AMPK activity and isoform protein expression are similar in muscle of obese subjects with and without type 2 diabetes


AMPK activity and isoform protein expression are similar in muscle of obese subjects with and without type 2 diabetes. Am J Physiol Endocrinol Metab 286: E239–E244, 2004. First published October 7, 2003; 10.1152/ajpendo.00326.2003.—Acute or chronic activation of AMP-activated protein kinase (AMPK) increases insulin sensitivity. Conversely, reduced expression and/or function of AMPK might play a role in insulin resistance in type 2 diabetes. Thus protein expression of the seven subunit isoforms of AMPK and activities and/or phosphorylation of AMPK and acetyl-CoA carboxylase-β (ACCβ) was measured in skeletal muscle from obese type 2 diabetic and well-matched control subjects during euglycemic-hyperinsulinemic clamps. Protein expression of all AMPK subunit isoforms (α1, α2, β1, β2, γ1, γ2, and γ3) in muscle of obese type 2 diabetic subjects was similar to that of control subjects. In addition, α1- and α2-associated activities of AMPK, phosphorylation of α-AMPK subunits at Thr172, and phosphorylation of ACCβ at Ser212 showed no difference between the two groups and were not regulated by physiological concentrations of insulin. These data suggest that impaired insulin action on glycogen synthesis and lipid oxidation in skeletal muscle of obese type 2 diabetic subjects is unlikely to involve changes in AMPK expression and activity.

Kurt Højlund, Kurt, Kirsty J. Mustard, Peter Stehr, D. Grahame Hardie, Henning Beck-Nielsen, Erik A. Richter, and Jørgen F. P. Wojtaszewski. AMPK activity and isoform protein expression are similar in muscle of obese subjects with and without type 2 diabetes. Am J Physiol Endocrinol Metab 286: E239–E244, 2004. First published October 7, 2003; 10.1152/ajpendo.00326.2003.—Acute or chronic activation of AMP-activated protein kinase (AMPK) increases insulin sensitivity. Conversely, reduced expression and/or function of AMPK might play a role in insulin resistance in type 2 diabetes. Thus protein expression of the seven subunit isoforms of AMPK and activities and/or phosphorylation of AMPK and acetyl-CoA carboxylase-β (ACCβ) was measured in skeletal muscle from obese type 2 diabetic and well-matched control subjects during euglycemic-hyperinsulinemic clamps. Protein expression of all AMPK subunit isoforms (α1, α2, β1, β2, γ1, γ2, and γ3) in muscle of obese type 2 diabetic subjects was similar to that of control subjects. In addition, α1- and α2-associated activities of AMPK, phosphorylation of α-AMPK subunits at Thr172, and phosphorylation of ACCβ at Ser212 showed no difference between the two groups and were not regulated by physiological concentrations of insulin. These data suggest that impaired insulin action on glycogen synthesis and lipid oxidation in skeletal muscle of obese type 2 diabetic subjects is unlikely to involve changes in AMPK expression and activity.

AMPK is an αβγ heterotrimer that is activated by low cellular energy status, such as decreases in both the ATP/AMP ratio and the phosphocreatine content (16, 17, 23). Several isoforms of both the catalytic (α1, α2) and the two regulatory subunits (β1, β2, γ1, γ2, and γ3) have been identified in mammalian cells (7, 33, 34). Protein expression of AMPK subunit isoforms is changed in response to exercise training (8, 25, 30), which might contribute to the metabolic alterations induced by exercise training, e.g., enhanced peripheral insulin sensitivity. Mutations in γ2- and γ3-subunit isoforms cause glycogen storage disease in human heart (1) and glycogen accumulation in pig muscle (27), respectively, implying that these subunits have a role in glycogen synthesis. In addition, the whole body α2-deficient mouse is insulin resistant, possibly due to enhanced sympathetic nervous activity (35). Thus evidence exists to suggest that altered AMPK activity caused by changes in expression or function of different AMPK subunit isoforms might modulate the metabolic profile of specific tissues and might influence whole body metabolism.

In a recent study of the AMPK system in muscle of nonobese type 2 diabetic subjects (28), exercise-induced α2-associated AMPK activity and protein expression of the α1-, α2-, and β1-subunits were found to be normal. Furthermore, metformin treatment was reported to increase basal α2-associated AMPK activity in muscle of nonobese type 2 diabetic subjects (29). These findings argue against functional defects of the AMPK system in diabetic muscle, at least in the fasting and exercised states. However, we (18) have recently demonstrated that the mechanism responsible for impaired insulin-induced nonoxidative glucose metabolism and impaired activation of glycogen synthase in muscle of obese subjects with type 2 diabetes is likely to involve a hyperphosphorylation of glycogen synthase at the NH2-terminal sites Ser7 and Ser10 (18). AICAR treatment of isolated skeletal muscles and human myoblast in vitro leads to inactivation of glycogen synthase (2, 14, 39), most likely induced by AMPK-mediated phosphorylation of glycogen synthase at Ser1 (6), which primes the protein for subsequent phosphorylation at Ser6 by casein kinase I (10, 11). Thus the possibility exists that AMPK is involved in the hyperphosphorylation of glycogen synthase that occurs during insulin stimulation in obese type 2 diabetic subjects.

Another consistent metabolic feature of insulin resistance in type 2 diabetes is the failure of insulin to suppress lipid
oxidation in skeletal muscle (22, 26). Although not consistent (4), an inhibitory effect of insulin on AMPK activity has been reported in hepatoma cells and in rat cardiac and skeletal muscle (3, 13, 37, 38), and this appears to involve decreased phosphorylation of Thr\(^{172}\) in α-AMPK (3). This effect has been proposed to mediate activation of acetyl-CoA carboxylase and, hence, suppression of lipid oxidation by insulin (13, 38). Thus, despite normal activity of AMPK in the fasting and exercised states in muscle of nonobese type 2 diabetic subjects (28), there are data that could indicate that changes in the expression or function of AMPK subunit isoforms other than α1, α2, and β1 could be involved in the failure of insulin to activate glycogen synthase and to suppress lipid oxidation in the muscle of type 2 diabetic subjects.

To explore these possibilities, we measured basal AMPK subunit expression of all of the seven recognized subunit isoforms (α1, α2, β1, β2, γ1, γ2, and γ3) and the effect of insulin on AMPK activity in resting skeletal muscle of obese type 2 diabetic subjects and well-matched healthy control subjects.

MATERIALS AND METHODS

Study subjects. Ten obese subjects with type 2 diabetes and 10 healthy, obese control male subjects, matched according to age (51.1 ± 2.0 vs. 50.4 ± 1.6 yr) and body mass index (29.6 ± 0.9 vs. 31.1 ± 1.1 kg/m\(^2\)), participated in the study (18). Subjects with type 2 diabetes were treated either by diet alone or by diet in combination with sulfonylurea or metformin, which was withdrawn 1 wk before the study. The patients were all negative for antibodies to glutamic acid decarboxylase 65 (GAD65) and without signs of diabetic retinopathy, nephropathy, neuropathy, or macrovascular complications. The control subjects had normal glucose tolerance and no family history of diabetes. All subjects had normal results on screening blood tests of hepatic and renal function. All subjects were instructed to refrain from strenuous physical activity for a period of 48 h before the experiment. Informed consent was obtained from all subjects before participation. The study was approved by the local ethics committee and was performed in accord with the Helsinki Declaration.

Study design. All study subjects were admitted to the Diabetes Research Centre at Odense University Hospital (Odense, Denmark). After an overnight fast, all subjects underwent a euglycemic-hyperinsulinemic clamp (4 h of insulin infusion, 40 μU·m\(^{-2}\)·min\(^{-1}\)), as described in detail previously (20). In type 2 diabetic subjects, plasma glucose was allowed to decline to ∼5.5 mmol/l before glucose infusion was initiated. Total glucose disposal rates (GDR) were calculated using Steels’s non-steady-state equations adapted for la-flow-through period with a modified Bergström needle by suction under local anesthesia [10–15 ml of lidocaine 2% (20 mg/ml) injected subcutaneously]. Muscle samples were immediately blotted free of blood, fat, and connective tissue and were frozen in liquid nitrogen within 10–15 s.

AMPK α-isoform-specific activity. Muscle lysates were prepared by homogenization of muscle tissue (1:20, w/v) in a buffer containing 50 mM HEPES (pH 7.5), 150 mM NaCl, 20 mM Na-pyrophosphate, 20 mM β-glycerophosphate, 10 mM NaF, 2 mM Na-orthovanadate, 2 mM EDTA, 1% Nonidet P-40, 10% glycerol, 2 mM PMSF, 1 mM MgCl\(_2\), 1 mM CaCl\(_2\), 10 μg/ml leupeptin, 10 μg/ml aprotinin, and 3 mM benzamidine. Homogenates were rotated end over end for 1 h at 4°C and then cleared by centrifugation at 17,500 g at 4°C for 1 h. Protein content in the supernatants was measured by the bicinchoninic acid method (Pierce, Rockford, IL). AMPK α-isoform-specific activity was measured in immunoprecipitates from 200 μg of muscle lysate protein by use of anti-α1 or anti-α2 antibodies (40). A p81-filter paper assay, using SAMS peptide (HMRSAMSGLHLVKKR) (200 μM) as substrate was used to measure AMPK activity in the presence of saturated AMP concentration (0.2 mM) (40).

α-AMPK and acetyl-CoA carboxylase-β phosphorylation. The phosphorylation of the α-subunits (Thr\(^{172}\)) and acetyl-CoA carboxylase-β (ACCB; Ser\(^{212}\)) was evaluated by Western blotting using phosophospecific antibodies from Cell Signaling Technology and Upstate Biotechnology, respectively. The ACC-phosphospecific antibody is raised against a peptide corresponding to the sequence in rat ACCβ containing the Ser\(^{212}\) phosphorylation site, but the antibody also recognizes the human ACCβ when phosphorylated, most likely at the corresponding Ser\(^{212}\). For the detection of α-AMPK phosphorylation (Thr\(^{172}\)), muscle lysate protein was subjected to SDS-PAGE (7.5% Criterion gradient gel; Bio-Rad Laboratories, Richmond, CA), followed by semi-dry transfer to PVDF membranes (Immobilon Transfer Membrane, Millipore, Milpore, Glostrup, DK). Immunoreactive bands were visualized with enhanced chemiluminescence (ECL-plus, Amershams Biosciences, Little Chalfont, UK) and detected and quantified using a charge-coupled device-image sensor and 1D software (Kodak Image Station, E440CF, Kodak, Glostrup, DK).

Western blotting for the AMPK subunit isoforms was performed as described previously (8) except in the case of γ1. In this latter case, a “pan”-γ antibody was generated in sheep to the peptide CRAAPLWDSDKQSFVG (residues 69–83 of rat γ1, a sequence highly conserved in human γ2 and γ3), coupled to keyhole limpet hemocyanin, and affinity purified as described previously (41).

Calculations and statistical analysis. Control samples were added to all activity assays and loaded on all gels in duplicates, and assay-to-assay variation was accounted for by expressing data relative to these samples. Data calculation and statistical analysis were performed using the SigmaStat for MS Windows version 2.0 software. Data are presented as means ± SE. Two-way ANOVA analysis for repeated measures and Student’s t-test for unpaired data were used as appropriate to detect any significant differences. Significance was accepted at the P < 0.05 level.

RESULTS

Metabolic characteristics. The metabolic profile of the subjects on glucose metabolism during basal conditions as well as during the clamp were recently published in a paper describing the phosphorylation profile of muscle glycogen synthase (18). Briefly, fasting levels of glycosylated hemoglobin, plasma glucose, serum insulin, and C-peptide were significantly higher in the diabetic group, whereas fasting levels of serum FFA did not differ between the groups (18). During the insulin-stimulated steady-state period (the 210- to 240-min period), euglycemia at a plasma glucose concentration of ∼5.5 mmol/l and physiological hyperinsulinemia at a serum insulin concentration of ∼400 pmol/l were obtained in both groups. In this period, serum FFA concentrations were significantly higher in the diabetic group vs. the control group (18).

In the basal state, GDR were significantly higher, whereas in the insulin-stimulated state GDR were significantly lower in the diabetic group compared with the control group (Table 1). Indirect calorimetry data showed no significant differences in
glucose oxidation, nonoxidative glucose metabolism (glucose storage), or lipid oxidation in the basal state between the groups. During the insulin-stimulated state, both glucose oxidation and glucose storage were significantly lower, whereas lipid oxidation was significantly higher in type 2 diabetic subjects compared with control subjects (Table 1). The reduction in insulin-stimulated GDR in type 2 diabetic subjects was primarily accounted for by impaired glucose storage (75%).

**Table 1. Euglycemic hyperinsulinemic clamp data**

<table>
<thead>
<tr>
<th></th>
<th>Control Subjects</th>
<th>Diabetic Subjects</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total glucose disposal</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Basal</td>
<td>2.43±0.08</td>
<td>2.92±0.10</td>
<td>0.001</td>
</tr>
<tr>
<td>Clamp</td>
<td>9.80±0.94†</td>
<td>5.97±0.88*</td>
<td>0.001</td>
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<tr>
<td>Glucose oxidation</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Basal</td>
<td>1.49±0.11</td>
<td>1.71±0.29</td>
<td>NS</td>
</tr>
<tr>
<td>Clamp</td>
<td>3.53±0.21†</td>
<td>2.57±0.29*</td>
<td>0.007</td>
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<tr>
<td>Lipid oxidation</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Basal</td>
<td>1.62±0.04</td>
<td>1.66±0.16</td>
<td>NS</td>
</tr>
<tr>
<td>Clamp</td>
<td>0.76±0.07†</td>
<td>1.21±0.11*</td>
<td>0.004</td>
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<tr>
<td>Nonoxidative glucose metabolism</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Basal</td>
<td>0.93±0.12</td>
<td>1.20±0.26</td>
<td>NS</td>
</tr>
<tr>
<td>Clamp</td>
<td>6.68±0.79†</td>
<td>3.89±0.78*</td>
<td>0.002</td>
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</table>

Characteristics of type 2 diabetic and control subjects during basal steady-state period (Basal) and insulin-stimulated steady state (Clamp). Data represent means ± SE expressed in mg-min⁻¹-kg FFM⁻¹. Data for glucose and lipid oxidation and nonoxidative glucose metabolism are based on indirect calorimetry on 8 control and 9 diabetic subjects. NS, not significant. *P < 0.05 and †P < 0.01 vs. basal.

**DISCUSSION**

Because of the apparent influence of AMPK on a variety of metabolic processes dysregulated in type 2 diabetic subjects, AMPK has been hypothesized to play a role in the pathogenesis of insulin resistance in patients with type 2 diabetes (16, 17, 36). In the present study, we found that muscle from obese type 2 diabetic subjects had a level of protein expression of all seven AMPK subunit isoforms that was similar to that of healthy but obese control subjects. We also observed similar AMPK activities associated with both the α1- and α2-catalytic isoforms, a similar degree of phosphorylation of Thr172 in the α-AMPK isoforms, and a similar phosphorylation of the AMPK target site on ACCβ (Ser221). This suggests that AMPK function is intact in muscle of obese type 2 diabetic subjects, at least in the fasted and insulin-stimulated states. These observations extend those previously reported in nonobese exercise-tolerant type 2 diabetic subjects (28).

The present findings, and the observation that nonobese type 2 diabetic subjects displayed normal AMPK activation during exercise (28) and showed increased AMPK activity in response to metformin treatment (29), argue against a functional defect in AMPK and suggest that pharmacological activation of the AMPK system is a feasible and attractive treatment of insulin resistance in type 2 diabetes. The finding that ACCβ Ser221 is phosphorylated to a similar extent in muscle of type 2 diabetic
and healthy control subjects in both the basal and insulin-stimulated states suggests that any regulation of lipid oxidation by AMPK (via ACC1/H925) might be intact and not regulated by insulin. However, studies of human muscle strips in vitro showed normal regulation of AMPK and ACC1 phosphorylation in response to AICAR treatment (24); however, despite this, AICAR-induced glucose transport was impaired, leading to the conclusion that downstream defects might still compromise actions of AMPK.

From a range of studies, it seems that altered AMPK activity might modify the metabolic profile of diabetic animals and might change insulin sensitivity of skeletal muscle (5, 9, 15, 21, 32). The data from our study and others of human skeletal muscle in vivo (28) indicate that type 2 diabetes per se is not associated with changes in AMPK expression or activity, but we cannot exclude the possibility that obesity-related insulin resistance might be associated with changes in AMPK expression. In addition, neither of these studies can exclude a role of altered AMPK function in other tissues in the pathogenesis of type 2 diabetes. From the present data, showing similar activity and expression of AMPK in muscle of type 2 diabetic and control subjects despite different insulin sensitivity, it appears that there is no simple relationship between these variables. This is in line with recent observations showing that lack of expression of the α2-AMPK isoform in knockout mice does not cause alterations in insulin sensitivity of isolated muscle, but rather causes insulin resistance due to central mechanisms (35).

We have recently published a subset of data from the present study showing that glycogen synthase in muscle from type 2 diabetic subjects becomes hyperphosphorylated on Ser7 and Ser10 in response to insulin stimulation and suggesting that this abnormality is involved in the impaired insulin activation of glycogen synthase found in this group of patients (18). Both biochemical and physiological studies in vitro suggest that AMPK is a glycogen synthase kinase, phosphorylating Ser7 at the NH2 terminus, which decreases glycogen synthase activity (2, 6, 14, 39). In cultured skeletal muscle cells, glucose deprivation has been shown to stimulate both of the catalytic subunit isoforms of AMPK, and this was associated with a significant decrease in the fractional velocity of glycogen synthase activity.
(14). A regulatory role of AMPK in vivo is suggested from findings in patients with glycogen phosphorylase deficiency (McArdle’s disease) in whom the exercise-induced AMPK activation is correlated with a decreased glycogen synthase activity (31). However, the present data suggest that AMPK is not the kinase leading to the dysregulation of glycogen synthase, because AMPK activity in muscle of type 2 diabetic subjects is not increased in the basal state or in response to insulin.

In summary, the present data suggest that changes in AMPK activity or protein expression of AMPK subunit isoforms are not present in muscle of obese type 2 diabetic subjects compared with healthy obese controls. Therefore, AMPK does not appear to be a major contributor to the altered metabolic profile in type 2 diabetes. Importantly, the data also demonstrate that AMPK activity in resting human skeletal muscle is not regulated by physiological concentrations of insulin. Thus failure of insulin to activate glycogen synthesis and to suppress lipid oxidation in muscle of type 2 diabetic subjects is unlikely to involve changes in expression or activity of AMPK.

REFERENCES

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