Original Articles

Dissociation of AMP-Activated Protein Kinase Activation and Glucose Transport in Contracting Slow-Twitch Muscle

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5′AMP-activated protein kinase (AMPK) has been suggested to be a key regulatory protein in exercise signaling of muscle glucose transport. To test this hypothesis, we investigated whether muscle glycogen levels affect AMPK activation and glucose transport stimulation similarly during contractions. Rats were preconditioned by a combination of swimming exercise and diet to obtain a glycogen-supercompensated group (high muscle glycogen content [HG]) with ~3-fold higher muscle glycogen levels than a glycogen-depleted group (low muscle glycogen content [LG]). In perfused fast-twitch muscles, contractions induced significant increases in AMPK activity and glucose transport and decreases in acetyl-CoA carboxylase (ACC) activity in both HG and LG groups. Contraction-induced glucose transport was nearly 2-fold ($P < 0.05$) and AMPK activation was 3-fold ($P < 0.05$) higher in the LG group compared with the HG group, whereas ACC deactivation was not different between groups. Thus, there was a significant positive correlation between AMPK activity and glucose transport in contracting fast-twitch muscles ($r = 0.80$, $P < 0.01$). However, in slow-twitch muscles with HG, glucose transport was increased 6-fold ($P < 0.05$) during contractions, whereas AMPK activity did not increase. In contracting slow-twitch muscles with LG, the increase in AMPK activity (315%) and the decrease in ACC activity (54 vs. 34% at 0.2 mmol/l citrate, LG vs. HG) was higher ($P < 0.05$) compared with HG muscles, whereas the increase in glucose transport was identical in HG and LG. In conclusion, in slow-twitch muscles, high glycogen levels inhibit contraction-induced AMPK activation without affecting glucose transport. This observation suggests that AMPK activation is not an essential signaling step in glucose transport stimulation in skeletal muscle. *Diabetes* 49:1281–1287, 2000

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2DG, 2-deoxy-glucose; ACC, acetyl-CoA carboxylase; AICAR, 5-aminoimidazole-4-carboxamide ribonucleoside; AMPK, 5′AMP-activated protein kinase; CrP, creatine phosphate; HG, high muscle glycogen content; LG, low muscle glycogen content.

The ability of exercise to stimulate muscle glucose uptake is intact in patients with peripheral insulin resistance, obesity, and type 2 diabetes (1). Therefore, a better insight into the mechanism of contraction-induced glucose transport may lead to the development of drugs to help treat peripheral insulin resistance. It is known that muscle contractions evoke the translocation of GLUT4 glucose transporters from intracellular storage sites to the muscle surface membrane, thereby increasing the glucose uptake rate of the muscle cell. It has been found that the GLUT4 storage compartments that are recruited by contractions are different from those recruited by insulin (2–4). The signal that leads to contraction-induced GLUT4 translocation is also known to be different from the insulin signal (5), but little is known about which proteins are involved in the contraction-induced signaling. Recently, however, it has been proposed that 5′AMP-activated protein kinase (AMPK) is a key mediator of the contraction signal, based on the observation that activation of AMPK by 5-aminoimidazole-4-carboxamide ribonucleoside (AICAR) is paralleled by an increase in muscle glucose uptake (6) and GLUT4 translocation (7). In addition, AICAR and contractions stimulate glucose transport in a similar insulin- and wortmannin-independent manner, and the stimuli are not additive (8). It was therefore suggested that AMPK is involved in the exercise signal for glucose transport activation. The next logical step to test this hypothesis would be to investigate whether inhibition of AMPK during contractions results in reduced glucose transport rates. Although the unspecific protein kinase inhibitor iodotubercidin (9) and adenine 9-β-D-arabinofuranoside have been used for this purpose (10), specific AMPK inhibitors have not yet been identified. When searching for an alternative to chemical inhibition of AMPK, we hypothesized that contraction-induced AMPK activation can be modulated by alteration of pre-exercise muscle glycogen levels. AMPK is activated by a decrease in ATP:AMP and creatine phosphate (CrP):Cr ratios (11–13). Loading the cell abundantly with glycogen before exercise might reduce the contraction-induced decrease in these ratios and concomitantly the activation of AMPK. Conversely, muscle glycogen depletion before contractions could possibly intensify the contraction-induced activation of AMPK. If AMPK is an essential intermediary in the exercise signaling pathway, then changes in exercise-induced AMPK activation are expected to be paralleled by changes in glucose transport.

AMPK activity can be measured with an in vitro phosphorylation assay using SAMS peptide as a substrate. The assay reflects the phosphorylation status of the enzyme (14). However, because AMPK is also allosterically activated, it is possible that a more complete picture of the in vivo activity of AMPK can be obtained by also assaying the activity of acetyl-CoA carboxylase (ACC). This is so because active AMPK inhibits ACC activity (15), which then can be considered as an intracellular reporter of AMPK activity in vivo.

We have previously shown the importance of muscle glycogen levels in regulating the magnitude of contraction-induced glucose transport in skeletal muscle (16). In addition, in fast-twitch muscles with high glycogen content, GLUT4 translocation in response to contraction has been shown to be diminished compared with muscles with normal glycogen content, whereas in muscles with low glycogen content, contraction-induced GLUT4 translocation was enhanced compared with muscles with normal glycogen content (17). In line with the above-mentioned reasoning, it is tempting to speculate that in fast-twitch muscles, glycogen regulates contraction-induced glucose transport through AMPK.

In the present study, we have altered muscle glycogen levels in rats by a combination of swimming exercises and diet. We have investigated whether different muscle glycogen levels affect contraction-induced glucose transport and AMPK and ACC activity in slow-twitch and fast-twitch muscles of the perfused rat hind limb preparation.

RESEARCH DESIGN AND METHODS

Animals. All experiments were approved by the Danish Animal Experiments Inspectorate. Male Wistar rats (200–250 g) were preconditioned, as described previously (16), to obtain 2 subgroups with varying muscle glycogen concentrations. In short, rats were subjected to 2 h of swimming in water maintained at 32–35°C with weights (~4% of body weight) attached to their tails. After swimming, they were fed ad libitum with either butcher's lard and tap water (low muscle glycogen content [LG]) or normal rat diet, tap water, and a 20% glucose drinking solution (high muscle glycogen content [HG]). Rats were perfused between 18 to 24 h after the swimming bout and were deprived of food 3–5 h before perfusions.

Perfusion procedure. The rats were anesthetized by an intraperitoneal injection of pentobarbital sodium (5 mg/100 g body wt). Surgery was performed as described by Ruderman et al. (18) for isolated hindquarter perfusion. All perfusions were carried out using a cell-free perfusate consisting of Krebs-Ringer bicarbonate buffer solution, 4% bovine serum albumin (fraction V; Sigma, St. Louis, MO), 0.15 mmol/l pyruvate, and 4.2 U/ml heparin, as previously described (19). For measurement of glucose transport, 8 mmol/l of 2-deoxyglucose (2DG) (Sigma) and 1 mmol/l mannitol (Sigma) together with radioactive-labeled tracers 2-[2,6-^{3}H]deoxy-D-glucose (specific activity 51 Ci/mmol) and D-[1-^{14}C]mannitol (specific activity 57 mCi/mmol) (Amersham International, U.K.) yielding an activity of 0.075 and 0.05 μCi/ml, respectively, were added to the medium. The perfusion medium (200 ml) was constantly gassed with a mixture of 95% oxygen–5% carbon dioxide, and the perfusion flow was maintained at 20 ml/min throughout all perfusions. With respect to the viability of the presently used muscle preparation, we have previously shown that muscle ATP and creatine phosphate values during 45 min of basal perfusion with a cell-free medium do not change compared with values obtained from rested anesthetized rats (20). During the initial equilibration period, both legs were perfused for 10 min, after which the left common iliac artery and vein were ligated and muscles from the left leg were freeze-clamped to obtain resting values. After 5 min of one-leg perfusion, the right leg was made to contract isometrically for 10 min by electrical stimulation of the sciatic nerve with supramaximal trains (25 V) of 100 ms of duration at 2-s intervals and an impulse duration and frequency within the train of 0.1 ms and 100 Hz, respectively. During the last 4 min of contractions, the hind limb was perfused with the hexose- and tracer-containing perfusate without recirculation. Immediately after perfusion, the white most superficial part of the gastrocnemius (0% slow-twitch fibers), the red deep medial portion of gastrocnemius (~50% slow-twitch fibers), and the soleus (>80% slow-twitch fibers) were cut out and freeze-clamped (21). Basal glucose transport was measured in separate experiments using 20-min exposure times to the hexose- and tracer-containing medium to obtain an adequate number of counts at these low glucose transport rates. In a subset of experiments, arterial and venous perfusate samples were taken and oxygen uptake was calculated as described previously (22).

Glycogen, ATP, CrP, and glucose transport measurements. Muscle glycogen content was measured as glucose residues by a hexokinase method after acid hydrolysis and expressed per gram wet weight of muscle. Soleus muscle concentrations of ATP and CrP were determined by standard enzymatic methods (23). The 2DG taken up by the different muscles was determined in perchloric acid extracts and corrected for labeling in the extracellular space as determined by the [^{14}C]mannitol counts, as previously described (19).

AMPK activity. For AMPK activity determination, muscles were homogenized with a Polytron PT 3100 (Kinematica, Littau-luzern, Switzerland) at maximum speed for 10–15 s in ~20 volumes of ice-cold buffer (210 mmol/l sucrose,1 mmol/l EDTA, 5 mmol/l sodium pyrophosphate, 50 mmol/l NaF, 1 mmol/l dithiothreitol, 1.5 mmol/l phenylmethylsulfonyl fluoride, and 50 mmol/l HEPES, pH 7.4). The homogenate was centrifuged for 45 s at 15,000*g*, and the supernatant was stored in aliquots at –80°C for later determination of protein concentration and AMPK activity. AMPK activity was determined directly on the postnuclear supernatant without ammonium sulfate precipitation using SAMS peptide as a substrate in the presence of 200 μmol/l AMP as described (15). The standard assay was initiated by adding 4 μl of appropriately diluted sample in homogenization buffer to 21 μl of assay buffer, the latter yielding final concentrations (in 25 μl) of 50 mmol/l PIPES (pH 6.6), 80 mmol/l NaCl, 8% glycerol (vol/vol), 5 mmol/l MgCl, 0.8 mmol/l EDTA, 0.8 mmol/l dithiothreitol, 0.2 mmol/l ATP, 0.2 mmol/l AMP, 0.2 mmol/l SAMS peptide, and 2 μCi [^{32}P]ATP (PB168, Amersham Pharmacia). After 10 min at 37°C, the reaction was terminated by adding 12.5 μl 40% tricholoroacetic acid. Then, 30 μl was spotted on Whatman P81 paper, which was washed 6 times in 1% phosphoric acid and once in acetone. The papers were air-dried and radioactivity was quantitated in 4 ml scintillation liquid (Ultima Gold; Packard).

We found that AMPK activity in the absence of AMP is not decreased compared with activity in the presence of AMP. This finding is in agreement with earlier findings by Davies et al. (24), who could not demonstrate AMP dependence of kinase activity in crude preparations because, apparently, endogenous AMP remained bound to the enzyme. In the present study, a 50 mmol/l PIPES buffer (pH 6.6) was used instead of a 40 mmol/l HEPES buffer (pH 7.0) because, as shown in Fig. 1*A*, the use of a pH of 6.6 provides better linearity of the assay with increasing sample amounts compared with a pH of 7.0, possibly because of the removal of the inhibitory action of CrP (12). Fig. 1*B* shows that when using our assay protocol, AMPK activity is reasonably linear up to 15 min of assay duration. For background subtraction, we have substituted a sample with homogenization buffer. This substitution gives similar results compared with using heat-inactivated (3 min at 95° C) homogenate. In the absence of SAMS peptide and with noninactivated homogenate present, activity is similar (within 20%) to that obtained with homogenization buffer and SAMS peptide present.

To further validate our modified AMPK activity assay, we compared it with the conventional method with ammonium sulfate precipitation (144 mg ammonium sulfate/ml) and assay pH 7.0, as described by Winder and Hardie (15). The results are shown in Fig. 2. In our lab, the difference between basal and contracted muscle samples is 5-fold without precipitation and 16-fold with precipitation. Therefore, by using the cited protocol for ammonium sulfate precipitation, there is apparently a huge loss of activity. This result is most likely explained by insufficient precipitation of the enzyme and not by the different assay conditions (pH 6.6 in unprecipitated samples vs. pH 7.0 in precipitated samples), since it can be seen from Fig. 1*A* that activity is only slightly affected by the different pH value. Actually, because 3.3 μg protein was used in all assays in Fig. 2, which translates to ~2 μl in Fig. 1*A*, it can be seen that, if anything, activity is actually higher at pH 7.0 than at pH 6.6. It remains to be determined if this loss of activity means that different isoforms of AMPK are precipitated at the chosen ammonium sulfate concentration or that the phosphorylation state of AMPK affects precipitation efficiency. We are not aware of any previously published validation of the cited ammonium sulfate precipitation procedure in skeletal muscle.

ACC activity. ACC activity was measured on ACC-β immunoprecipitates, as described elsewhere (25), with minor modifications. Frozen muscle (20 mg) was homogenized in ice-cold buffer A (30 mmol/l HEPES, 3 mmol/l EDTA, 30 mmol/l KCl, 250 μmol/l phenylmethylsulfonyl fluoride, 1 mmol/l dithiothreitol, 10% glycerol, and 1% Igepal, pH 7.4) and centrifuged at 20,000*g* for 30 min (4°C). Equal amounts of supernatant protein (1 mg) were incubated for 3 h with 6 μg of the monoclonal antibody 7AD3 (26) and with 50 μl agarose protein G beads (Pharmacia, Sweden) overnight. The beads were washed once in buffer A, twice in buffer A containing 250 mmol/l NaCl, and once more in buffer A. They were then divided into 2 portions and assayed for ACC activ-

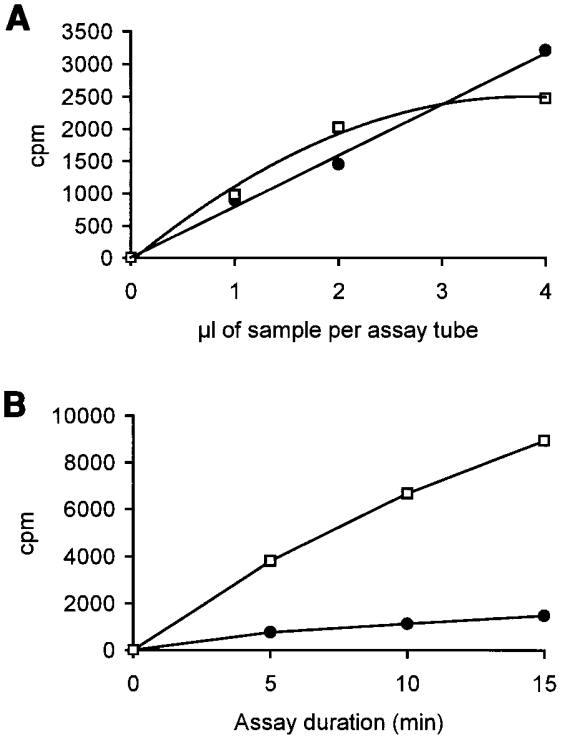

FIG. 1. *A*: Effect of sample amount and pH on AMPK activity. Either 1, 2, or 4 µl of a postnuclear supernatant (protein concentration 1.8 mg/ml) from in vitro incubated and contraction-stimulated rat soleus muscles (35) was assayed in triplicate for AMPK activity (radioactivity of product in counts per minute) in a total volume of 25 µl at either pH 6.6 (●) or 7.0 (□), as described in RESEARCH DESIGN AND METHODS. *B*: Effect of assay time on AMPK activity. Four microliters of a postnuclear supernatant (protein concentration 2.0 mg/ml) from in vitro incubated and basal (●) and contraction-stimulated (□) rat soleus muscles was assayed in triplicate for AMPK activity (cpm) at pH 6.6 for 5, 10, or 15 min.

ity by the $^{14}CO_2$ fixation assay (27) in the presence of either 0.2 or 10 mmol/l citrate. Results are expressed as picomoles of $^{14}CO_2$ incorporated into acid-stable products per minute per milligram of extract protein subjected to immunoprecipitation.

Statistical analysis. Statistical evaluation of the data was done by paired or unpaired Student's *t* tests. Correlations were calculated with the Pearson Product Moment test. Data are presented as means ± SE, and the level of significance was set at 0.05.

RESULTS

Fast-twitch muscles. Because of the effects of treatment on glycogen levels, glucose transport rates and AMPK activities were nearly identical in red and white gastrocnemius; therefore, we have chosen to show only the data from white gastrocnemius for reasons of clarity and brevity. In the white gastrocnemius, resting muscle glycogen content in glycogen-supercompensated rats (HG) was 3-fold higher compared with glycogen-depleted (LG) rats ($P < 0.05$; Fig. 3*A*). The 10-min stimulation period depleted muscle glycogen content by 44% in the HG group and by 82% in the LG group (Fig. 3*A*). Basal glucose transport, as measured by 2DG uptake, was ~2-fold higher ($P < 0.05$) in LG groups compared with HG groups (Fig. 3*B*). Electrical stimulation (10 min) increased glucose transport ~6-fold over basal in both groups, resulting in 80% higher ($P < 0.05$) glucose transport rates in LG compared with HG muscles. In glycogen-supercompensated muscles, contractions resulted in a 1.8-fold increase in AMPK activity, whereas in glycogen-depleted muscles, contractions induced a 4.6-fold increase in AMPK activity ($P < 0.05$, HG vs. LG; Fig. 3*C*). AMPK activity was positively correlated with glucose transport rate during contractions in white gastrocnemius ($r = 0.80$, $P < 0.01$). Contractions caused a significant decrease in ACC activity in both glycogen groups, which was more prominent when measured at submaximal (0.2 mmol/l) citrate levels (3- to 4-fold decrease) than at maximal (10 mmol/l) citrate levels (30–40% decrease; Fig. 3*D*). There was no significant difference in ACC deactivation between HG and LG muscles.

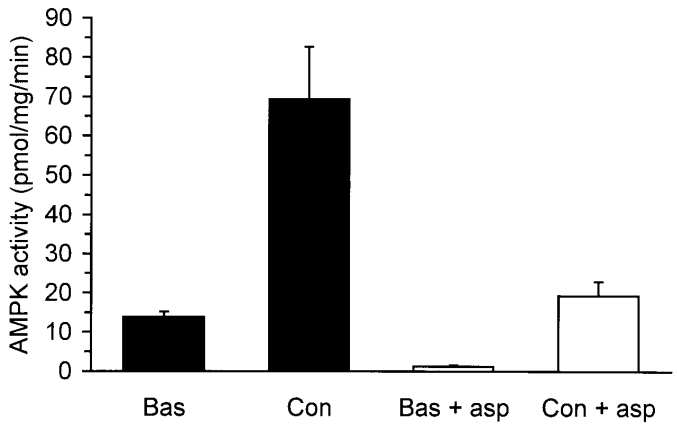

FIG. 2. Effect of ammonium sulfate precipitation (asp) on AMPK activity. Aliquots of a postnuclear supernatant from in vitro incubated and contraction-stimulated (Con) and basal (Bas) rat soleus muscles (35) homogenized in the buffer given in RESEARCH DESIGN AND METHODS were assayed in triplicate for AMPK activity at pH 6.6 (■) and after asp at pH 7.0 (□), as described by Winder and Hardie (15). Activity is expressed as picomoles of phosphate incorporated into SAMS peptide per milligram of protein in the postnuclear supernatant before asp. Data are means ± SE of 3 independent experiments.

Slow-twitch muscles. The basal glycogen content in soleus muscles was 2.6-fold higher ($P < 0.05$) in the HG group compared with the LG group (Fig. 4*A*). Muscle glycogen stores were decreased by 18% (HG) and 44% (LG) during stimulation (Fig. 4*A*). Basal 2DG uptake and AMPK activity were not influenced by glycogen levels in soleus muscle (Fig. 4*B* and *C*). Contraction-induced glucose transport was increased ~6-fold over basal in HG and LG groups. Interestingly, AMPK activity in the HG group was not increased by contractions, whereas it increased over 3-fold in the LG group (Fig. 4*C*). In soleus muscle, AMPK activity did not correlate significantly with glucose transport (Fig. 5). ACC activity decreased during contractions by 33% ($P < 0.05$) and 15% (NS; $P = 0.18$, $n = 10$) in HG muscles and by 54% ($P < 0.05$) and 32% ($P < 0.05$) in LG muscles, measured in the presence of 0.2 and 10 mmol/l of citrate, respectively (Fig. 4*D*). In HG muscles, the decrease in ACC activity at 0.2 mmol/l citrate was significantly smaller than that in LG muscles ($P < 0.05$). In the contracting soleus muscle, ATP and CrP concentrations decreased by ~20 and ~60%, respectively, independent of muscle glycogen levels (Table 1). Hind limb oxygen uptake ($n = 6$) in HG and LG muscle, respectively, was 12 ± 1 and 15 ± 2 µmol · g^{-1} · h^{-1} at rest, increasing ($P < 0.05$) to 31 ± 11 and 34 ± 11 µmol · g^{-1} · h^{-1} during contractions.

DISCUSSION

In agreement with previous studies (16,17), the present data indicate that in fast-twitch muscles (white and red gastroc-

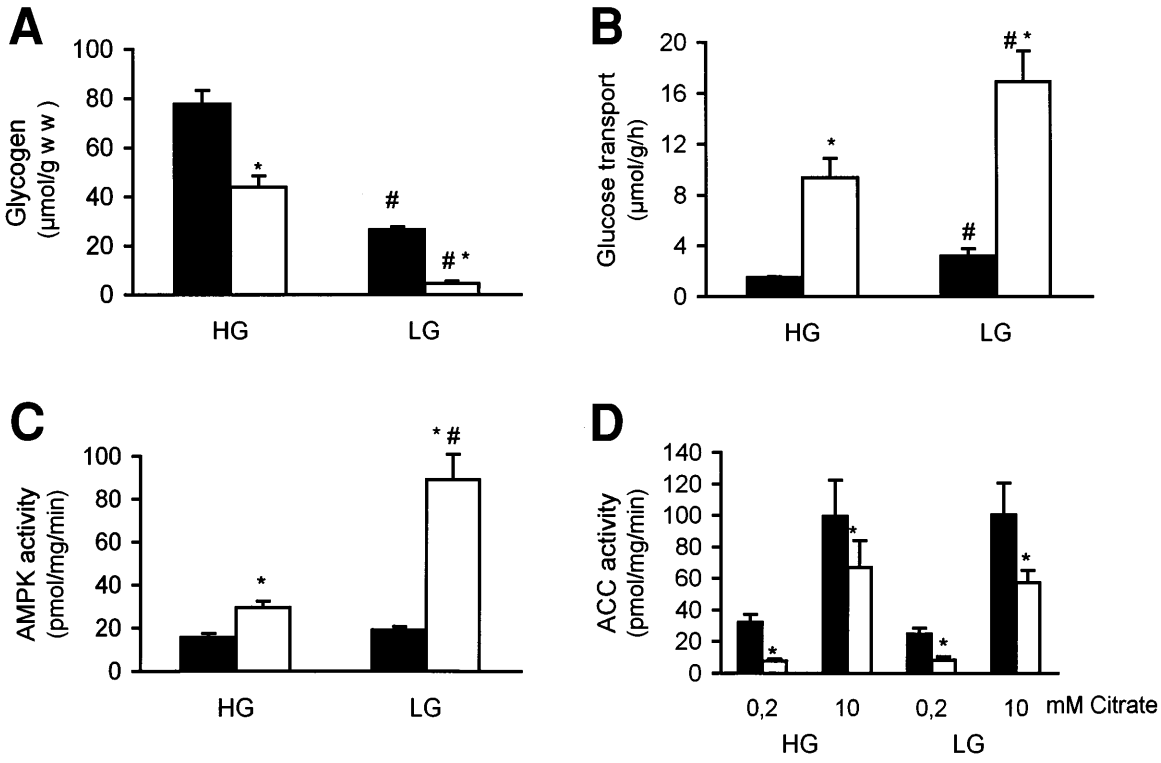

FIG. 3. Muscle glycogen content (*A*), glucose transport (*B*), AMP kinase activity (*C*), and ACC activity (*D*) in fast-twitch muscles (white gastrocnemius). Muscles with high and low glycogen content were perfused, and measurements were done at rest (■) and after 10 min of contractions (□). Data are given as means ± SE (n = 7–10). *Different from resting values ($P < 0.05$); #different from HG ($P < 0.05$). ww, wet weight.

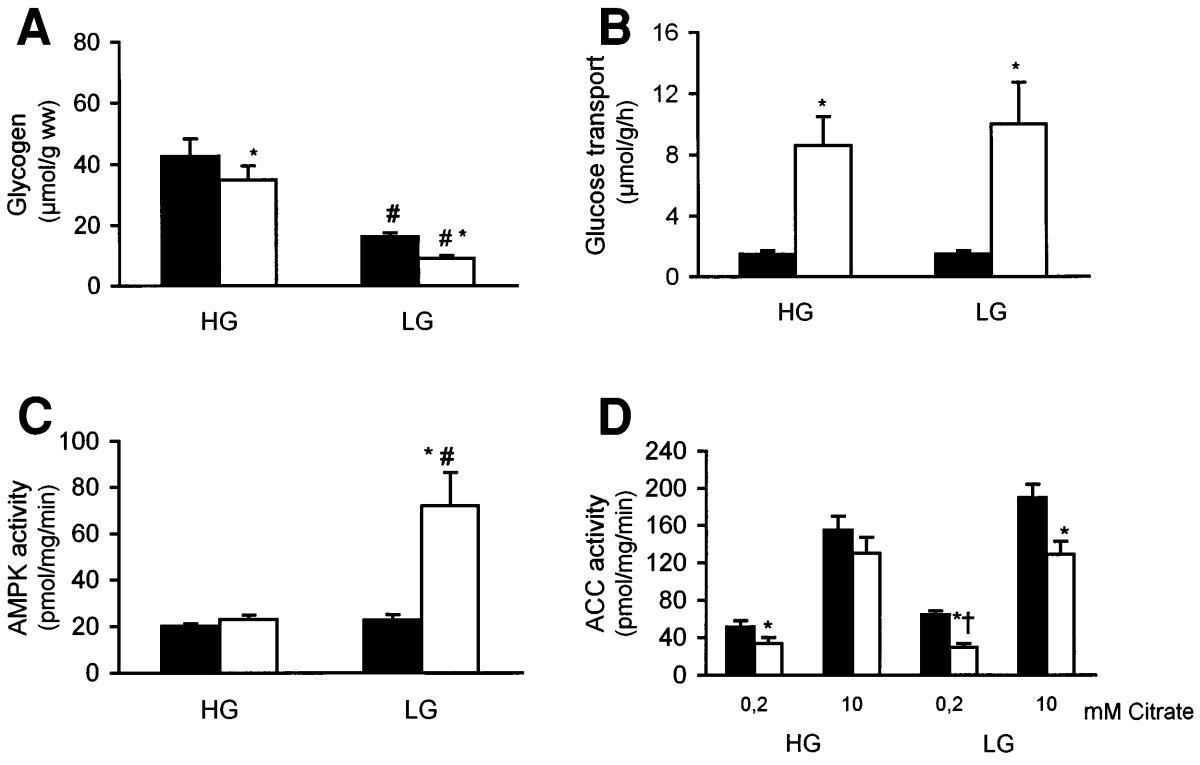

FIG. 4. Muscle glycogen content (*A*), glucose transport (*B*), AMP kinase activity (*C*), and ACC activity (*D*) in slow-twitch muscles (soleus). Muscles with high and low glycogen content were perfused, and measurements were done at rest (■) and after 10 min of contractions (□). Data are given as means ± SE (n = 7–10). *Different from resting values ($P < 0.05$); #different from HG ($P < 0.05$); †different contraction-induced decrease vs. HG ($P < 0.05$). ww, wet weight.

TABLE 1
Soleus muscle concentrations of ATP and CrP in HG and LG groups at rest (basal) and after 10 min of contractions

	ATP		CrP	
	Basal	Contractions	Basal	Contractions
HG	3.4 ± 0.2	2.6 ± 0.2*	6.2 ± 0.9	2.4 ± 0.7*
LG	3.1 ± 0.2	2.7 ± 0.2*	6.5 ± 0.8	2.9 ± 0.9*

Data are means ± SE of 7–8 observations and are given as micromoles per gram wet weight. *Different from basal ($P < 0.05$).

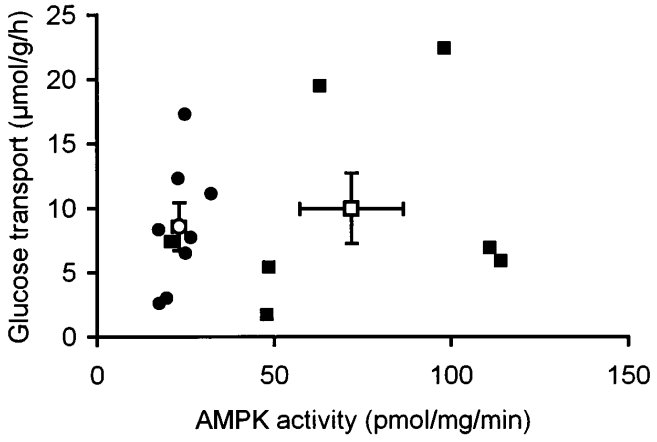

FIG. 5. The relationship between AMPK activity and glucose transport during contractions in slow-twitch muscles (soleus). Individual data of HG (●; $n = 8$) and LG (■; $n = 7$) are shown as filled symbols, and means ± SE are given as open symbols.

nemius), the glucose transport rate in response to a standardized contraction stimulus is higher when muscle glycogen levels are low compared with when glycogen levels are high. This result may indicate that the contraction signal for glucose transport stimulation is influenced by an energy-sensing mechanism. For the first time, the present data indicate that contraction activation of AMPK is dependent on the pre-exercise muscle glycogen content, i.e., it is significantly higher in LG muscles compared with HG muscles. This observation supports the recently raised hypothesis (6,8) that AMPK provides the link between energy status and glucose transport in fast-twitch muscle. There is little doubt that AMPK is a potent sensor of the intracellular energy status (13) because of its sensitivity to decreases in ATP:AMP and CrP:Cr ratios. However, the data linking AMPK activation to glucose transport stimulation are all merely correlative (6–8), and direct evidence for the link between AMPK activation and glucose transport is lacking. In addition, the pathway downstream of AMPK that might activate glucose transport is unknown. It could be hypothesized that ACC (a well-described substrate of AMPK) and thereby intracellular malonyl-CoA concentrations may be involved in this process by analogy with their role in the regulation of fatty acid metabolism in muscle (14). The present data, however, are not compatible with a role for ACC in glucose transport regulation because ACC activity at both submaximal and maximal citrate levels was not affected by glycogen levels in contracting fast-twitch muscles and could therefore not account for the differences in glucose transport rate.

The data in slow-twitch muscles do not support a role for AMPK in glucose transport stimulation during muscle contractions. In glycogen-depleted versus glycogen-supercompensated soleus muscles, contraction-stimulated AMPK activity was >3-fold higher ($P < 0.01$), whereas respective glucose transport rates were not different. Even more convincingly, contractions did not measurably increase AMPK activity in HG soleus muscles, whereas glucose transport was increased 6-fold. This result indicates that an activation of AMPK that can be retained in the in vitro assay (such as phosphorylation of AMPK by, e.g., AMPK kinase) is not essential for glucose transport stimulation in contracting slow-twitch muscles. It could be argued that increased soleus glucose transport is related to a direct effect of glycogen supercompensation on GLUT4 translocation. However, we have recently shown that a glycogen-loaded state is not associated with an increased GLUT4 cell surface content but instead is associated with a reduced GLUT4 cell surface content in both basal and contracting plantaris muscles (17). Furthermore, that study showed a close positive correlation between glucose transport rate and cell surface GLUT4 content (17). Thus, it seems safe to conclude that in contracting soleus muscles with HG, increased glucose transport is due to an increased recruitment of GLUT4, likely through a mechanism independent of AMPK.

It could also be argued that activity of AMPK, measured in vitro, may not necessarily accurately reflect activity in vivo, since AMPK is also activated by allosteric mechanisms such as decreased ATP:ADP and CrP:Cr ratios and accumulation of AMP. To investigate this possibility, we assayed the activity of ACC, an endogenous reporter substrate for AMPK that is inhibited by phosphorylation (15). In the HG soleus, contractions caused a small decrease in ACC activity at submaximal but not at maximal citrate levels (V_{max}), whereas in LG muscles, V_{max} was also decreased significantly, and the decrease at submaximal citrate was significantly larger than in the HG soleus. The observation that ACC inhibition by contractions is more easily detected at submaximal than at maximal citrate concentrations is consistent with the notion that ACC phosphorylation mainly affects the citrate sensitivity of the enzyme. The partial contraction-induced decrease in ACC activity in HG muscles indicates that ACC was partly phosphorylated. Because we observed no AMPK activation in these muscles, we have concluded that phosphorylation of ACC may result from the action of allosterically activated AMPK but could also be the result of other kinases. AMPK is thought to be the major physiological regulator of ACC-β (28), but a possible role for cAMP-dependent protein kinase or another unknown kinase cannot be ruled out at present (29). Therefore, it is difficult to determine whether the deactivation of ACC in HG soleus muscle is due to AMPK activation, which cannot be detected in the assay, or to another ACC kinase.

Whether the allosteric activation of AMPK is different in HG and LG groups is hard to assess because the free AMP concentration cannot be measured accurately (30). However, the equal postcontraction ATP and CrP concentrations measured in HG and LG groups suggest that allosteric activation of AMPK was not markedly different in the 2 conditions. Thus, whereas we cannot totally rule out that AMPK in HG soleus was somewhat activated during contractions in

the perfused hind limb through a mechanism other than phosphorylation, AMPK still must have been activated markedly less than in LG soleus. Nevertheless, glucose transport increased identically in HG and LG soleus muscles during contractions. It might then be argued, however, that this possible small activation of AMPK in HG soleus could be sufficient to induce full activation of glucose transport. But then it becomes difficult to understand why in the HG fast-twitch muscles a solid 2-fold increase in AMPK activity and a 4-fold decrease in ACC activity (at 0.2 mmol/l citrate) was not sufficient to induce full activation of glucose transport (Fig. 3). Furthermore, all previous positive evidence for involvement of AMPK in glucose transport stimulation (6–8) has conclusions based on positive correlations between glucose transport and AMPK activity in vitro (i.e., phosphorylation status). Therefore, our data suggest a dissociation between AMPK activation and glucose transport in contracting slow-twitch muscles, which in turn suggests that AMPK activation—at least in this fiber type—cannot be an essential factor involved in activation of glucose transport by muscle contractions.

AMPK is a heterotrimeric protein, and the catalytic α-subunit and regulatory β- and γ-subunits also exist in different isoforms in rat skeletal muscle (31,32). During muscle contractions or exercise, the catalytic activity of α2 but not α1 is increased (25). In the present study, we measured total AMPK activity, and consequently, we did not take into account AMPK heterogeneity. Therefore, we cannot exclude the fact that a small increase in activity of one of the isoforms is missed by measuring total AMPK activity. With regard to the possibility that AMPK may be involved in contraction-induced glucose transport in fast-twitch but not slow-twitch muscle, it is interesting to note that Chen et al. (32) have recently shown that rat soleus muscle (slow-twitch) exclusively contains β1-subunits associated with the α2–catalytic subunit, whereas in EDL muscle (fast-twitch), α2 is associated with both β1 and β2 (32).

The molecular mechanism involved in contraction-induced glucose transport is thought to involve signaling through Ca^{2+} (1). Ca^{2+} release from the sarcoplasmic reticulum to the cytoplasm is presumably regulated by the frequency of depolarization of the muscle membrane. Thus, the rate of depolarization sets the average Ca^{2+} concentration in the myoplasm, and the rate of glucose transport is then linked to the stimulation frequency as originally shown in frog muscle by Holloszy and Narahara (33). This feed-forward activation of glucose transport by Ca^{2+} could then be modulated by feedback signals from the working muscles as originally proposed by Wojtaszewski et al. (22). Such feedback signals could involve activation of AMPK as proposed by Ihlemann et al. (34). Our findings in fast-twitch muscle are compatible with such a notion, whereas in slow-twitch muscle, neither glycogen levels nor AMPK activity seem to regulate glucose transport.

In conclusion, the present findings show that contraction-stimulated glucose transport in fast-twitch muscles is influenced by the glycogen concentration, setting off a signal that possibly (but not necessarily) involves AMPK. At the same time, our data indicate that in slow-twitch muscles, glucose transport can be highly stimulated without measurable increases in AMPK activity. This dissociation between AMPK activation and glucose transport suggests that AMPK activation may not be necessary for increasing glucose transport during contractions in skeletal muscle.

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