Effects of endurance training on activity and expression of AMP-activated protein kinase isoforms in rat muscles

PAULA E. DURANTE,1* KIRSTY J. MUSTARD,1* SOO-HYUN PARK,2 WILLIAM W. WINDER,2 AND D. GRAHAME HARDIE1

1Division of Molecular Physiology, School of Life Sciences, Dundee University, Wellcome Trust Biocentre, Dundee, DD1 5EH Scotland, UK; and 2Department of Physiology and Developmental Biology, Brigham Young University, Provo, Utah 84602

Received 12 September 2001; accepted in final form 12 March 2002.

The AMPK-activated protein kinase (AMPK) is the downstream component of a protein kinase cascade that is activated in an ultrasensitive manner by a drop in cellular energy charge (16, 17, 46, 48). The key signals that trigger this activation are a rise in AMP coupled with a fall in cellular ATP and phosphocreatine (28, 31). This suggested that cellular energy charge might be one of the key signals.

The AMPK-activated protein kinase (AMPK) is the downstream component of a protein kinase cascade that is activated in an ultrasensitive manner by a drop in cellular energy charge (16, 17, 46, 48). The key signals that trigger this activation are a rise in AMP coupled with a fall in cellular ATP and phosphocreatine (28, 31). This suggested that cellular energy charge might be one of the key signals.
nant negative mutant (32), whereby the effect of contraction on glucose uptake was partly abolished. As well as these acute effects on muscle metabolism, there is increasing evidence that chronic activation of the AMPK system might underlie some of the long-term effects of endurance exercise training. These studies have mainly been performed by chronic treatment of rats with 5-aminimidazole-4-carboxamide (AICA) riboside, an agent that is converted inside the cell to AICA ribotide (ZMP), which activates the AMPK system without disturbing AMP or ATP levels (8, 29). This treatment results in increased expression of GLUT4, increased activity of hexokinase and mitochondrial enzymes, and increased glycogen content (23, 49, 54), all of which are also seen in response to endurance training. AICA riboside treatment in vivo also prevented the decrease in GLUT4 content induced by denervation (35) and increased insulin-stimulated glucose transport in isolated muscle (4). In genetically obese mice, AICA riboside treatment also caused increased expression of GLUT4 and hexokinase and improved glucose tolerance (33). These observations, and the finding that the antidiabetic drug metformin activates AMPK in vivo (55), greatly strengthen our previous proposals (48) that activation of the AMPK system is a promising target for treatment of type 2 diabetes.

Although these findings suggest that AMPK is intimately involved in both the acute and the chronic effects of exercise in skeletal muscle, little is known about the effects of endurance training on the expression or response of the AMPK system itself. In this study, we have addressed this in rat red and white quadriceps and soleus muscles, and we find that the response is dependent on the muscle type. Intriguingly, endurance training caused a threefold increase of expression of the γ3-subunit of AMPK in red quadriceps muscle, although not in soleus or white quadriceps. This is one of the first reports of regulation of AMPK at the level of protein expression.

MATERIALS AND METHODS

Animal care and training protocol. Male Sprague-Dawley (SAS:VAP) rats (Sasco, Wilmington, MA) were housed in individual cages in a temperature-controlled (21°C) room with a 12:12-h light-dark cycle. Rats assigned to the trained group were run on motor-driven rodent treadmills, 5 days/wk, in two 1-h sessions, morning and afternoon. The initial treadmill speed was 16 m/min (grade 15%). The speed was gradually increased so that after 4 wk, these rats were running at 31 m/min (grade 15%). They were maintained at this training intensity and duration for at least three additional weeks. Rats assigned to the untrained group were run for 5 min/day (same speed as trained group) to accustom them to the treadmill. Rats in the trained group were allowed to eat ad libitum, whereas untrained rats had their food intake restricted so that they gained weight at the same rate as the trained rats. Trained rats weighed 319 ± 9 g, and nontrained rats weighed 320 ± 11 g at the end of the experiment. Preliminary analysis of a group of untrained rats fed ad libitum indicated that their AMPK activities were similar to those of the untrained food-restricted group, so their further analysis was not pursued.

Three days before the final exercise test, trained rats were run for 2 h, and untrained rats were run for 5 min. Jugular catheters were installed with rats under ether anesthesia. The next day, trained rats were run for 1 h in the morning and 1 h in the afternoon. The next day, these rats ran 1 h in the morning only. Untrained rats ran 5 min/day during this period. All rats were given 25 g of rat chow on the evening before being killed. On the third day after catheterization, rats from trained and untrained groups were anesthetized by intravenous injection of pentobarbital sodium via the catheter, either at rest or after running on the treadmill for 5 min at 16 m/min and 5 min at 31 m/min (15% grade). The soleus muscles and the red and white regions of the quadriceps (vastus lateralis) muscle were quickly removed and immediately freeze-clamped between stainless steel blocks at liquid nitrogen temperature. Tissues were kept frozen below −80°C until analysis. A blood sample was also collected via the abdominal aorta. For glucose and lactate determination, 0.5 ml of blood was added to 1 ml of 10% perchloric acid. After centrifugation, the supernatant was frozen for later analyses.

Antibodies. Sheep anti-α1, anti-α2, anti-γ1, anti-γ2, and anti-PT172 antibodies have been described previously (7, 41, 53). Sheep anti-β2 and anti-γ3 antibodies were raised against the synthetic peptides CSVFSLPDPDSKLPGDK and CPGGLEGAGQSGPAA [residues 43–56 of rat β2 and rat sequence equivalent to 72–84 of human γ3 (D. Carling, personal communication), respectively, plus NH2-terminal cysteines for coupling]. Sheep antibodies were raised and affinity purified as described previously (53). A rabbit antisera raised against the bacterially expressed COOH-terminal region of rat β1 has been described previously (52).

Measurement of glucose, lactate, and glycogen. Glucose (1) and lactate (13) were measured by spectrophotometric methods on neutralized perchloric acid extracts of blood. Muscle glycogen was determined as described in Ref. 34.

Analysis of metabolic enzymes and GLUT4. Citrate synthase (39) and hexokinase (43) were determined as described previously. GLUT4 was determined by Western blotting (23) with polyclonal anti-GLUT4 (84 of human, Biogenesis, SanDiego, NH) and horseradish peroxidase-conjugated anti-rabbit IgG (Amersham Pharmacia Biotech, Arlington Heights, IL). Muscles for ACC determination were ground to powder under liquid nitrogen. The powder was weighed and then homogenized in a buffer containing 100 mM mannitol, 50 mM NaF, 10 mM Tris, 1 mM EDTA, 10 mM β-mercaptoethanol, pH 7.5, and proteolytic enzyme inhibitors (10 ml/l aprotonin, 10 mg/l leupeptin, and 10 mg/l antitrypsin). The homogenate was immediately centrifuged (48,000 g, 20 min). The pellet was dissolved in 10% of the original volume of the homogenate buffer and was centrifuged again to remove insoluble protein. ACC activity was determined on the supernatant at citrate-magnesium acetate concentrations ranging from 0 to 20 mM, as described previously (47). The ACC activity data were fitted to the Hill equation \( \nu = (V_{\max} - V_0) \cdot C^n / (K_c + C^n) \), where \( V_{\max} \) is the maximum velocity, \( V_0 \) is activity in the absence of citrate, \( K_c \) is the activation constant for citrate, \( C \) is citrate concentration, and \( n \) is the Hill coefficient) by use of the Grafit program (Sigma, St. Louis, MO). Expression of ACC was determined by analyzing 15 µg of extract protein per lane by SDS-PAGE in 3–8% Tris-acetate gels (NuPAGE, Invitrogen), with detection by ExtrAvidin (Sigma) and enhanced chemiluminescence.
EFFECT OF TRAINING ON AMPK

Muscles were subjected to a 7-wk program of endurance training involving two 1-h bouts of treadmill exercise per day. Untrained controls were run for 5 min per day to accustom them to the treadmill. Trained or untrained rats were either killed at rest or immediately after 10 min of treadmill exercise. Table 1 shows blood glucose and lactate levels for the four groups. The glucose levels were rather constant except that there was a moderate increase in trained rats (but not in untrained rats) after 10 min of exercise ($P < 0.001$). There was a marked increase in blood lactate ($P < 0.001$) in untrained animals after 10 min of exercise that was not observed in trained animals. Table 2 shows glycogen contents of the three muscle types with and without training and acute exercise. The glycogen content of muscle from trained animals was significantly higher than that from untrained animals in all three muscle types (before acute exercise, 1.6-fold, 1.4-fold, and 1.3-fold higher in red quadriceps, white quadriceps, and soleus, respectively). In all muscle types, treadmill exercise caused decreases in glycogen content, although these were small in magnitude in white quadriceps, and in this case the decrease was significant only in trained animals. Table 3 shows data for the activities of citrate synthase and hexokinase and for the expression of GLUT4. Training caused significant increases in citrate synthase in all three muscle types, in hexokinase in both red quadriceps and soleus, and in GLUT4 in red quadriceps only. Similar effects have been demonstrated previously, and they confirm that the training regimen was effective.

**Table 2. Effect of training and acute exercise on muscle glycogen contents**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Red Quadriceps</th>
<th>White Quadriceps</th>
<th>Soleus</th>
</tr>
</thead>
<tbody>
<tr>
<td>Untrained, rest</td>
<td>42.9 ± 2.2</td>
<td>14.7 ± 2.4</td>
<td>39.5 ± 3.9</td>
</tr>
<tr>
<td>Trained, rest</td>
<td>23.2 ± 2.1*</td>
<td>35.9 ± 3.6</td>
<td>10.5 ± 1.3*</td>
</tr>
<tr>
<td>Trained, exercised</td>
<td>69.9 ± 4.0†</td>
<td>57.2 ± 3.7‡</td>
<td>51.1 ± 4.3‡</td>
</tr>
</tbody>
</table>

Values are means ± SE for 6 or 7 rats in muscle units/g wet weight. *Effect of exercise significant ($P < 0.001$); †effect of training significant ($P < 0.01$); ‡effect of training significant ($P < 0.001$).
pletely remove all AMPK activity from the supernatant.

**Effects of endurance training on activity of AMPK isoforms in red quadriceps muscle.** Figure 1A shows that, in red quadriceps muscle, the activity of AMPK complexes containing the α2-isofom was ~10-fold higher than the activity of complexes containing the α1-isofom. There may have been small increases in α1-activity after acute exercise, but they were not statistically significant. In untrained animals there was a large (2.6-fold, *P < 0.001*) activation of α2-complexes in response to an acute bout of treadmill exercise. However, in trained animals, the effect of acute exercise on α2-activity appeared to be greatly reduced, and any increases were no longer statistically significant. The α2-activities measured after acute exercise were significantly lower (54%, *P < 0.001*) in trained animals compared with untrained animals. Because of the low α1-activity in this tissue, the results for the total activity essentially mirrored those for α2-activity.

Because we found evidence for a change in expression of the γ3-subunit isoform on training (Figs. 3 and 4), we also immunoprecipitated from extracts of red quadriceps using anti-γ1, anti-γ2, and anti-γ3 antibodies and measured the activity in the pellets. For reasons that are not clear, the total activity recovered by the anti-γ antibodies tended to be slightly higher (134 ± 21%, means ± SD, *n* = 12) than the total recovered with the anti-α antibodies. In animals killed at rest, expressed as a percentage of the sum of the activities recovered by all anti-γ antibodies, the proportions were 89 ± 6 (γ1), 8 ± 5 (γ2), and 3 ± 3 (γ3) (means ± SD, *n* = 12). There were no significant differences between trained and untrained animals.

**Effects of endurance training on activity of AMPK isoforms in white quadriceps muscle.** In white quadriceps muscle, a second pattern was seen. Figure 1B shows that, as in red quadriceps, the activity of AMPK complexes containing the α2-isofom were almost 10-fold higher than those containing the α1-isofom, and there were no effects of either acute exercise or endurance training on α1-activity. In both untrained and trained animals, any activation of α2-complexes in response to acute exercise was also small and not statistically significant. A difference from the results in red quadriceps was that the α2-activity was elevated after training whether it was measured in animals that had (2.0-fold, *P < 0.01*) or had not (2.2-fold, *P < 0.05*) been subjected to a bout of acute exercise. Because of the low α1-activity in this tissue, the results for the total activity essentially mirrored those for α2-activity.

This increase in AMPK activity in the white quadriceps muscle of trained animals did not appear to be due to changes in protein expression (Figs. 3 and 4). To address whether the increase was due to phosphorylation, we analyzed equal loadings of extract protein by Western blotting, using an antibody that recognizes the α1- or α2-subunits only in the form in which they are phosphorylated at the activating site (Thr172) (41). The results (Fig. 2) correlate well with those for total AMPK activity (see Fig. 1B). Although there appeared to be an increase in phosphorylation after acute exercise in trained animals, this was not statistically significant. However, when the data for animals with or without acute exercise were pooled, there was a highly
significant increase in phosphorylation of Thr^{172} in trained compared with untrained animals (2.0 ± 0.4-fold; P < 0.001).

**Effects of endurance training on activity of AMPK isoforms in soleus muscle.** Figure 1C shows that, in marked contrast to red or white quadriceps, the activities of AMPK complexes containing the α1- and α2-isoform were almost equal, due to a slightly higher α1-activity and to a much lower α2-activity. There appeared to be small increases in both α1- and α2-activity in response to acute exercise, although by analysis of variance these were significant only for α2 after training (3.6-fold, P < 0.001) and for total activity (α1 plus α2) both with (2.9-fold, P < 0.001) and without (1.8-fold, P < 0.01) training. In soleus muscle, prior training appeared to have no significant effects.

**Effect of endurance training on the activity of ACC.** As a marker of AMPK activity in vivo, we also measured the activity of ACC. Table 4 shows the effect of an acute bout of exercise on the citrate dependence (K_0) and V_{max} of ACC in the three muscle types with animals that either had or had not been subjected to prior endurance training. We have shown previously that phosphorylation of rat muscle ACC by AMPK causes a marked increase in K_0 and a more modest decrease in V_{max} (50). In red quadriceps of untrained animals, there was a robust inactivation of ACC in response to acute exercise due to both a decrease in V_{max} and an increase in K_0 (Table 4). By contrast, in trained animals, there was no significant effect on K_0, and the effect on V_{max} was much reduced. In white quadriceps, there were no significant effects of exercise on ACC activity in either the trained or untrained state. In soleus muscle, there appeared to be a small inactivation of ACC in response to acute exercise in untrained animals, as evidenced by a significant increase in K_0 (Table 4). In trained animals, acute exercise had no effect on ACC activity in soleus muscle.

By probing blots of muscle extracts with peroxidase-conjugated avidin, we confirmed that training did not produce a significant change in ACC expression in any of the three muscle types (not shown).

**Effect of endurance training on the expression of AMPK subunit isoforms in the different muscles.** Figure 3 shows Western blot analysis of equal amounts of protein from the 14,000-g supernatant fraction of the three muscle types before and after the training protocol. All of the extracts shown in Fig. 3 were from animals killed at rest, although similar results were obtained when muscles were obtained after a bout of acute exercise (not shown). Blots were probed with affinity-purified antipeptide antibodies against α1, α2, β2, γ1, γ2, and γ3, and with an anti-β1 antiserum raised against bacterially expressed β1 that also cross-reacts with β2. All polypeptides migrated with the approximate molecular mass expected from the amino acid sequence (α1, 63 kDa; α2, 63 kDa; β1, 32 kDa; β2, 30 kDa; γ1, 35 kDa; γ2, 63 kDa; and γ3, 55 kDa). The results revealed several interesting features regarding the expression of AMPK subunit isoforms in these muscle types, which will be discussed further. Quantification of the results (Fig. 4) showed that any minor changes in expression of α1, α2, β1, β2, γ1, or γ2 in response to endurance training were not significant in any of the muscle types. However, there was a threefold increase in γ3-expression in red quadriceps that was highly significant (P < 0.001). No data are shown for γ3 in white quadriceps, because the 55-kDa γ3-polypeptide was not detectable in extracts of this muscle type.

**DISCUSSION**

The effects of endurance training observed in this study are clearly dependent on the muscle type, and we will discuss the results for each type in turn.

Red quadriceps. The red quadriceps is a muscle that would be utilized particularly during running exercise of the moderate intensity utilized in this study. In untrained animals, α2-complexes were activated 2.6-
fold by acute exercise, and this correlated with a marked inactivation of ACC. Both of these effects of acute exercise were greatly reduced in the trained animals. The attenuation of the exercise-induced activation of AMPK is likely to be the mechanism for the blunting of the decline in ACC activity and malonyl-CoA content of red quadriceps reported previously to occur in response to endurance training in rats (25). During the training period, this muscle would have experienced changes in gene expression that would bring about adaptation to aerobic exercise. We believe, therefore, that a bout of treadmill exercise of the same intensity would cause less of a metabolic stress in the trained animals, as indicated by the lack of an increase in blood lactate in response to the exercise bout in these animals. This lower degree of metabolic stress would result in more modest effects on AMP, ATP, and phosphocreatine levels that would account for the lower degree of activation of AMPK and inactivation of ACC. Pilot measurements of ATP and phosphocreatine on red quadriceps samples from each treatment group failed to detect significant differences (not shown). However, it takes a minimum of 2–3 min to dissect out the muscles, and changes in ATP and phosphocreatine may not be stable for that long. Effects on AMPK activity are due to phosphorylation, and the differences appear to persist, although we cannot completely rule out the possibility that some changes occurred during

Fig. 3. Western blotting of the isoforms α1, α2, β1, β2, γ1, γ2, and γ3 of AMPK in extracts of red quadriceps (A), white quadriceps (B), and soleus (C) muscles from untrained and trained animals. All animals were killed at rest, although similar results were obtained when the animals were killed after 10 min of treadmill running. Extract protein (α1, α2, or β1: 2.5 μg; β2, γ1, γ2 or γ3, 5 μg) was analyzed by SDS-PAGE, as for Fig. 2.

Fig. 4. Quantitation of the Western blots shown in Fig. 3. Results are expressed relative to the mean intensity for that isoform in the same muscle type in untrained animals. The γ3-isoform was not detectable in white quadriceps (see Fig. 2). Open bars, untrained animals; hatched bars, trained animals. Symbols above bars indicate significant effects of training (§§§P < 0.001) compared with equivalent untrained animals.
dissection of the muscles. Another problem with detection of changes in nucleotides is our finding that the AMPK system is ultrasensitive (19), so that a very small change in the AMP-to-ATP ratio can produce a large change in AMPK activity. However, in agreement with our interpretation, endurance training has been reported to attenuate the falls in phosphocreatine and ATP and the rise in AMP in rat red gastrocnemius in response to electrical stimulation, where more rapid freezing of the tissue is possible (11). A second explanation of the reduced response to the same acute bout of exercise in trained animals compared with untrained animals is that the glycogen content of the muscle was higher (Table 2). AMPK activation by contraction has been reported to be markedly attenuated by a high muscle glycogen content in two different studies (10, 26), although the mechanism remains unclear.

White quadriceps. The fibers of the white quadriceps muscle are probably not recruited to a large extent during the moderate exercise intensity used in this study. It is therefore not surprising that there were no significant effects of acute exercise on either AMPK or ACC in this muscle, either with or without prior training. Citrate synthase was the only one of the three markers of training to show a small increase in activity. In white quadriceps, glycogenolysis and glycolysis are major pathways that provide ATP for contraction. These pathways can be controlled by direct allosteric effects of AMP and ATP on phosphorylase and phosphofructokinase (as well as by phosphorylation of the former in response to elevated Ca$^{2+}$ and/or cAMP), and there is currently no evidence that AMPK has any role in controlling these enzymes. The only effect observed on AMPK activity in white quadriceps was that the activity of α2-complexes, and the total activity, were significantly increased after training. This correlated with increased phosphorylation of the activating site (Thr172) on the α-subunits, as judged by Western blotting with a phosphospecific antibody that does not distinguish between α1 or α2 (Fig. 2). No changes in expression of any AMPK subunits were evident. The mechanism behind the increased phosphorylation of AMPK in white quadriceps remains unclear, but our results show that the basal activity of AMPK is increased after training. Because long-term activation of AMPK has been found to increase glycogen content in gastrocnemius/plantaritis muscles (23), the higher basal activity of AMPK might contribute to the higher glycogen content observed in white quadriceps after training (Table 2).

Soleus. In rats, this muscle contains predominantly type I, slow, oxidative fibers. The activity of α1-containing AMPK complexes was slightly higher, and that of α2-complexes markedly lower, than in red or white quadriceps. There appeared to be small increases in both α1 and α2 in response to acute exercise, although these were significant only for α2 after training and for the total activity with or without training. Acute exercise also had a small effect on ACC activity in soleus muscle of untrained but not trained animals. There were no significant effects of training on the expression of any AMPK subunit isoforms.

Figure 3 represents a comprehensive analysis of the expression of all known AMPK subunit isoforms in the three muscle types. The isoforms α1, α2, β1, and γ1 seem to be expressed at similar levels in all three muscles. The β2-isoform is also detectable in all three when a β2-specific antibody is used. However, with the anti-β1 antiseraum, which was raised against the bacterially expressed COOH-terminal region of rat β1 protein and which cross-reacts with β2, it is clear that β2 is expressed more highly relative to β1 in white quadriceps than in the other two muscle types. Chen et al. (5) previously reported that β2 co-precipitated with α2 from extracts of extensor digitorum longus muscle, which contains predominantly type IIB fibers, but not from soleus, which contains predominantly type I fibers. Thus there appears to be an association of β2 with muscles containing predominantly fast-twitch, glycolytic fibers. The γ2-isoform appears to be expressed most highly in white quadriceps, with lower levels in red quadriceps and soleus. The γ3-isoform, on the other hand, was present in red quadriceps and soleus but was not detectable in white quadriceps. Thus there is an association between γ3 and muscles that utilize predominantly oxidative metabolism.

Perhaps the most interesting finding in this study was that the expression of the γ3-isoform was markedly (threefold) increased in red quadriceps muscle in response to a period of endurance training. Smaller apparent changes in expression of other subunit isoforms (e.g., decreases in α2 in red quadriceps and γ1 in white quadriceps) were not significant. It is normally considered that AMPK is expressed constitutively, and the effect on γ3 is one of the first reports that expression of an AMPK subunit isoform is regulated at the level of protein expression. Given the tissue-specific expression of AMPK subunit isoforms, it is clear, of course, that their expression changes during differentiation and/or development, and it could be argued that the long-term adaptation that occurs in red quadriceps in response to endurance training represents a form of developmental change. At present, the mechanism for the increased expression of γ3 remains unknown, although it is tempting to speculate that there might be a positive feedback loop in which AMPK itself activates expression from the γ3-promoter.

The specific functions of AMPK complexes containing γ3 are unfortunately not yet clear. A curious feature of γ3-complexes is that they are only allosterically activated to a rather modest extent (<50%) by AMP (7). Taken together with our present results, this suggests that, for complexes containing γ3, regulation of expression might be as important, if not more important, than regulation by AMP. In a previous study using whole rat quadriceps, γ3-complexes were found to contribute only a very small proportion (<5%) of total AMPK activity (7), and this was confirmed in the present study in red quadriceps. Although the results in Figs. 3 and 4 show that the expression of γ3-protein increased after training, we were unable to detect a
significant increase in γ3-activity by immunoprecipitate kinase assays. However, because the γ3-activity was extremely low, any change would be very difficult to detect with this methodology. Even though γ3-complexes represent a very small proportion of total muscle AMPK, the effect of increased γ3-expression could still be very significant if these complexes were highly localized, either in specific fiber types and/or at the subcellular level. For example, because γ3 was undetectable by Western blotting in white quadriceps (Fig. 3), the proportion of activity due to γ3 might be higher in type I or IIA muscle fibers.

Perhaps the most interesting clue to γ3-function comes from the recent report that a point mutation (R200Q) in γ3 accounts for the high muscle glycogen content in a strain (RN−) of Hampshire pigs (30). Although the effects of this mutation on AMPK activity remain unclear, the arginine residue affected is conserved in γ1, and the equivalent mutation in γ1 (R70Q) gives rise to AMPK complexes that are more highly phosphorylated and more active under basal conditions (15). If the mutation in γ3 also caused activation, this would explain why the effect of the mutation in the RN− pigs is dominant. It is intriguing that a mutation that may cause a persistent activation of the γ3-isoform of AMPK in pigs leads to a high glycogen content, and we now report that endurance training causes an increase in expression of γ3 that is also associated with a high glycogen content.

After the first version of this paper was submitted, Tian et al. (42) made the interesting observations that there was an increase in expression of the α1-subunit of AMPK, and a small decrease in expression of α2, in response to pressure overload hypertrophy caused by restriction of the aorta in rat heart. The expression of subunit isoforms β and γ was not addressed.

We thank Dustin S. Rubink and Jared Bernotski for training the rats.

This study was supported by Project Grant RD99/001901 from Diabetes UK (to D. G. Hardie) and by National Institute of Arthritis and Musculoskeletal and Skin Diseases Grant AR-41438 (to W. W. Winder). K. J. Mustard was supported by a studentship from the Biotechnology and Biological Sciences Research Council (UK) and by a grant from Novo-Nordisk.

REFERENCES


41. Sugden C, Crawford RM, Halford NG, and Hardie DG. Regulation of spinach SNF1-related (SnRK1) kinases by protein kinases and phosphatases is associated with phosphorylation of the T loop and is regulated by 5′-AMP. Plant J 19: 1–7, 1999.