conference on ‘nutrition and health: cell to community’

plenary lecture
energy sensing by the AMP-activated protein kinase and its effects on muscle metabolism

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The AMP-activated protein kinase (AMPK) is a sensor of cellular energy status, and a regulator of energy balance at both the cellular and whole body levels. Although ubiquitously expressed, its function is best understood in skeletal muscle. AMPK contains sites that reversibly bind AMP or ATP, with an increase in cellular AMP:ATP ratio (signalling a fall in cellular energy status) switching on the kinase. In muscle, AMPK activation is therefore triggered by sustained contraction, and appears to be particularly important in the metabolic changes that occur in the transition from resistance to endurance exercise. Once activated, AMPK switches on catabolic processes that generate ATP, while switching off energy-requiring processes not essential in the short term. Thus, it acutely activates glucose uptake (by promoting translocation of the transporter GLUT4 to the membrane) and fatty acid oxidation, while switching off glycogen synthesis and protein synthesis (the later via inactivation of the mammalian target-of-rapamycin pathway). Prolonged AMPK activation also causes some of the chronic adaptations to endurance exercise, such as increased GLUT4 expression and mitochondrial biogenesis. AMPK contains a glycogen-binding domain that causes a sub-fraction to bind to the surface of the glycogen particle, and it can inhibit glycogen synthesis by phosphorylating glycogen synthase. We have shown that AMPK is inhibited by exposed non-reducing ends in glycogen. We are working on the hypothesis that this ensures that glycogen synthesis is rapidly activated when glycogen becomes depleted after exercise, but is switched off again as soon as glycogen stores are replenished.

Muscle: Exercise: Type 2 diabetes: Nutraceuticals

Animal cells take up fuel molecules such as glucose or fatty acids, and oxidise them to CO₂ via the process of catabolism. Much of the energy released during this process is used to convert ADP to ATP, which can be likened to the chemicals in a rechargeable battery. Extending this analogy, catabolism charges up the battery by converting ADP to ATP, whereas most other cellular activities (e.g. growth, division, secretion and movement) require energy and are driven by the conversion of ATP back to ADP, thus draining the battery. Just as machines that utilise rechargeable batteries (such as laptop computers or electric cars) require systems to monitor the state of the battery, cells require systems to monitor their ATP:ADP ratio and match the rates of uptake and consumption of carbon nutrients to the rate of ATP utilisation. The topic of this review is the AMP-activated protein kinase (AMPK), which is the major system responsible for achieving this task in eukaryotes. Researchers who wish to understand disorders of energy balance, such as obesity and type-2 diabetes, have been particularly interested in the system.

Protein kinases, including AMPK, are signalling enzymes that modify the function of target proteins by transferring phosphate groups from ATP to side chains of specific amino acids, usually serine or, less frequently,
AMPK and muscle metabolism

Fig. 1. Regulation of the AMP-activated protein kinase (AMPK) system. Numbers below the three forms of AMPK indicate their relative kinase activity. The upstream kinase (LKB1) continually converts AMPK to its phosphorylated form, increasing the activity 100-fold. However, in the absence of AMP it is rapidly converted back to the dephosphorylated, inactive form by protein phosphatases. Binding of AMP causes a conformational change that increases the activity a further 10-fold via an allosteric effect, and also prevents dephosphorylation. The activating signal, AMP, rises during metabolic stress because a rise in ADP causes displacement of the adenylate kinase reaction towards AMP. The system can also be activated by a rise in intracellular Ca\(^{2+}\), due to phosphorylation catalysed by the Ca\(^{2+}\)-activated kinase calmodulin-dependent kinase-\(\beta\) (CaMKK\(\beta\)).

Regulation, structure and evolution of AMP-activated protein kinase

Addition of AMP causes almost instant activation of AMPK by up to 10-fold\(^2\).\(^4\). Since this is caused by binding of AMP at a site distinct from the active site, it is referred to as allosteric activation. However, it is not the whole story: the author discovered that phosphorylation of AMPK at a specific site within the kinase domain (Thr-172), catalysed by distinct upstream protein kinases, causes \(>100\)-fold activation of AMPK\(^5\) (Fig. 1). This multiplies with the allosteric effect to give up to 1000-fold activation overall. Phospho-specific antibodies recognising AMPK phosphorylated at Thr-172, and antibodies recognising the downstream target ACC phosphorylated at the AMPK site (Ser-79) are now widely used as biomarkers to monitor AMPK activation. We and others identified the major upstream kinase phosphorylating Thr-172 to be a complex containing the protein kinase LKB1\(^(6,7)\). This was an exciting discovery, because LKB1 had been previously identified as a tumour suppressor, i.e. a gene product that inhibits the development of cancer. It now seems clear that AMPK mediates many of the tumour suppressor effects of LKB1, although discussion of this is beyond the scope of this review. The LKB1 complex appears to phosphorylate Thr-172 continuously under basal conditions, although the phosphate is normally immediately removed by protein phosphatases. However, the binding of AMP to AMPK causes a structural change that prevents dephosphorylation of Thr-172, thus triggering a net switch to the phosphorylated form, causing an activation that is further amplified by the allosteric mechanism. Thr-172 can also be phosphorylated by the Ca\(^{2+}\)-activated kinase calmodulin-dependent kinase-\(\beta\), which can trigger the activation of AMPK in response to agents that increase intracellular Ca\(^{2+}\) (Fig. 1), even in the absence of a rise in AMP\(^8\).\(^9\). AMPK occurs universally as heterotrimeric complexes, i.e. complexes formed from three non-identical protein subunits, these being the catalytic \(\alpha\) subunit (which contains Thr-172) and the regulatory \(\beta\) and \(\gamma\) subunits\(^10\). In human subjects each subunit is encoded by multiple genes, giving rise to up to twelve heterotrimeric combinations whose expression varies between cell types. The \(\beta\) subunits should cells express a protein kinase activated by AMP? A high ATP:ADP ratio, which is maintained by catalolysis in cells that have adequate supplies of glucose and oxygen, drives the reversible reaction catalysed by adenylate kinase (ATP + AMP \(\rightleftharpoons\) 2ADP) from left to right, keeping the AMP concentration low (up to 100-fold lower than ATP). However, any metabolic stress that causes a drop in the ATP:ADP ratio will cause some displacement of the adenylate kinase reaction to the left, producing AMP (Fig. 1). A rise in AMP is therefore a signal that the energy status of the cell has been compromised. Since deprivation for glucose or oxygen are two of the metabolic stresses that can cause an increase in AMP, the AMPK system acts as a nutrient sensor and is a key player in cellular sensing mechanisms for both glucose and oxygen.
contain carbohydrate-binding modules whose regulatory function will be discussed later, whereas the γ subunits contain two sites that bind the regulatory nucleotide, AMP(11,12). Why there should be two sites is not yet clear, but one explanation is that one is required for the allosteric effect of AMP and the other for the effect on dephosphorylation. Both sites also bind ATP in competition with AMP, but this fails to trigger the activating effects. Thus, AMPK is activated by an increase in the cellular AMP:ATP ratio, a signal that the energy status of the cell is compromised.

Genes encoding the α, β and γ subunits of AMPK are readily found in the genomes of all eukaryotes, ranging from unicellular organisms such as protozoa (e.g. Giardia lamblia) and fungi (e.g. budding yeast, Saccharomyces cerevisae), to multicellular organisms such as nematodes, insects, plants and mammals. In lower eukaryotes, the function of the AMPK relatives (orthologues) has been studied by genetics, and the results give interesting clues as to the probable function of AMPK during early evolution. Budding yeast lacking the complex are viable if always grown in a medium containing high glucose, but cannot grow in glucose-deprived media (13). In the nematode worm Caenorhabditis elegans, the AMPK orthologue is involved in response to dietary energy restriction. This organism was one of the model systems where the effect of dietary energy restriction to enhance longevity, which is now thought to occur also in mammals, was first defined. Restricting the diet during early development delays sexual maturation of the worms but also considerably extends their lifespan, and the AMPK orthologue is required for this response (14,15). In the plant Physcomitrella patens (a moss), the AMPK orthologue is not required for growth in continuous light, but is required for growth in alternate light–dark cycles (16). Since darkness for a plant is the equivalent of a period of starvation or fasting, this reinforces the view that the ancestral role of the AMPK system was in response to starvation for carbon nutrients.

**Activation of mammalian AMP-activated protein kinase by metabolic stresses**

If mammalian cells are deprived of glucose in culture, AMPK is rapidly activated (as in yeast) due to a reduced rate of glucose catabolism and a consequent increase in the AMP:ATP ratio (17). Most mammalian cells express GLUT1 or GLUT4, and the first step in subsequent glucose metabolism is catalysed by hexokinase. GLUT1, GLUT4 and hexokinase all exhibit high K_m values for glucose, and are thus saturated at normal plasma glucose concentrations. In these cells, the activation of AMPK therefore only occurs at very low glucose concentrations that may not be physiologically relevant. However, specialised ‘glucose-sensing’ cells, such as the β cells of the pancreas and specific neurones in the hypothalamus, express high K_m transmitters and glucose phosphorylating enzymes, i.e. GLUT2 and glucokinase, and fluctuations in glucose within the normal physiological range alter the rate of glucose metabolism and consequently modulate the AMPK activity. In these cells, the activation of AMPK appears to play important roles in the physiological responses to hypoglycaemia, such as increased secretion of glucagon and adrenaline by the pancreas and adrenal medulla (17–20).

Deprivation of oxygen (hypoxia) is another stress that can limit catabolism, thus causing increases in AMP:ATP and AMPK activation. This occurs, for example, in cardiac muscle in response to ischaemia, an interruption in the blood supply (21–23). Similar to what has just been described for hypoglycaemia, in most cells the oxygen tension has to drop to pathologically low levels (such as occurs during ischaemia) before AMPK is activated. However, just as there are specialised glucose-sensing cells, there are also specialised oxygen-sensing cells, such as the Type 1 cells in the carotid body, in which AMPK is activated by more physiological levels of hypoxia. Type 1 cells release neurotransmitters onto afferent nerves, signalling to the brain to regulate breathing in response to the oxygen content of the blood reaching it via the carotid artery. Other oxygen-sensing cells include the smooth muscle cells lining pulmonary arteries, which (opposite to what happens in most arteries) contract in response to hypoxia, in order to divert blood flow to more oxygenated regions of the lung. In both cell types, mitochondrial catabolism appears to be particularly sensitive to decreasing oxygen tension so that AMPK is activated, triggering changes in Ca^{2+} movement that promote either neurotransmitter release (Type 1 cells) or contraction (pulmonary smooth muscle) (24–26).

The stresses discussed thus far activate AMPK by inhibiting ATP synthesis. A metabolic stress that activates AMPK by accelerating ATP consumption, and which occurs under normal physiological conditions, is muscle contraction. With Will Winder, the author found that AMPK is activated in rodent skeletal muscle during treadmill exercise (27), and this has been confirmed many times using biopsies of human muscle. In a later section, I will focus on the metabolic effects that occur downstream of AMPK in contracting muscle.

**Regulation of AMP-activated protein kinase by adipokines and other cytokines**

As discussed earlier, the ancestral role of AMPK in unicellular eukaryotes appears to have been in response to starvation for a carbon source. In mammals, plasma glucose is maintained within tight limits by hormonal homoeostasis, and so glucose deprivation is less of an issue. Nevertheless, AMPK is still ubiquitously expressed. It appears that during the evolution of multicellular organisms, adaptations occurred to allow other types of input to interact with the system. This is illustrated by the effects of the adipokines, cytokine-like substances released by adipocytes, of which leptin is the classical example. Leptin activates AMPK in skeletal muscle, accounting for its ability to stimulate fatty acid oxidation (see the next section) and hence energy expenditure (28). The more well-known role of leptin is to repress appetite (and hence energy intake) via effects on the hypothalamus, and Kahn...
and co-workers have suggested that this is mediated by inhibition of AMPK (29). Results with knock-out mice that lack AMPK in hypothalamic neurones have not fully supported this model, but do support the idea that AMPK is involved in glucose sensing in the hypothalamus (19). There is also widespread agreement that treatments which activate AMPK in the hypothalamus, such as infusion with the nucleoside 5-aminoimidazole-4-carboxamide riboside, treatment with the gut hormone ghrelin or cannabinoids, or hypoglycaemia, all increase food intake in rodents (30–32).

The other well-known adipokine is adiponectin, whose plasma concentration (in contrast to leptin) is paradoxically reduced in obese or insulin-resistant individuals. Adiponectin, acting via the AdipoR1 receptor (33), activates AMPK in the liver and other tissues, and this appears to be responsible for many of its effects, including suppression of gluconeogenesis in the liver (34) and enhancement of food intake via effects on the hypothalamus (35). Finally, a number of cytokines, hormones and nutrients have been reported to modulate AMPK activity, accounting for diverse effects in different cell types, including IL-6 (36), ciliary neurotrophic factor (37,38), macrophage inhibitory factor (39) and lipoic acid (40). In most of these cases, the exact mechanisms by which these agents activate AMPK, whether through changes in AMP:ATP or some other mechanism, remain unclear.

**Metabolic effects of AMP-activated protein kinase activation during muscle contraction**

The metabolic consequences of AMPK activation have been particularly well studied in skeletal muscle. AMPK is responsible not only for acute responses to exercise, occurring within seconds or minutes, but also for longer-term adaptations to repeated exercise caused by changes in gene expression. AMPK is mainly activated during prolonged exercise and may not be involved in responses to short bouts of resistance exercise, such as weight-lifting. In this case, much of the ATP is generated by the conversion of phosphocreatine to ATP, so that there is little change in the content of ATP, ADP or AMP. As phosphocreatine becomes depleted during an event such as a sprint, glycogen breakdown and anaerobic glycolysis then become important for the generation of ATP. However, once again these effects do not require AMPK, because the key regulatory enzymes involved (phosphorylase and phosphofructokinase) directly bind AMP and are activated by increases in the AMP:ATP ratio independent of AMPK. AMPK does become crucial in the switch from anaerobic to aerobic metabolism that is required for endurance exercise, for example, in running events of more than 200 m. During prolonged exercise, the use of aerobic metabolism and blood-borne fuels such as glucose and fatty acids becomes increasingly important, and this is where AMPK plays a key role. Intriguingly, one of the roles of the AMPK orthologue in budding yeast is to trigger the switch from fermentation (anaerobic metabolism) to the more efficient oxidative metabolism when the glucose in the medium starts to become depleted (33).

The acute increase in glucose uptake in skeletal muscle in response to contraction is caused by a translocation of the GLUT4 from intracellular storage vesicles to the plasma membrane. The first evidence that this might be mediated by AMPK came from experiments by Winder and the author using the nucleoside, 5-aminoimidazole-4-carboxamide riboside. This is taken up by cells and converted to the nucleotide 5-aminoimidazole-4-carboxamide riboside monophosphate, which mimics the activating effects of AMP on the AMPK system. When rat skeletal muscle was perfused with 5-aminoimidazole-4-carboxamide riboside, AMPK was activated and this was associated with increased glucose uptake (41). Subsequent studies with GM mice, in which AMPK activation during contraction was rendered defective by various means, confirmed that AMPK was involved in this effect (42, 43). There may be multiple mechanisms by which AMPK promotes GLUT4 translocation to the plasma membrane, but one appears to be the phosphorylation of a protein called TBC1D1 (TRE2/BUB2/CDC16 domain family member 1), which enhances GLUT4 translocation by promoting the conversion of small guanine nucleotide-binding proteins called Rabs from their inactive (Rab:GDP) to their active (Rab:GTP) forms (44, 45). This is similar to the mechanism by which insulin stimulates glucose uptake in resting muscle, when the insulin-activated protein kinase B phosphorylates TBC1D1 or other members of the same family. In insulin-resistant individuals, insulin-dependent activation of protein kinase B is defective, but the mechanism by which contraction stimulates glucose uptake via AMPK remains unaffected (46). This is one reason why regular exercise is particularly beneficial in insulin-resistant subjects.

Another catabolic pathway activated during low-intensity exercise is fatty acid oxidation. Muscle expresses the ACC2 isoform of ACC which, like the ACC1 isoform expressed in most other cells, is phosphorylated and inactivated by AMPK (47). ACC2 is associated with mitochondria and produces malonyl-CoA, an inhibitor of the enzyme carnitine-palmitoyl transferase-1 located on the outer mitochondrial membrane. Carnitine-palmitoyl transferase-1 is required for the uptake of fatty acids into mitochondria, and is inhibited in resting muscle by the malonyl-CoA produced by ACC2 (48). Inhibition of ACC2 by AMPK during contraction therefore promotes fatty acid uptake into mitochondria and hence oxidation (41). Interestingly, excessive storage of fatty acids as TAG in muscle, as in other tissues, is associated with insulin resistance. Stimulation of fat oxidation by AMPK is therefore another reason why exercise is beneficial in insulin-resistant subjects.

As well as these acute effects on muscle metabolism, AMPK is also responsible for some of the longer-term adaptations induced by repeated endurance exercise training. This includes increased expression of GLUT4 (49), which may be mediated in part by phosphorylation of a histone deacetylase that modifies the chromatin structure at the GLUT4 promoter (50). AMPK activation also up-regulates mitochondrial biogenesis by up-regulating the transcriptional co-activator PPARα co-activator-1α, which promotes the expression of mitochondrial genes encoded in both nuclear and mitochondrial DNA. AMPK has been reported to directly phosphorylate PPARα co-activator-1α (51), but the mechanism may also involve deacetylation
of PPARγ co-activator-1α by Siruatin-1, which is activated downstream of AMPK(52).

These longer-term adaptations to endurance exercise would ensure that, as exercise is repeated regularly, it becomes easier because glucose is taken up more rapidly and mitochondrial ATP production is faster. Because both glucose uptake and mitochondrial function tend to be reduced in individuals at risk of developing type-2 diabetes(53), AMPK can also help to explain the ability of regular exercise to provide protection against the development of diabetes.

Muscle contraction causes very rapid ATP consumption and it therefore makes sense that other ATP-requiring processes, such as biosynthesis, should be switched off. Another target for AMPK in skeletal muscle is glycogen synthase, the enzyme catalysing the last step in glycogen synthesis that also controls its rate(54,55). AMPK phosphorylates glycogen synthase at site 2, reducing its activity(64). This may help to ensure that when glucose uptake increases during contraction, the increased flux of glucose into the cell is diverted towards catabolic breakdown of glucose rather than glycogen synthesis. Paradoxically, however, immediately after exercise glycogen synthase is found to be dephosphorylated immediately following exercise(56,62). This mechanism would ensure that, if glycogen became significantly depleted, it would be rapidly replenished as soon as exercise ceased. If this model is correct, AMPK not only monitors the short-term availability of energy in the form of ATP and ADP, but also the medium term reserves in the form of glycogen. It also means that AMPK is involved in the rapid glucose uptake that occurs following exercise to replenish glycogen, another of the beneficial outcomes of exercise in individuals who are insulin resistant or hyperglycaemic.

Another biosynthetic pathway that is switched off, at least temporarily, during exercise is translation of mRNA into protein. An important signalling pathway that stimulates translation has as its central component the mammalian target-of-rapamycin (mTOR). mTOR is a protein kinase that is stimulated by insulin and by amino acids, and it promotes protein translation by regulating its initiation(63). AMPK activation, on the other hand, inhibits mTOR by phosphorylating multiple upstream regulators(64,65), and thus inhibits protein synthesis. There is evidence that this mechanism does partly account for a temporary cessation of translation in muscle during contraction(66,67). Interestingly, mTOR is also activated during resistance exercise training(68) as well as by insulin and by feeding amino acids, and this may account for the protein synthesis and hence muscle hypertrophy induced by all of these inputs. Since AMPK opposes mTOR activation, this may help to explain why endurance exercise training does not cause muscle hypertrophy, and why endurance athletes have less bulky muscles compared to weight-lifters or sprinters.

Regulation of AMP-activated protein kinase by drugs and ‘nutraceuticals’

In the previous section, I stressed the numerous metabolic changes caused by AMPK activation in muscle, which are particularly beneficial in individuals at risk of developing insulin resistance and/or type-2 diabetes. AMPK activation in the liver, which occurs in response to adiponectin, is also beneficial by repressing production of glucose by gluconeogenesis(34,69), which is at least partly responsible for the tendency of insulin-resistant individuals to hyperglycaemia. AMPK activation in the liver also promotes fat oxidation(34), thus reducing TAG accumulation, a metabolic state that is strongly associated with insulin resistance. It is therefore not surprising that AMPK has become an exciting target for new drugs aimed at the treatment of type-2 diabetes(70). In fact, the biguanide metformin, which was developed from an ancient herbal remedy and is now the first choice drug for treatment of type-2 diabetes, activates AMPK(71), and there is good evidence from rodents that this accounts for its ability to lower plasma glucose(72).

The author has recently shown that metformin activates AMPK indirectly by causing a mild inhibition of complex I of the respiratory chain, thus increasing cellular AMP:ATP(73). Despite this rather crude mechanism of action, metformin is a safe drug that is now prescribed to >100 million patients worldwide. A number of other xenobiotics or ‘nutraceuticals’ derived from foods and beverages, or from traditional herbal medicines, also activate AMPK and have been claimed to have beneficial effects in obesity, type-2 diabetes and even cancer. These include resveratrol, present in small quantities in red wine, quercetin present in many fruit and vegetables and berberine, a yellow dye derived from plants of the genus *Berberis* used in...
traditional Chinese medicine. We have recently reported that all of these activate AMPK due to their ability to cause inhibition of mitochondrial ATP production (73). They are all secondary metabolites of plants, and an interesting speculation is that they may be produced by the plant to prevent or discourage grazing by animals, or infection by micro-organisms. In support of this, the natural product galegine (from which metformin is derived and which itself is a potent AMPK activator (73)), is produced by the plant Galega officinalis, which also goes by the name of Goat’s Rue and is classed as a noxious weed in USA because it is poisonous to herbivores. Also, resveratrol is produced by grapes in response to fungal infection, which is why its concentration is often higher in organic wines where the grapes have not been treated with fungicides.

Conclusions

AMPK is an ancient system that arose during early eukaryotic evolution, where its role may have been in response to starvation for a carbon source. In mammals, by monitoring the rate of catabolism, it can act in specialised cells as a sensor of either glucose or oxygen. In skeletal muscle it is activated by contraction and appears to be responsible for many of the acute metabolic changes and the longer-term adaptations induced by exercise. Through its regulation by adipokines and other signal molecules, AMPK is also a regulator of energy balance at the whole body level, and drugs that are used to treat disorders of energy balance, such as metformin, now appear to work by activating AMPK.

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