogen particles are directly involved in the translocation process of GLUT4-containing vesicles. It has long been hypothesised that glycogen particles are structurally attached to GLUT4 vesicles, making the latter unavailable for translocation as long as glycogen is amply present in muscle and – conversely – available for translocation as soon as glycogen is broken down. Still, despite extensive efforts by us and others, no study has so far been able to provide biochemical evidence for such a physical link. It is therefore possible that glycogen exerts its regulatory role on glucose transport by interacting on the activation of signal transduction pathways, as will be discussed below.

Feed-forward control by Ca^{2+}. It was established years ago that the rise in intracellular [Ca^{2+}] as a result of membrane depolarisation is a contributing factor to enhanced glucose uptake during muscle contractions (Holloszy & Narahara, 1967). Thus, Ca^{2+} probably activates a signal transduction pathway leading to GLUT4 translocation. Several protein kinases, such as the conventional and novel PKC isoforms (cPKC and nPKC isoforms), are Ca^{2+} sensitive and could therefore serve as signalling intermediates for contraction-induced glucose transport. It has been known for some time that muscle contraction is associated with translocation of PKC from the cytosol to the particulate fraction (Richter *et al.* 1987; Cleland *et al.* 1989), and recently it was clarified that inhibition of PKC by calphostin C inhibits contraction- but not insulin-stimulated glucose transport (Wojtaszewski *et al.* 1998; Ihlemann *et al.* 1999*a*). Which of the PKC isoforms is/are involved is still unclear, but cPKC-β has recently been suggested to be involved in Ca^{2+}-dependent stimulation of muscle glucose transport (Khayat *et al.* 1998; Kawano *et al.* 1999). The cPKC isoforms also require diacylglycerol (DAG) for optimal activation. Several studies indicate that the intracellular concentration of diacylglycerol is increased during contraction (Cleland *et al.* 1989) and that it may play a role in stimulation of glucose transport since phorbol esters, which are functional analogues of DAG, may stimulate muscle glucose transport (Hansen *et al.* 1997).

The metabolic feedback signal. If glucose transport were only activated by a feed-forward Ca^{2+}-sensitive mechanism, the regulatory and adaptive capacity of the system would be limited. Rather than events occurring early in the excitation–contraction coupling, parameters of energy status, fuel depletion, tension development and fatigue may be more accurate indicators of the need for glucose inside the muscle and the degree to which glucose transport stimulation should be activated. Ihlemann *et al.*

(1999*b*, 2000) showed that glucose transport rate in response to muscle contractions in incubated rat soleus is more dependent on the tension development (metabolic stress) than on the stimulation frequency. This indicates that metabolic stress is monitored during muscle contractions and leads to acceleration or deceleration of glucose transport rate. This function is possibly fulfilled by 5′-AMP-activated protein kinase (AMPK). This kinase is activated during muscle contractions (Winder & Hardie, 1996; Hutber *et al.* 1997), and its activation is larger the lower the ATP/ADP ratio, CrP/Cr ratio and glycogen concentration become during contractions, which makes it a valuable intracellular fuel gauge (Hardie & Carling, 1997; Ponticos *et al.* 1998; Derave *et al.* 2000*a*). Moreover, stimulation of AMPK by 5-aminoimidazole-4-carboxamide ribonucleoside (AICAR) activates glucose transport in resting rat muscle *in vivo* and *in vitro* (Merrill *et al.* 1997; Bergeron *et al.* 1999). This AICAR-activation of glucose transport is wortmannin insensitive and is additive to the effects of insulin, but not to that of contraction (Hayashi *et al.* 1998), indicating that AICAR mimics the effect of contractions on glucose transport. This has therefore rendered AMPK a likely candidate for metabolic signalling to glucose transport. Still, not all studies support a role for AMPK in glucose transport stimulation. Recent data in incubated rat epitrochlearis muscle indicate that AICAR and contractions have partially additive effects on glucose transport (T. Ploug, personal communication). Furthermore, evidence has been presented that glucose transport activation in glycogen-loaded contracting slow-twitch rat muscles can occur in the absence of measurable AMPK activation, indicating that – at least in slow-twitch fibres – AMPK is not essential for glucose transport stimulation (Derave *et al.* 2000*a*). Presently, no specific pharmacological inhibitors of contraction-stimulated AMPK activity are available to clarify the role of AMPK in contraction signalling to glucose transport. Musi *et al.* (2001) recently showed that 9-β-D-arabinofuranoside and iodotubercidin are potent inhibitors of AICAR-stimulated α2-AMPK activity and glucose transport in isolated rat epitrochlearis muscles, but these compounds had no effect on contraction-stimulated AMPK activity. More definitive answers, however, can be provided by experiments with AMPK-deficient animals. Thus, it was recently shown in mouse muscle that overexpression of a dominant negative mutant of AMPK completely blocked the effect of hypoxia and partially (~30–40 %) blocked the effect of muscle contractions on glucose transport (Mu *et al.* 2001). This might indicate that AMPK activation is partially involved in contraction-induced glucose transport, but its involvement is limited to relatively intense contractions/exercise during which some degree of hypoxia occurs and the CrP/Cr ratio and possibly the ATP/AMP ratio decrease.

Recently, several studies have investigated the activity of AMPK in human working muscle. Fuji *et al.* (2000) and Wojtaszewski *et al.* (2000*b*) showed that 1 h of bicycle exercise at an intensity of 75 % of $\dot{V}_{O_2,max}$ increases the activity of the α2- but not the α1-isoform of AMPK in human thigh muscle. The activity was still increased 30 min following the exercise bout, but had returned to baseline after 3 h of recovery. Exercise at lower intensity (50 % of $\dot{V}_{O_2,max}$) did not activate either of the AMPK isoforms (Fujii *et al.* 2000; Wojtaszewski *et al.* 2000*b*). Exercise at higher intensity (supramaximal all-out bicycling for 30 s), however, seems to activate both α1 and α2 isoforms of AMPK in human thigh muscle (Chen *et al.* 2000). By analogy, some studies with rat muscles showed that both α1 and α2 isoforms of AMPK are activated during contractions *in vitro* (Hayashi *et al.* 2000), whereas others found that only α2-AMPK is activated during contractions *in situ* (Vavvas *et al.* 1997). In conclusion, with progressive exercise intensities in humans, increasing muscle glucose uptake rates (Katz *et al.* 1986) are associated with progressive activation of AMPK isoforms. Whether there is any causal link between AMPK activation and glucose transport during exercise in humans is, however, not clear at present. Since in humans application of molecular biology techniques and specific blockers of AMPK is not possible, the types of experiments available to establish a link between AMPK activation and glucose uptake in man are less definitive. One approach is to examine whether exercise-induced AMPK activation correlates with exercise-induced muscle glucose uptake. Such a recent experiment failed to provide support for AMPK in the regulation of glucose uptake in humans during exercise because no correlation was found between AMPK activity in muscle and glucose uptake during bicycling exercise at 70 % of $\dot{V}_{O_2,max}$ (Richter *et al.* 2001).

Nitric oxide. With respect to glucose transport, nitric oxide synthase (NOS) may play a role in signalling to GLUT4 translocation. In fact it has been suggested that AMPK signals to increased glucose transport via activation of NOS (Fryer *et al.* 2000). This conclusion was based on experiments in cell culture as well as in incubated rat muscle. Furthermore AMPK can phosphorylate and activate endothelial NOS (eNOS) in rat hearts (Chen *et al.* 1999). In human skeletal muscle, phosphorylation of neuronal NOS (nNOSμ) has been observed when AMPK is activated during exercise (Chen *et al.* 2000). Activated NOS increases NO production in muscle. Indeed, rat experiments have shown that acute exercise increases NOS activity and NO release (Balon & Nadler, 1994; Roberts *et al.* 1999). In line with this hypothesis, it would then be expected that inhibition of NOS attenuates exercise-induced glucose uptake. However, conflicting opinions exist about this issue in the literature. The administration of a NOS inhibitor (N^G-nitro-L-arginine methyl ester; L-NAME) during dynamic knee-extensor exercise in humans had no effect on muscle glucose uptake (Y. Hellsten, personal communication) whereas Bradley *et al.* (1999) report a

48% reduction in glucose uptake during bicycle exercise when N^G-monomethyl-L-arginine (L-NMMA) was used as a NOS inhibitor. When comparing rat studies, Roberts *et al.* (1997) showed that NOS inhibition completely blocks glucose transport stimulation during exhaustive treadmill running, whereas Etgen *et al.* (1997) observed no effect of NOS inhibition on glucose transport in incubated contracting epitrochlearis muscles. Higaki *et al.* (2001) have suggested that the mechanism by which NO stimulates glucose transport is distinct from the exercise/contraction mechanism. This suggestion was based on their recent findings that (1) the effect of sodium nitroprusside, a NO donor, on glucose uptake is fully additive to the effect of contractions, and (2) the NOS inhibitor L-NMMA did not inhibit contraction-induced glucose transport in isolated rat soleus and EDL muscles (Higaki *et al.* 2001). Thus, the role of NO in exercise-induced muscle glucose uptake is undefined at present.

Hypoxia as part of the contraction stimulus. For more than 40 years it has been known that hypoxia is a potent stimulus of *in vitro* glucose uptake in skeletal muscle (Randle & Smith, 1958; Özand *et al.* 1962). For some time, it was believed that muscle contraction is in fact an identical stimulus to hypoxia, based upon the observation that in incubated rat epitrochlearis muscles, the effects of contractions and hypoxia are not additive (Cartee *et al.* 1991). However, as shown in Fig. 2, more recent experiments performed with rat hindlimb perfusions have shown that contractions and hypoxia can additively stimulate muscle glucose transport (Derave & Hespel, 1999; Fluckey *et al.* 1999), although the effect was not evident in fast-twitch glycolytic muscle (Fluckey *et al.* 1999). Furthermore, when the maximal effects of the stimuli are compared, contractions appear to stimulate greater multiples of increase in muscle glucose uptake than hypoxia (Wojtaszewski *et al.* 1998). Finally, the involvement of PKC and adenosine in contraction-induced glucose transport is not evident in the hypoxia stimulus (Wojtaszewski *et al.* 1998; Derave & Hespel, 1999). It can thus be concluded from these recent studies that hypoxia can only partly mimic the contraction stimulation of muscle glucose transport *in vitro*. Since hypoxia causes decreased CrP and ATP concentrations and increased AMP concentrations, AMPK would be expected to be activated by hypoxia, which indeed has recently been demonstrated (Hayashi *et al.* 2000). As mentioned above, the pivotal role of AMPK in hypoxia-induced but much less so in contraction-induced glucose transport was recently demonstrated in mice overexpressing a dominant negative mutant of AMPK (Mu *et al.* 2001).

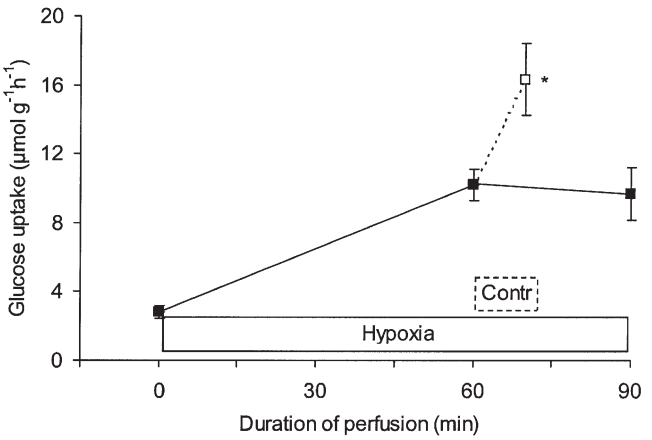

Figure 2. Additive effect of contractions and hypoxia on glucose uptake in perfused rat hindquarters

Glucose uptake was measured at rest and after 60 min of hypoxia ($n = 17$). Thereafter, hypoxic perfusion was either continued for another 30 min (continuous line and filled symbol, $n = 3$) or a 5 min electrical stimulation was started (dotted line and open symbol, $n = 14$). Values are means ± S.E.M. * $P < 0.05$ compared with 60 min of hypoxia. Data are reproduced from Derave & Hespel (1999) with permission.

Integrative hypothesis of the different factors. When muscles start to contract, at least two intracellular mechanisms cause an increased glucose transport rate through translocation of GLUT4 vesicles. The first one is dependent on the intensity and frequency of neural stimulation and is triggered by the rise in intracellular Ca^{2+}. This pathway possibly involves PKC and other unknown signalling proteins. The second pathway, which is a feedback mechanism, is activated upon metabolic stress, when muscle cells start to fail in homeostasis of ATP, CrP, glycogen and/or oxygen. This pathway is likely to involve AMPK as a monitor of energy status, and more downstream maybe NOS. The pathway is probably also activated when muscles become hypoxic and increase glucose transport. There may exist additional factors involved in contraction-induced glucose transport, such as adenosine (Vergauwen *et al.* 1994; Han *et al.* 1998), β-endorphin (Evans *et al.* 1997) and bradykinin (Kishi *et al.* 1998). However, their mechanisms are poorly understood at present. In addition to the intracellular mechanisms, an important part of the integrated response to exercise is increased muscle blood flow and capillary recruitment thereby ensuring that the interstitial glucose concentration during exercise is maintained high (MacLean *et al.* 1999).

Glucose metabolism in the post-exercise state

An increase in the metabolic action of insulin has been considered an important benefit of exercise for healthy people as well as for patients with insulin resistance. Understanding the molecular mechanism behind the phenomenon is important and it has been researched in many laboratories for years.

In the period after prolonged and heavy physical activity glycogen synthesis is of high priority for the previously exercised muscles. In accordance with this, muscle

glycogen synthase activity and glucose transport are increased following exercise. In addition, an enhanced metabolic action of insulin in skeletal muscle (glucose transport, glycogen synthase activity, glycogen synthesis) is usually also observed after exercise (Richter *et al.* 1982, 1989; Mikines *et al.* 1988; Perseghin *et al.* 1996). The duration of this period with enhanced insulin sensitivity may last up to 48 h (Mikines *et al.* 1988) probably depending upon the rate of muscle glycogen repletion (see below). Enhanced insulin sensitivity in skeletal muscle contributes to the restoration or even super-compensation of the glycogen stores.

One-legged exercise experiments *in vivo* and electrical stimulation *in vitro* have demonstrated that exercise-induced alteration in insulin action is restricted to the muscle actually performing the work (Richter *et al.* 1984, 1989). Using the *bis*-mannose surface labelling technique in rodent muscle or isolation of plasma membranes from human muscle biopsies, it has been shown that the increased insulin action after exercise on glucose transport involves enhanced recruitment of the glucose transporter protein GLUT4 to the plasma membrane (Hansen *et al.* 1998; Thorell *et al.* 1999).

Glycogen content and glucose availability *per se* exert a regulatory role in the resynthesis of glycogen after exercise. For example, during euglycaemic clamp conditions in humans, the ability of physiological levels of insulin to stimulate muscle glucose uptake after exercise is positively correlated to the amount of glycogen used during the prior exercise bout (Fig. 3) (Wojtaszewski *et al.* 1997, 2000*a*). In a more physiological setting, we also observed that food intake and accompanying hyperinsulinaemia 3 h after exercise activate glycogen synthase in an inverse relationship to the muscle glycogen content. In accordance with this, a negative correlation was also evident between glycogen content before food intake and the increase in muscle glycogen in the following 3 h (Wojtaszewski *et al.* 2001) (Fig. 4).

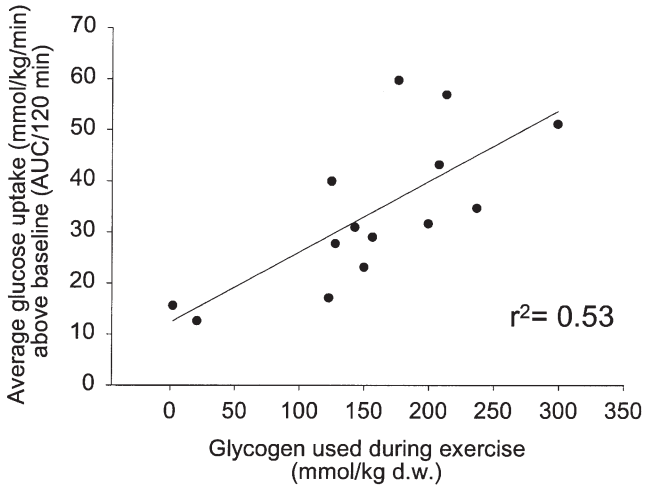

Figure 3. Correlation between the degree of glycogen depletion and the insulin-stimulated glucose uptake in thigh muscles of healthy men

Subjects performed 60 min one-legged knee-extensor exercise and glycogen depletion was measured as the difference in glycogen content between the rested and exercised leg 3–4 h following exercise. The insulin-stimulated glucose uptake was measured as the area under the curve (AUC) after baseline subtraction for glucose uptake (A–V difference × flow) in the exercised leg during a 120 min hyperinsulinaemic (~100 μu ml^{-1}) euglycaemic clamp starting 3–4 h post-exercise. $n = 14$ and $r^2 = 0.53$. Data are combined from Wojtaszewski *et al.* (1997) and Wojtaszewski *et al.* (2000*a*).

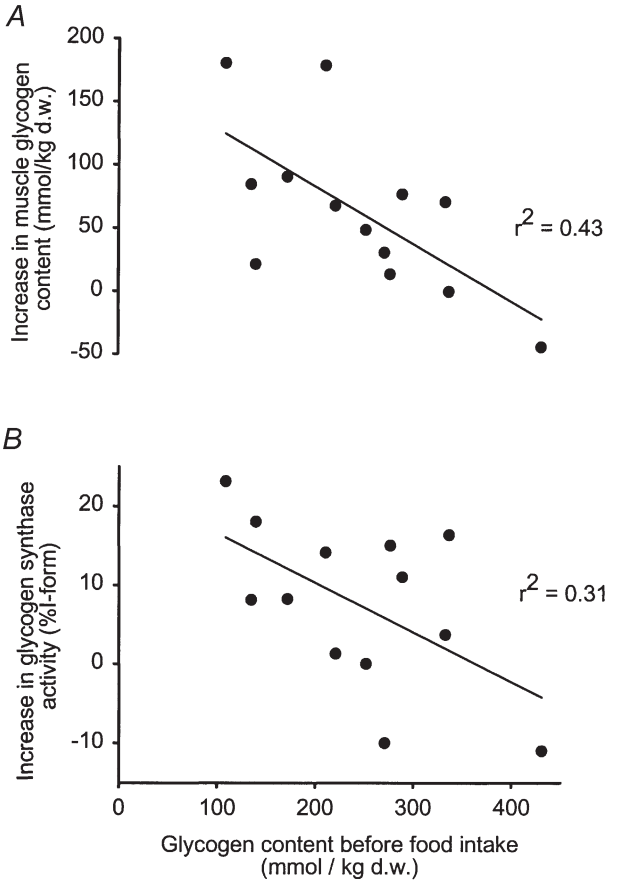

Figure 4. Correlations between the post-exercise glycogen content and the increase in muscle glycogen content (*A*) and muscle glycogen synthase activity (*B*) in response to food intake in healthy men

Subjects performed either a high (75% $\dot{V}_{O_2,max}$) or a low (50% $\dot{V}_{O_2,max}$) intensity exercise bout on a bicycle ergometer. After 3 h of rest a carbohydrate rich meal was taken, and the subjects rested for another 3 h. Biopsies from vastus lateralis 3 h after exercise (before food intake) and 3 h after food intake were analysed for glycogen content and glycogen synthase activity ($n = 13$). Data are reproduced from Wojtaszewski *et al.* (2001) with permission.

In line with these observations, we and others have recently found that the ability of a variety of factors (insulin, contractions and AICAR) to regulate glycogen synthase activity and glucose transport is increased in glycogen-depleted *vs.* glycogen-loaded fast-twitch rat muscle (Jensen *et al.* 1997; Derave *et al.* 1999; Kawanaka *et al.* 2000; Nielsen *et al.* 2001; and authors' unpublished observations). The glycogen-dependent glucose transport in response to insulin and contraction is linked to an increased cell surface localisation of GLUT4 (Derave *et al.* 1999, 2000*b*). Similarly, the changes in glycogen synthase activity may relate to a change in subcellular localisation as we have found this also to be dependent on muscle glycogen levels (Nielsen *et al.* 2001).

These above-mentioned observations indicate that the glycogen content after exercise or the amount of glycogen used during exercise is linked to the enhanced metabolic action of insulin in the following period. Studies in rodents have shown that the reversal of the enhanced insulin sensitivity to stimulate muscle glucose transport is also linked to glucose metabolism. Thus, carbohydrate deprivation in the post-exercise period is associated with a prolonged increase in insulin sensitivity compared to the carbohydrate fed state (Cartee *et al.* 1989). In fact, replacement of glucose by the non-metabolised 2-deoxy-glucose analogue in the incubation medium significantly prolonged the period of increased insulin sensitivity (Gulve *et al.* 1990). In contrast, muscle incubation in conditions conducive to glucose uptake results in a faster normalisation of muscle insulin sensitivity (Gulve *et al.* 1990).

Both the increased recruitment of GLUT4 and activation of glycogen synthase after exercise could theoretically be explained by changes in the cellular signalling events activating these processes. Since exercise or contractions activate these metabolic processes in the absence of insulin, several mechanisms could be involved at different time points after exercise. We have conducted experiments looking at the cellular insulin signalling at a point where many acute effects of exercise are no longer present. Three to four hours after a single bout of exercise, no differences in insulin-induced signalling in human muscle could account for the increased metabolic action of insulin assessed during euglycaemic hyper-insulinaemic clamp conditions. Thus, at this time point, insulin receptor activation, insulin receptor substrate 1 (IRS-1) phosphorylation, IRS-1-associated phosphatidyl-inositol 3-kinase (PI3K) activation, Akt activation and phosphorylation, as well as glycogen synthase kinase 3 (GSK3) phosphorylation and inactivation are not increased in exercised compared to rested muscle (Wojtaszewski *et al.* 1997, 2000*a*, 2001). In fact, a decreased IRS-1-associated PI3K activation was evident in these studies. Similarly, in rodent experiments, insulin signalling following exercise was either unchanged or decreased at the level of the insulin receptor and IRS-1 (Goodyear *et al.* 1995; Hansen *et al.* 1998). At least within the first 3–4 h after exercise, changes in metabolic action also precede measurable changes in expression of key proteins (Wojtaszewski *et al.* 2000*a*). However, at later time points increased expression of key proteins in the insulin-signalling cascade may play a role (Chibalin *et al.* 2000; Wadley *et al.* 2001). Still, so far no study has shown a relationship between changes in insulin signalling and the metabolic effects of insulin a few hours after exercise.

Also the mechanism by which glycogen affects post-exercise insulin sensitivity is unclear. Thus, in the studies of glycogen-depleted *vs.* glycogen-loaded rat muscle, an increased ability of insulin to activate Akt and of contractions and AICAR to activate AMPK is evident in the glycogen-depleted muscle (Derave *et al.* 2000*a*,*b*; Kawanaka *et al.* 2000; and authors' unpublished observations). Thus, glycogen can clearly affect cellular signalling, but since changes in insulin signalling are not seen in the post-exercise period, the mechanisms by which glycogen affects insulin action in the post-exercise period are unclear. Finally, it should be noted that *in vivo*, increased capillary recruitment may be an important part of the effect of insulin in increasing muscle glucose uptake both at rest and in the post-exercise period (Rattigan *et al.* 1997).

When insulin is administrated immediately after treadmill running, an enhanced insulin-activated signalling has been observed in rat muscle at the level of phosphotyrosine-associated PI3K activity and in human muscle at the level at Akt-Ser^{473} phosphorylation (Zhou & Dohm, 1997; Thorell *et al.* 1999). At first, this could be due to an enhanced flow and insulin delivery in the exercised compared to the rested state. However, neither insulin receptor (IR) tyrosine phosphorylation nor IRS-associated PI3K activity was affected under these conditions, indicating that another, as yet unknown, tyrosine-phosphorylated protein may associate with PI3K (Zhou & Dohm, 1997; Wojtaszewski *et al.* 1999). Even mice lacking the insulin receptor in the skeletal muscle display enhanced effects of insulin after treadmill exercise at downstream (but not upstream) signalling events (Akt and GSK3) and glucose transport (Wojtaszewski *et al.* 1999). Although the mechanism by which this occurs is still unknown, it illustrates the point that neither IR nor IRS-1 apparently take part in the alterations necessary for enhanced metabolic action immediately after exercise. Nevertheless, the signalling alterations are not long lasting and thus cannot account for the prolonged increase in insulin sensitivity after exercise. For example, insulin signalling is apparently normal 3–4 h after exercise in humans (Wojtaszewski *et al.* 1997, 2000*a*, 2001), 3–4 h after swimming (Hansen *et al.* 1998) or running exercise in rats (Richter *et al.* 1982) and 30 min after treadmill exercise in mice (Wojtaszewski *et al.* 1999; J. F. P. Wojtaszewski & L. J. Goodyear, unpublished observations).

Conclusion

During contractions, muscle glucose transport seems to be regulated by a feed-forward Ca^{2+}-dependent signal as well as a metabolic feedback signal possibly via AMPK. Contraction-, AICAR- and insulin-stimulated glucose transport are, however, influenced by muscle glycogen content. Yet, the molecular mechanism behind the effect of glycogen is not well characterised. So far, it has been shown in rats that a high glycogen content may decrease Akt activation upon insulin stimulation and decrease AMPK activation during muscle contractions and AICAR treatment. In the post-exercise period increased insulin sensitivity is observed. It is noteworthy that at least after the immediate post-exercise period this is not related to increased proximal insulin signalling but is dependent upon muscle glycogen levels. The lower the glycogen content, the stronger the response to insulin. Muscle glycogen content thus emerges as an important regulator of contraction- and insulin-induced muscle glucose transport in addition to its well-known effect on glycogen synthase activity. In skeletal muscle glycogen content influences glycogen synthase subcellular localisation as well as activity (Nielsen *et al.* 2001). Glycogen content may therefore also influence subcellular localisation and thereby possibly the activity of signalling intermediates in glucose transport stimulation – a research area that deserves increased attention.

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Acknowledgements

This work was supported by grants from the Danish National Research Foundation (no. 504-14) and from Novo-Nordisk. W.D. is a postdoctoral fellow of the Fund for Scientific Research–Flanders (Belgium) (F.W.O.–Vlaanderen). J.F.P.W. is a postdoctoral fellow of the Danish Medical Science Research Council.

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