Skeletal muscle fatty acid oxidation is not directly associated with AMPK or ACC2 phosphorylation

Hakam Alkhateeb, Graham P. Holloway, and Arend Bonen

Abstract: Rescue of palmitate-induced insulin resistance has been linked with improvements in fatty acid oxidation, but importantly, not always with concurrently altered AMP-activated protein kinase (AMPK) or acetyl-CoA carboxylase-2 (ACC2 phosphorylation). Therefore, we examined the interrelationships among AMPK, ACC2, and fatty acid oxidation under 12 controlled conditions in isolated muscle. Incubation of soleus muscle (0–12 h) did not alter fatty acid oxidation, but did increase AMPK and ACC2 phosphorylation (24%–30%). Muscle incubation with palmitate (2 mmol·L⁻¹) inhibited palmitate oxidation (~55%), but paradoxically, this was associated with increased AMPK and ACC2 phosphorylation (~50%). Addition of an AMPK activator (thujone) to control (no palmitate) muscle increased AMPK and ACC2 phosphorylation (~25%) but did not alter palmitate oxidation. Addition of AMPK inhibitors, compound C (50 µmol·L⁻¹) or adenine 9-β-D-arabinofuranoside (Ara; 2.5 mmol·L⁻¹), to thujone-treated muscles (no palmitate) did not alter palmitate oxidation but reduced AMPK phosphorylation (32%–42%), while ACC2 phosphorylation remained above basal levels (+14%–18%). Finally, in palmitate-treated muscle, thujone increased AMPK (+100%) and ACC2 phosphorylation (+52%) and restored palmitate oxidation. Compound C or Ara, administered along with thujone in palmitate-treated muscle, only partly blunted palmitate oxidation recovery despite inhibiting AMPK phosphorylation (~22%), although ACC2 phosphorylation remained upregulated (~33%). Among these experiments, AMPK phosphorylation and ACC2 phosphorylation were positively correlated. However, AMPK phosphorylation was not correlated with palmitate oxidation, and unexpectedly, palmitate oxidation was negatively correlated with ACC2 phosphorylation. Our study, in accordance with a growing body of evidence, indicates that neither AMPK phosphorylation nor ACC2 phosphorylation is by itself an appropriate marker of fatty acid oxidation, and further serves to question their regulatory role.

Key words: AMPK, ACC2, fatty acid oxidation, AMPK inhibitors.


Mots-clés : AMPK, ACC2, oxydation des acides gras, inhibiteurs de l’AMPK.

[Traduit par la Rédaction]
Introduction

Defects in skeletal muscle fatty acid metabolism, namely increased intramuscular lipid accumulation, are thought to be risk factors for the pathogenesis of insulin resistance (Boden and Shulman 2002; Roden 2004). For some time the general opinion has been that a reduction in fatty acid oxidation accounted for the intramuscular accumulation of reactive lipids, which interfere with insulin signaling. However, more recent work has not supported this speculation, as muscles can also accumulate lipids when fatty acid oxidation is increased (Alkhateeb et al. 2009; Perdomo et al. 2004; Holloway et al. 2009). In isolated muscle, a high concentration of palmitate can readily induce insulin resistance within 6 h (Alkhateeb et al. 2007, 2009). This is linked with both an increase in intramuscular reactive lipids and a reduction in palmitate oxidation (Alkhateeb et al. 2009; Alkhateeb and Bonen 2010). In this isolated muscle model the rescue of insulin sensitivity can be induced rapidly (<6 h) by stimulating fatty acid oxidation, despite maintaining increased concentrations of intramuscular reactive lipids (Alkhateeb et al. 2009; Alkhateeb and Bonen 2010). Taken together, it appears that reductions in fatty acid oxidation are not always necessary for lipid accumulation and insulin resistance to occur; nevertheless, stimulating fatty acid oxidation remains a beneficial approach to recovering insulin signaling. Understanding the pathways contributing to the regulation of fatty acid oxidation is therefore important for identifying potential therapeutic targets for the treatment of insulin resistance in obesity and type 2 diabetes.

A large body of evidence has shown that AICAR-induced activation of AMP-activated protein kinase (AMPK) stimulates fatty acid oxidation by phosphorylating and thereby inhibiting acetyl-CoA carboxylase-2 (ACC2). This ACC2 phosphorylation is generally thought to prevent the production of malonyl-CoA, thereby releasing the inhibitory effect exerted by malonyl-CoA on carnitine-palmitoyl-CoA transferase 1 (CPT-I)-facilitated uptake of fatty acyl CoA into mitochondria and allowing an increased rate of fatty acid oxidation (McGarry and Foster 1980, 1981; Ruderman et al. 1999; Kahn et al. 2005). This scheme has, however, been questioned. Recently, there has been evidence suggesting that ACC2 phosphorylation and fatty acid oxidation can be upregulated in the absence of AMPK activation (Fedicu et al. 2006; Roepstorff et al. 2006; Dzamko et al. 2008) and that fatty acid oxidation can be impaired despite increased ACC2 phosphorylation and (or) AMPK phosphorylation in L6 myotubes (Pimenta et al. 2008) and isolated muscle (Alkhateeb et al. 2007, 2009). Moreover, ACC2 phosphorylation can be maintained despite a lack of AMPK activation in muscle (Dzamko et al. 2008; Alkhateeb and Bonen 2010). In addition, recent work in muscle-specific ACC2 knockout mice has shown that neither malonyl-CoA content nor rates of fatty acid oxidation were altered (Olson et al. 2010). Taken together, it appears that the classic view of some of the mechanisms involved in regulating fatty acid oxidation in muscle is in doubt.

Examining the relationship between fatty acid oxidation and AMPK and ACC2 phosphorylation in skeletal muscle in vivo is difficult, as it is known that many factors can phosphorylate AMPK (for review see Kahn et al. 2005). Hence, experimental perturbations in vivo designed to examine fatty acid oxidation and its regulation are easily confounded. Also, genetic studies provide limited insight, as whole-body and tissue-specific ablation or unphysiological overexpression of selected metabolic genes can provoke metabolic changes in multiple organs, as well as unexpected adaptive responses of unknown origin (Lehman et al. 2000; Norris et al. 2003; Lin et al. 2004). An alternative experimental approach is provided by studies with isolated small (<20 mg) muscle tissue in vitro, a model which is viable for at least 18 h and in which it is possible to alter carbohydrate and lipid metabolism in a highly controlled manner (Alkhateeb et al. 2007, 2009; Alkhateeb and Bonen 2010). In this model we have been able to rapidly induce changes in fatty acid oxidation and glucose transport with a variety of perturbations (Alkhateeb et al. 2007, 2009; Alkhateeb and Bonen 2010). Therefore, the isolated soleus muscle preparation offers an experimental system in which the regulation of fatty acid oxidation can be examined.

Recently, we found that a number of factors (AICAR, leptin, thujone) rescued palmitate-induced insulin resistance (Alkhateeb et al. 2009; Alkhateeb and Bonen 2010). These positive effects were associated with improvements in fatty acid oxidation, but not always with AMPK or ACC2 phosphorylation (Alkhateeb et al. 2009; Alkhateeb and Bonen 2010). To explore the relationships among fatty acid oxidation and AMPK and ACC2 phosphorylation in skeletal muscle, we examined these parameters in control and palmitate-treated muscle under conditions in which fatty acid oxidation was experimentally reduced and (or) increased. We found that rapidly induced changes in skeletal muscle fatty acid oxidation were not attributable to concurrent changes in either AMPK or ACC2 phosphorylation. Indeed, despite the fact that AMPK phosphorylation and ACC2 phosphorylation are positively associated, we found, in contrast to the conventional view, that ACC2 phosphorylation was negatively correlated with rates of fatty acid oxidation.

Materials and Methods

Materials

[1-14C]Palmitate was purchased from Amersham Life Science (Oakville, Ont., Canada). Insulin (Humulin-R) was purchased from Eli-Lilly (Toronto, Ont., Canada). Penicillin and streptomycin were purchased from Invitrogen Corporation (Grand Island, N.Y., USA). Total and phosphorylated proteins were determined with commercially available antibodies from the following sources: anti-phospho-AMPK Thr 172 and anti-AMPK from Upstate (Millipore, Lake Placid, N.Y., USA); anti-phospho-ACC2 Ser 79 and anti-ACC2 from Cell Signaling Technology (Danvers, Mass., USA); goat-anti-rabbit secondary antibodies from Chemicon International (Temecula, Calif., USA). Thujone mixture (50% a and 50% b) and all other reagents were obtained from Sigma–Aldrich (St. Louis, Mo., USA).

Animals

All experiments were approved by the Committee on Animal Care at the University of Guelph. Male Sprague–Dawley rats (55–75 g) were used in this study. These animals consumed normal laboratory chow and were given water ad libi-
tum. For each experiment rats were anesthetized with Somnotol (6 mg·(100 g body weight)$^{-1}$, i.p.), and the soleus muscles were dissected.

**Muscle incubation**

Soleus muscles were incubated as we have previously described (Alkhateeb et al. 2007). Briefly, intact soleus muscles (~20 mg) were preincubated (15 min) and then incubated with palmitate (2 mmol·L$^{-1}$) or without palmitate (control) for 0–12 h. All incubations were performed in 10 mL of warmed (30 °C), pregassed (95% O$_2$ – 5% CO$_2$) Medium 199 containing 5 mmol·L$^{-1}$ glucose supplemented with 4% bovine serum albumin V, penicillin (100 IU·mL$^{-1}$), and streptomycin (0.1 mg·mL$^{-1}$). To maintain muscle viability, low concentrations of insulin (14.3 µU·mL$^{-1}$), which do not alter the rates of glucose transport (Alkhateeb et al. 2006, 2007) were also included. Incubation vials were shaken at 110 cycle·min$^{-1}$, and the gas phase and temperature were maintained at 95% O$_2$ – 5% CO$_2$ and 30 °C, respectively. After 6 h of incubation with or without palmitate, muscles were incubated for an additional 6 h in fresh media with or without palmitate. During this last 6 h of incubation, some muscles were treated with thujone (0.01 mg·mL$^{-1}$), a medicinal herb that can improve fatty acid oxidation (Alkhateeb and Bonen 2010). Studies were also performed in which thujone (0.01 mg·mL$^{-1}$) was administered along with selected AMPK inhibitors, namely adenine 9-β-D-arabinofuranoside (Ara) (2.5 mmol·L$^{-1}$) or compound C (50 µmol·L$^{-1}$). The quantities of thujone (0.01 mg·mL$^{-1}$), Ara (2.5 mmol·L$^{-1}$), and compound C (50 µmol·L$^{-1}$) were established in pilot studies, as these concentrations provided the optimal effects (data not shown).

**Palmitate oxidation**

The rates of palmitate oxidation were determined as we (Alkhateeb et al. 2007, 2009; Alkhateeb and Bonen 2010) have previously described. Briefly, at the end of the incubation period (0–12 h) muscles were transferred to glass vials containing 2 mL pregassed (95% O$_2$ – 5% CO$_2$) Medium 199 supplemented with 4% bovine serum albumin V and palmitate (2 mmol·L$^{-1}$, 0.5 µCi·mL$^{-1}$ of [1-14C]palmitate. Palmitate oxidation occurred at 30 °C for 40 min, and the $^{14}$CO$_2$ released was captured in a benzothium hydroxide trap (400 µL, 1.0 mol·L$^{-1}$). In addition, at the end of the 40-min incubation period, dissolved CO$_2$ was also captured in this manner by adding sulfuric acid (1.0 mL, 1 mol·L$^{-1}$) to a 1.0 mL aliquot of the incubating medium. Finally, water-soluble $^{14}$C-labeled intermediates were extracted from muscles homogenized after their incubation. After scintillation counting, the palmitate oxidation rate was determined by summing the 3 sources of $^{14}$C palmitate (Alkhateeb et al. 2007, 2009; Alkhateeb and Bonen 2010).

**Muscle protein analysis with Western blotting**

In the foregoing experiments the content of total and phosphorylated AMPK and ACC2 were determined in soleus muscle at 0, 6, and 12 h. At each time point the muscles were rapidly blotted, frozen in liquid nitrogen, and stored at −80 °C until analyzed.

Western blotting was used to determine AMPK and ACC2 protein content and phosphorylation, as we have previously described (Alkhateeb et al. 2007, 2009; Alkhateeb and Bonen 2010). Briefly, soleus muscles were homogenized in 2 mL of homogenizing buffer (210 mmol·L$^{-1}$ sucrose, 2 mmol·L$^{-1}$ EGTA, 40 mmol·L$^{-1}$ NaCl, 30 mmol·L$^{-1}$ Hepes, 5 mmol·L$^{-1}$ EDTA, and 2 mmol·L$^{-1}$ PMSF, pH 7.4). Protein concentrations were determined using the bicinchoninic acid assay. Thereafter, proteins were separated using SDS–PAGE (10% v/v gel). Western blotting was performed as reported previously (Alkhateeb et al. 2007, 2009; Alkhateeb and Bonen 2010). Equal quantities of protein (30 µg) were loaded in each lane, and this was confirmed with Ponceau staining.

**Statistics**

Because of the complexities of the study design, various statistical treatments were employed, including two-way analyses of variance (i.e., no palmitate treatment (0, 6, 12 h) vs. palmitate treatments (0, 6, 12 h), a one-way analysis of variance within these studies, and at selected time points across the various treatments, followed by appropriate post hoc tests when this was warranted (Fisher’s least significant difference post hoc analysis). All data are reported as means ± SE.

**Results**

The parameters under consideration, palmitate oxidation, AMPK phosphorylation, and ACC2 phosphorylation, were readily altered with selected perturbations in isolated soleus muscles (Fig. 1). No changes in total muscle protein content of AMPK or ACC2 were observed in these studies (data not shown).

**Effects of treatments on palmitate oxidation and AMPK and ACC2 phosphorylation**

**Control experiments**

Under basal conditions, rates of palmitate oxidation were stable for up to 12 h (Fig. 1A). However, at 6 and 12 h, the phosphorylation of AMPK (+30%) and ACC2 (+24%) increased ($p < 0.05$, Figs. 1B and 1C).

**Palmitate treatment**

Incubation of muscle with palmitate markedly reduced palmitate oxidation after 6 h (~63%) and 12 h (~52%) ($p < 0.05$, Fig. 1A). However, relative to basal phosphorylation levels at $t = 0$ (100%), AMPK phosphorylation increased at 6 h (+50%) and 12 h (+51%) ($p < 0.05$, Fig. 1B). Similar increases above basal levels (100%) were observed in ACC2 phosphorylation at 6 h (+48%) and 12 h (+44%) ($p < 0.05$, Fig. 1C). Notably, these increases in AMPK and ACC2 phosphorylation at 6 and 12 h in the palmitate-treated muscles were greater than in the control muscles (no palmitate) at 6 and 12 h ($p < 0.05$).

**No Palmitate plus thujone and AMPK inhibitors**

Provision of thujone alone or with AMPK inhibitors (compound C and Ara) did not alter the rates of palmitate oxidation (Fig. 1A). However, relative to basal levels, addition of thujone increased both AMPK phosphorylation (+31%) ($p < 0.05$, Fig. 1B) and ACC2 phosphorylation (+24%) ($p < 0.05$, Fig. 1C). Reductions in AMPK phosphorylation occurred when compound C (~32%) and Ara (~42%) were added to thujone-treated muscles ($p < 0.05$, Fig. 1B). In contrast,
with these inhibitors, ACC2 phosphorylation remained unaltered and was elevated above basal levels (compound C, +14%; Ara, +18%) \((p < 0.05, \text{Fig. 1C})\).

Addition of thujone with compound C and thujone with Ara reduced AMPK phosphorylation from that observed after 12 h of incubation (no palmitate) with thujone (thujone plus compound C, −32%; thujone plus Ara, −42%) \((p < 0.05, \text{Fig. 1B})\), while ACC2 phosphorylation remained elevated above basal levels (compound C, +14%; Ara, +18%) \((p < 0.05, \text{Fig. 1C})\).

**Palmitate plus thujone and AMPK inhibitors**

In palmitate-treated muscles, thujone treatment for the last 6 h of incubation fully restored palmitate oxidation \((p < 0.05, \text{Fig. 1A})\). This was accompanied by a thujone-induced increase in the phosphorylation of AMPK (+100%) \((p < 0.05, \text{Fig. 1B})\) and ACC (+52%) in the palmitate-treated muscles \((p < 0.05, \text{Fig. 1C})\).

Presenting palmitate-treated muscles with AMPK inhibitors, as well as thujone, reduced AMPK phosphorylation by 12% (Ara) and 25% (compound C) to below levels observed under basal conditions \((100\% \text{ at } t = 0 \text{ in the absence of any treatments}) \((p < 0.05, \text{Fig. 1B})\). In contrast, treatment with compound C and Ara, as well as thujone, did not fully inhibit ACC phosphorylation, which remained above basal levels \((100\%) \text{ for both compound C (+34\%) and Ara (+31\%}) \((p < 0.05, \text{Fig. 1C})\). Despite this, the thujone-mediated restoration of palmitate oxidation was partly inhibited when muscles were treated with thujone and the AMPK inhibitors compound C (−22%) and Ara (−23%) \((p < 0.05, \text{Fig. 1A})\).

**Relationships between AMPK phosphorylation, ACC2 phosphorylation, and fatty acid oxidation**

In the present study, the rates of fatty acid oxidation and the phosphorylation of AMPK and ACC2 were examined under 12 different conditions. This allowed us to compare the interrelations among these variables. In the present experiments, AMPK phosphorylation and ACC2 phosphorylation were positively correlated \((\text{Fig. 2A})\). However, AMPK phosphorylation was not correlated with palmitate oxidation \((\text{Fig. 2B})\). In addition, palmitate oxidation was negatively correlated with ACC2 phosphorylation \((\text{Fig. 2C})\).
In this study we examined the interrelationships among AMPK phosphorylation, ACC2 phosphorylation, and palmitate oxidation in isolated soleus muscle incubated for up to 12 h under highly controlled conditions. In this model we were able to alter fatty acid oxidation by prolonged (0–12 h) treatment with palmitate and selected agents. We found that chronic incubation of soleus muscles with palmitate (i) inhibited palmitate oxidation, but paradoxically, this was associated with (ii) increased AMPK and ACC2 phosphorylation. On the other hand, treatment with thujone (iii) completely rectified palmitate oxidation and (iv) improved AMPK and ACC2 phosphorylation. To explore whether thujone enhanced fatty acid oxidation by an AMPK-dependent mechanism, we performed experiments with AMPK inhibitors, namely Ara (2.5 mmol·L⁻¹) and compound C (50 µmol·L⁻¹). While Ara or compound C (v) completely inhibited AMPK phosphorylation, neither fatty acid oxidation nor ACC2 phosphorylation was inhibited. Taken together, it appears that AMPK phosphorylation was not correlated with fatty acid oxidation, and ACC2 phosphorylation was negatively associated with fatty acid oxidation. Therefore, this study casts doubt on the classic view that activation of the AMPK–ACC2 pathway necessarily indicates promotion of fatty acid oxidation.

Our observations parallel those of Pimenta et al. (2008). However, this study was conducted in L6 muscle cells, in which the mechanisms regulating fatty acid oxidation may not be entirely similar to those in mature mammalian muscle. Nevertheless, some common themes emerged from our study and that of Pimenta et al. (2008). Specifically, exposure to palmitate reduced the rate of fatty acid oxidation (present study and Pimenta et al. 2008), an effect that is dose dependent in L6 cells (Pimenta et al. 2008) and in soleus muscle (Alkhateeb et al. 2007) and is also time dependent in soleus muscle (Alkhateeb et al. 2007, 2009).

Our present result showing that there was no relationship between fatty acid oxidation and AMPK phosphorylation is in agreement with the findings in L6 muscle cells (Pimenta et al. 2008), namely that palmitate treatment induced the phosphorylation of AMPK, while paradoxically fatty acid oxidation was concomitantly reduced. Conversely, others have shown that palmitate oxidation remained upregulated when AMPK activation was inhibited (Dzamko et al. 2008) and that exercise-induced fatty acid oxidation was increased in the absence of AMPK activation (Roepstorff et al. 2006). Collectively, these studies have begun to question the need to activate AMPK to increase fatty acid oxidation.

The relationship between ACC2 phosphorylation and fatty acid oxidation has also been brought into question by a number of recent studies, including the present report and those of Pimenta et al. (2008) and Olson et al. (2010). Our results in mammalian muscle demonstrated a negative relationship between ACC2 phosphorylation and fatty acid oxidation, comparable to the inverse relationship between these parameters in palmitate-treated L6 muscle cells (Pimenta et al. 2008). The necessity for ACC2 activation in upregulating fatty acid oxidation has been also questioned in muscles of ACC2 knockout mice. In these knockout animals, the rate of fatty acid oxidation was not altered (Olson et al. 2010). We did not assess CPT-I activity in muscle homogenates, as both CPT-I and II activity are detected in tests. Measurement of CPT-I activity can be done in isolated mitochondria, as we have shown (Holloway et al. 2007, 2009), but such studies in isolated mitochondria to complement the 12 experimental conditions would have required an additional 300 animals, as we would have needed to pool substantial quantities of small soleus muscles. However, others have shown, in L6 muscle cells treated with palmitate, that fatty acid oxidation can be...
that the nonspecific effects necessarily interfere with the upregulation of fatty acid oxidation in skeletal muscle. In view of recent research, it is perhaps not entirely surprising that in the present study the expected relationship between AMPK–ACC2 activation and fatty acid oxidation in skeletal muscle was not as tightly linked as has been assumed. While fatty acid oxidation has long been thought to be entirely regulated by CPT-I, it now appears that fatty acid oxidation in skeletal muscle is regulated at several locations. For example, the uptake of fatty acids into muscle and delivery into mitochondria is regulated by plasma membrane fatty acid transporters (for review, see Glatz et al. 2010) and CPT-I (Eaton et al. 2001; Eaton 2002), respectively, as well as other unknown regulators yet to be identified (Olson et al. 2010). Impaired or upregulated transport of fatty acids into muscle concomitantly alters their delivery to, and oxidation in, mitochondria. It is clear that these processes can be independently altered, as has been shown in obese Zucker rats (Holloway et al. 2009). It has also been shown that changes in fatty acid oxidation are not necessarily attributable to changes in the activity of CPT-I, as has been widely believed (Holloway et al. 2009a, 2009b; Koves et al. 2005). Moreover, questions have also been raised as to whether there is necessarily a reduction in malonyl-CoA to release the inhibition on CPT-I and to allow fatty acid oxidation to increase, such as during exercise. Specifically, in rat skeletal muscle, malonyl-CoA levels decrease during exercise, releasing the inhibition of CPT-I and increasing long-chain fatty acid oxidation (Winder et al. 1989). However, in human skeletal muscle, malonyl-CoA levels do not change either during moderate-intensity exercise, when fatty acid oxidation is increased, or during high-intensity exercise, when fatty acid oxidation is reduced (Odland et al. 1998), suggesting that malonyl-CoA is not the sole regulator of increased CPT-I activity during exercise. Other work has shown that FAT/CD36 in mitochondria may be important for contributing to the upregulation of fatty acid oxidation in this organelle (Campbell et al. 2004; Glatz et al. 2010; Holloway et al. 2009) and that decreased CPT-I malonyl-CoA sensitivity and increased mitochondrial FAT/CD36 protein are both important for increasing whole-body fatty acid oxidation during prolonged exercise (Holloway et al. 2006). Taken together, it is becoming clear that no single enzymatic step is necessarily a determinant of fatty acid oxidation. Under normal physiological circumstances it is the orderly integration of multiple selected biochemical regulatory steps that must be combined to regulate the rate of skeletal muscle fatty acid oxidation.

Inhibition of AMPK activity has typically involved the use of nonspecific inhibitors such as compound C or Ara (Russell et al. 1999; Musi et al. 2001; Ojuka et al. 2002; Chabowski et al. 2006; Feduc et al. 2006). These agents were also used in the present study. Lack of complete inhibition and (or) specificity of compound C or Ara is a caveat that applies to a great many pharmacological inhibitors. Yet this limitation is frequently not addressed explicitly, in part because all the nonspecific effects are not fully known, nor is it certain that the nonspecific effects necessarily interfere with the mechanisms being examined in a particular study. In the present study, inhibitors were used to alter the phosphorylation state of AMPK. We recognize that other unknown kinases may have been affected by these inhibitors, some of which may perhaps contribute to regulating fatty acid oxidation. At present, which kinases contribute to the regulation of muscle fatty acid oxidation remains uncertain, and this has only been explored in very limited detail (Dzamko et al. 2008). Nevertheless, our intent was to examine the relationship between fatty acid oxidation and AMPK phosphorylation per se. For these purposes, compound C and Ara are useful for altering the phosphorylation state of AMPK. We recognize that it is possible that the nonspecificity of these compounds affected fatty acid oxidation in some unknown manner. If so, this reinforces our observations, namely that fatty acid oxidation can be regulated in an AMPK-independent manner.

Conclusion

We have shown that in skeletal muscle AMPK phosphorylation and ACC2 phosphorylation are positively related. However, AMPK phosphorylation is not correlated with the rate of fatty acid oxidation, while ACC2 phosphorylation and fatty acid oxidation are negatively associated. These observations add to a growing body of evidence indicating that neither AMPK phosphorylation nor ACC2 phosphorylation is by itself an appropriate marker of fatty acid oxidation and further serves to question their regulatory role.

Acknowledgements

These studies were supported by the Natural Sciences and Engineering Research Council of Canada (NSERC), the Canada Research Chair program, and Hashemite University. A. Bonen is the Canada Research Chair in Metabolism and Health.

References


