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The Role of AMP-Activated Protein Kinase in Obesity

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Abstract

AMP-activated protein kinase (AMPK) is a major regulator of energy metabolism at both the cell and at the whole body level. Numerous genetic and obesity models as well as human studies have suggested a role for AMPK in the physiological regulation of fatty acid and glucose metabolism, and in the regulation of appetite. Changes in AMPK activity have been reported in obesity, type 2 diabetes, the metabolic syndrome and cardiovascular disease, which jointly represent a major health and economical problem worldwide. Whether AMPK changes are one of the causes or the consequence of these pathological conditions remains a matter of debate, but AMPK clearly represents a major potential pharmacological target in the treatment of these conditions.

Obesity is a major health and economic problem in both Western and developing societies. Its continuing rise in prevalence, 20% in England and 30% in USA [1, 2] seems to be unstoppable despite multiple efforts to attempt to halt this trend. Obesity is characterised by multiple metabolic changes such as insulin resistance, dyslipidaemia and hypertension. The diseases arising as a consequence of obesity such as type 2 diabetes (T2D), cardiovascular disease and certain cancers, are increasingly important causes of morbidity and mortality. In the last decades, a huge amount of research has been dedicated to the study of the complex pathophysiology of obesity and to the research for new medical therapies.

AMP-activated protein kinase (AMPK) has emerged in the last years as a major regulator of cell and whole body metabolism. Numerous papers have reported evidence for its role in the regulation of appetite, of body weight and of metabolism [3–5]. Therefore, it is natural to consider AMPK as a major player in the development of obesity. The AMPK complex is an evolutionally conserved serine/threonine heterotrimer kinase complex consisting of α-, β- and γ-subunits [for detailed reviews see 5, 6]. AMPK is

activated by cellular stress, which depletes cellular ATP leading to a concomitant rise in AMP. AMP activates AMPK by three distinct mechanisms: (a) allosteric activation, (b) stimulation of phosphorylation of the α-subunit on Thr172 by upstream kinase(s) [LKB1 and calmodulin kinase kinase-α or -β and recently a new possible AMPK kinase candidate, the transforming growth factor-β-activated kinase (TAK1), which phosphorylates AMPK on Thr-172 in HeLa cells [7], has been reported], and (c) inhibition of dephosphorylation by protein phosphatases [5, 8–10]. Cellular stresses that cause a rise in the AMP/ATP ratio include metabolic poisons (arsenite, oligomycin), oxidative stresses, hypoxia, low glucose, muscle contraction and nutrient deprivation. Osmotic stress also activates AMPK even without a change in the AMP/ATP ratio. Once activated, AMPK switches off anabolic pathways such as gluconeogenesis, glycogen, fatty acid, triglyceride, cholesterol and protein synthesis (mTOR-p70SK-E2 pathway), and switches on catabolic pathways such as glycolysis, glucose uptake, and fatty acid oxidation. It also leads to mitochondrial biogenesis, which improves the ATP synthesis capacity of the cell [11]. Metabolic changes induced by AMPK are both acute changes due to phosphorylation of key enzymes and longer-term effects on the expression of genes involved in metabolic regulation. AMPK, through several mediators, plays a role in various physiological and pathological processes in different tissues (fig. 1). Therefore, it was logical to hypothesise that abnormal AMPK activity would be present in conditions of deregulated energy balance, such as obesity and T2D.

Role of AMPK in Normal Physiology

Role of AMPK in Skeletal Muscle Metabolism

Skeletal muscle is the major site of glucose uptake [12], a process that is mainly stimulated by insulin but also by other alternative pathways. Exercise stimulates glucose uptake in the skeletal muscle independently of the insulin pathway and AMPK appears to be the mediator of this effect, primarily in the glycolytic white muscle. These conclusions derived from studies in which in vivo AMP-mimetic 5-aminoimidazole-4-carboxamide ribonucleoside (AICAR) treatment stimulated glucose uptake [13]. The effect was not inhibited by the inhibition of the insulin-dependent PI3K pathway and was additive to insulin-stimulated glucose uptake [14]. AICAR also stimulates glucose transporter GLUT4 expression [15, 16] and its translocation to the cell membrane in rat skeletal muscles [17]. Chronic AMPK activation also increases the expression of hexokinase II, the first enzyme of the glycolysis pathway [18] and inactivates glycogen synthase [19]. The effect of AMPK is fibre dependent and is different in resistance (weight lifting) or endurance (distance running) exercise. AMPK stimulates glucose uptake and GLUT4 expression/transport in fast-twitch (glycolytic, white) muscle but not in slow-twitch (oxidative, red) muscle [20]. AMPK in muscle is activated during exercise, probably as a result of the exercise-induced IL-6 release, a cytokine which activates AMPK in isolated rat muscles [21]. Moreover, it seems that

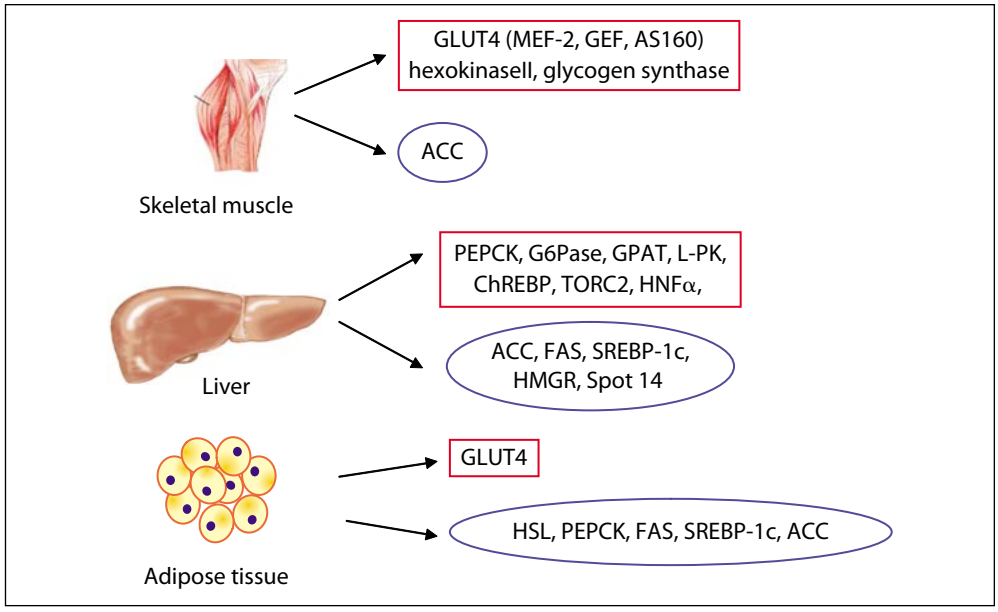

Fig. 1. Metabolic targets of AMPK in muscle, liver and adipose tissues. AMPK regulates the expression and phosphorylation of enzymes and genes involved in glucose and lipid metabolism. GLUT4 = Glucose transporter 4; MEF-2 = myocyte enhancer factor-2; GEF = GLUT4 enhancer factor; AS-160 = Akt-substrate-of-160 kDa; ACC = acetyl-coenzyme A carboxylase; PEPCK = phosphoenolpyruvate carboxykinase; G6Pase = glucose-6-phosphatase; GPAT = glycerol-3-phosphate acyltransferase; L-PK = L-pyruvate kinase; ChREBP = carbohydrate response element-binding protein; TORC2 = transducer of regulated CREB activity 2; HNFα = hepatic nuclear factor α; FAS = fatty acid synthase; SREBP-1c = sterol regulatory element binding protein-1; HMGR = 3-hydroxy-3-methylglutaryl-coenzyme A reductase; HSL = hormone-sensitive lipase.

only endurance exercise and not resistance exercise can induce AMPK activation [20, 22]. AMPK activation in endurance exercise could also explain the lack of muscle hypertrophy in distance running in contrast to weight lifting. This is possibly due to the effect of AMPK on the mTOR pathway [20]. The mTOR pathway stimulates protein synthesis and hence cell growth and hypertrophy in response to growth factors and amino acids. Therefore, AMPK inhibition of this pathway would result in inhibition of protein synthesis and lack of muscle hypertrophy. AMPK also stimulates fatty acid oxidation in muscle. This results in lower lipid deposition and increases the ability of the muscle to meet energy needs by increasing glucose uptake and fatty acid oxidation as well. Studies with transgenic animals (AMPK α1 and α2 knockout mice, muscle-specific over-expression of dominant negative AMPK α2, AMPK γ3 knockout, muscle-specific over-expression of AMPK γ3 and muscle-specific over-expression of AMPK γ3 R225Q overactive mutant and skeletal muscle-specific LKB1 knockout [for detailed descriptions, see 20, 23]), have provided further evidence for AMPK being the main mediator, although not the only one, of the adaptations (i.e. increased

glucose uptake, fatty acid oxidation, inhibition of glycogen synthesis) of skeletal muscle in response to exercise.

Role of AMPK in Liver Metabolism

The liver is the major site for storage and release of carbohydrates and for fatty acid synthesis. It responds to fasting with increased glucose output and increased fatty acid oxidation, while in post-prandial conditions liver glucose uptake increases with consequent glycogen and triglyceride synthesis [24]. AMPK regulates liver lipid and glucose homeostasis via phosphorylation of multiple enzymes (e.g. ACC1 – ↓ lipid synthesis, ACC2 – ↑ lipid oxidation, 3-hydroxy-3-methylglutaryl-coenzyme A reductase – ↓ cholesterol synthesis, glycerol-3-phosphate acyltransferase – ↓ glycerolipid synthesis), and influences the expression of genes involved in gluconeogenic, glycolytic and lipogenic processes and their upstream regulators [for a comprehensive review on the topic, see 25]. Therefore, overall AMPK activation in the liver results in inhibition of gluconeogenesis, fatty acid, triglyceride and cholesterol synthesis, and stimulation of fatty acid oxidation. Changes in hepatic metabolism are certainly present in obesity and T2D. Elevated glucose production by the liver is the major cause of fasting hyperglycaemia, and it is possible that AMPK activation by decreasing gluconeogenesis and cholesterol synthesis could be beneficial in these patients. Nevertheless, one needs to be cautious as AMPK activation, by increasing fatty acid oxidation and ketogenesis, might lead to ketoacidosis, and by inhibiting protein synthesis might lead to a negative nitrogen balance together with enhanced urea synthesis [25].

Role of AMPK in Adipose Tissue Metabolism

Adipose tissue has been considered for decades simply as an energy storage organ, while in the last years it has emerged as an active endocrine organ, which by secreting several proteins, known as adipokines, contributes to the regulation of appetite and metabolism. AMPK α1 subunit is the prevalent AMPK subunit expressed in the adipose tissue [26 and our own unpublished data]. AMPK regulates lipogenesis and lipolysis in adipose tissue. Activation of AMPK in rodent adipocytes leads to a decreased fatty acid uptake, decreased triglyceride synthesis and increased fatty acid oxidation via inhibition of ACC1 and ACC2 and, as in the liver, inhibition of the expression of lipogenic genes [27, 28].

During fasting, lipolysis is activated in adipose tissue in order to provide fatty acids and glycerol as fuels for peripheral tissues, but reports on the effect of AMPK activation on lipolysis are contradictory. There is evidence that AMPK activation, either by AICAR or by over-expression of a constitutively active AMPK isoform or by biguanide treatment, has an inhibitory effect on lipolysis [26, 29]. In conditions where lipolysis is activated, such as fasting and exercise, AMPK is also activated but as a feedback mechanism this activation leads to inhibition of lipolysis, which is an energy-consuming process for the adipocytes [27]. Furthermore, in the AMPK α1 knockout mice, the size of the

adipocytes is reduced and basal and isoprotenerol-induced lipolysis is higher than that of control adipocytes [26]. On the contrary, the study of Yin et al. [30] suggested a lipolytic action for AMPK and the study by Koh et al. [31] suggested that the adrenaline-induced lipolysis is due to AMPK activation. There are also contradictory findings related to the effect of AMPK on glucose transport in adipose tissue [32–34].

In conclusion, AMPK activation in adipose tissue, under conditions such as exercise, fasting or after stimulation with leptin, adiponectin or biguanides, decreases lipogenesis, triglyceride synthesis and lipolysis and increases fatty acid oxidation, contributing therefore to improved insulin sensitivity.

Role of AMPK in Endocrine Pancreas

The effects of AMPK activation in β-cells are complex: further data for the role of AMPK in endocrine pancreas are available in the chapter by Rutter and Parton [this vol., pp. 118–134]. AMPK might be involved in the expression of insulin receptor family members, such as the IGF-I receptor, insulin receptor and insulin receptor-related receptor, which are mandatory for several steps in insulin secretion [35], while AICAR increases the phosphorylation of insulin receptor substrate-1 (IRS-1) on Ser789 leading to increased IRS-1 activity [35]. On the other hand, AICAR and metformin inhibit rapid insulin release [35] and the activation of AMPK also enhances β-cell apoptosis; it remains to be determined if this is the cause or the consequence of the altered glucose metabolism [36–38]. AMPK appears to be a key regulator of hepatocyte nuclear factor-4α, which is linked to type 1 maturity-onset diabetes of the young [for further details, see 36]. The overall effect of AMPK on glucose homeostasis [6, 36] is determined by the joint effect on insulin secretion in addition to the prominent effects of AMPK activation on glucose transport, gluconeogenesis and glycogenolysis, in muscle and liver.

Role of AMPK in Hypothalamus

The role of AMPK in the regulation of body weight and energy homeostasis is not limited to its actions in the peripheral tissues. AMPK is a central regulator of food intake. AMPK mediates the effects of multiple orexigenic and anorexigenic signals in the hypothalamus [35]. Fasting increases and refeeding decreases the AMPK activity in the hypothalamus [39]. The downstream pathways of AMPK in the hypothalamus could involve the ACC-malonyl-CoA-CPT1 pathway [3] and the mTOR pathway [40, 41] (fig. 2). Leptin and changes in glucose concentration affect the activity of glucose-inhibited cells (40% of which are NPY-expressing neurons) in the hypothalamus via AMPK [42]. Actually, AMPK activity in the hypothalamus is probably responsible for some of the peripheral effects of leptin, of hypoglycaemia and of the FAS inhibitor C75 [3, 35], emphasising the complexity of the regulation of whole body metabolism and the role of AMPK, being not only a peripheral or a central mediator but also a key enzyme in coordinating the interaction between peripheral and central energy regulation.

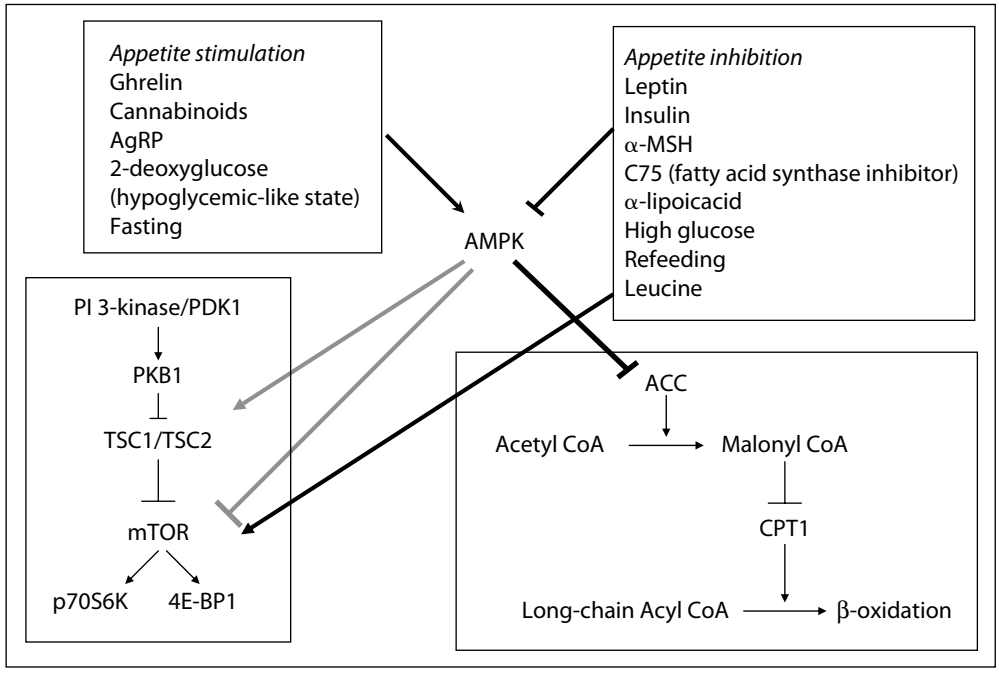

Fig. 2. Regulation of hypothalamic AMPK and possible downstream pathways. Black lines show pathways established in the hypothalamus, grey lines show pathways that have been described in rat muscle, rat liver, myotubes, hepatocytes, fibroblasts and lung carcinoma cells [97, 98] but not directly in the hypothalamus.

AMPK as a Mediator of Action of Metabolically Active Hormones

AMPK mediates the effects of many hormones/peptides/substances/drugs in numerous physiological and pathological processes. Insulin, leptin, adiponectin, cannabinoids and ghrelin influence peripheral metabolism at least partially via activation or inhibition of AMPK activity in the skeletal muscle, liver, adipose tissue and the hypothalamus (table 1) [35]. AMPK has been found to be the mediator of many hormones and its role in the interplay between these compounds and their metabolic effects is being actively investigated [for a detailed review on the topic, see 35].

AMPK in Animal Models of Obesity

Animal models of obesity and diabetes have provided evidence for implication of AMPK in the pathogenesis of these conditions and also provided evidence for a possible role of AMPK modulators in their treatment (table 2).

Table 1. Effect of hormones and drugs on AMPK activity in different tissues [modified and updated from 35]

Hormone/ substance	Hypothalamus	Skeletal Muscle	Liver	Adipose tissue	Pancreas: β-cells	Cardiac muscle
High glucose	⇓[39, 60, 61]	⇓[36]	⇓[62]		⇓[36, 63, 64]	
Insulin	⇓[39]	⇒[36]	⇒[50]	⇒[36] ⇓[30]	⇒[36]	⇓[65]
Leptin	⇓[39, 42, 66]	⇑[67, 68]	⇑[46, 50, 69, 70]	⇑[71]	⇒[63]	⇒[72, 73]
Ghrelin	⇑[66, 74]	⇒[74]	⇓[74, 75]	⇓[74]		⇑[74]
Adiponectin		⇑[76–78]	⇑[50, 76, 77]	⇑[33]	⇑[79]	⇑[80, 81]
Resistin		⇓[82]	⇓[83, 84]			
Glucagon			⇑[85]			
Cannabinoids	⇑[74]	⇒[74]	⇓[74]	⇓[74]		⇑[74]
Metformin	⇓[60]	⇑[86]	⇑[62, 86]	⇑[87]	⇑[37, 63]	⇑[88]
Rosiglitazone		⇑[54, 89, 90]				

References are listed in brackets. ⇑ = Stimulation; ⇓ = inhibition; ⇒ = no change.

Martin et al. [43] showed that diet-induced obesity (DIO) in mice alters the effect of leptin on AMPK activity both in skeletal muscle and in the hypothalamus. Leptin increases AMPK activity in the skeletal muscle of chow-fed mice and decreases it in the hypothalamus of the same animals but does not have an effect in the DIO mice. While, most interestingly, a ciliary neurotrophic factor analogue (CNTFAx15) given intracerebroventricularly not only reduces food intake in high-fat diet (HFD) mice but also suppresses hypothalamic AMPK activity, bypassing therefore diet-induced leptin resistance [44]. Rats on an HFD for 5 months exhibited decreased AMPK phosphorylation and expression in skeletal muscle associated with decreased levels of ACC and GLUT4 as well. Metformin treatment restored insulin sensitivity and increased AMPK activity [45].

In Zucker rats who do not respond to leptin treatment because of defects in the leptin receptor, administration of the AMPK activator AICAR results in leptino-mimetic effects, leading to the prevention of ectopic lipid deposition and diabetes [46]. Transgenic mice over-expressing leptin in liver are lean on a chow diet but despite the high pre-existing leptin levels become obese and insulin resistant on an HFD [47]. HFD for 15 weeks abolishes the increase in muscle AMPK activity observed in the same animals on a chow diet.

Short hepatic over-expression of a constitutively active form of AMPK decreased blood glucose levels in normal mouse, abolished hyperglycaemia in streptozotocin-induced and in *ob/ob* mice and also reduced gluconeogenic enzyme expression. The resulting low glucose levels led to a switch from glucose utilisation to fatty acid utilisation, associated with a decrease in white adipose tissue mass and development of fatty liver [48].

Table 2. AMPK changes in animal models of obesity

Animal model	AMPK-related changes	Reference
Obese Zucker *fa/fa* rat	AICAR increased muscle glucose transport and suppresses endogenous glucose production and lipolysis	[91, 92]
	Reduced AMPK and ACC phosphorylation LKB1 activity and PGC-1 content	[90]
	Rosiglitazone restores AMPK α2 activity in skeletal muscle	[46, 93]
	Chronic AICAR/exercise training prevented hyperglycaemia and increased whole-body insulin sensitivity	
ob/ob and *db/db* mice	AICAR and short hepatic over-expression of a constitutively active form of AMPK decreased blood glucose levels	[48, 94]
HFD in rats	Reduction of AMPK activity, ACC and GLUT4 levels in skeletal muscle. Metformin increases AMPK activity	[45]
	Rosiglitazone enhanced AICAR-stimulated glucose uptake in muscle and adipose tissue. Total AMPK and AMPK α2 activity increased in muscle	[95]
DIO mouse	AICAR administration blocked weight gain, reduced total content epididymal fat and lipid accumulation in adipocytes, restored adiponectin levels, improved glucose tolerance and insulin sensitivity	[96]
	DIO mice compared to chow-fed mice ate less, had lower respiratory exchange rate and lower ACC activity in muscle. Leptin did not improve either of these parameters or the AMPK α2 activity in muscle and hypothalamus of the DIO	[43]
	Ciliary neurotrophic factor analogue reduced food intake and AMPK hypothalamic activity, bypassing therefore diet-induced leptin resistance	[44]

Adiponectin inhibits glucose production in wild-type mouse and also in T2D mouse (*ob/ob*, non-obese diabetic or streptozotocin-treated mice) [49] and the effect of adiponectin is completely dependent on the presence of hepatic AMPK α2 subunit [50].

Studies on *ob/ob* and adiponectin double knockout mice or knockout only for adiponectin showed an impaired ability to improve glucose tolerance with rosiglitazone treatment and this was, at least partly, due to reduced activation of AMPK [51]. These results not only showed the role of adiponectin as a TZD mediator but also confirm the importance of AMPK activation in the mechanism of action of TZD type anti-diabetic drugs.

AMPK in Human Obesity

The majority of the research studies published have been performed on animals, and it is important to establish that their conclusions can be extrapolated to human physiology and pathology as the number of studies about AMPK activity in human diseases is much more limited. Skeletal muscle AMPK activity has been analysed in a limited number of obese vs. lean subjects, in obese diabetic versus obese non-diabetic patients and in healthy subjects before and after exercise. Obesity in humans is associated with leptin and insulin resistance and lipid accumulation. Adiponectin or AICAR activate muscle AMPK in obese rodents, which stimulates fatty acid oxidation, and it is reasonable therefore to hypothesise that pharmacological activation of AMPK might be of therapeutic benefit in human obesity. However, AMPK is not down-regulated in human skeletal muscle of obese females [52] and AMPK activity and specific isoform expression are similar in muscle of obese subjects with and without T2D [53]. These data suggest that impaired insulin action on glycogen synthesis and lipid oxidation in skeletal muscle of these patients is unlikely to involve changes in AMPK expression and activity. However, AICAR treatment of muscle biopsies stimulated AMPK $\alpha 2$ activity and fatty acid oxidation, suggesting that AMPK activation above basal levels may still be a valid therapeutic approach [52]. In contrast to the previous studies, Bandyopadhyay et al. [54] showed that there is a decrease in AMPK activity and an increase in ACC activity in insulin-resistant muscle from obese and from T2D patients that results in elevated intracellular levels of malonyl-CoA. Because, for the most part, the defects appear to be expressed equally in the obese subjects and in T2D subjects (who were also obese), the authors conclude that these differences from lean control subjects are caused by insulin resistance/obesity rather than hyperglycaemia/diabetes. Finally, when the T2D subjects were treated for 3 months with rosiglitazone, the various defects in fatty acid and mitochondrial metabolism reverted towards normal. The beneficial effect of AMPK activation in muscle was demonstrated in a study which showed that acute intensive exercise (3 h) increased AMPK and ACC phosphorylation altogether with an increase in expression of adiponectin receptor in the skeletal muscle of 5 healthy females [55]. Interestingly, Roepstorff et al. [56] showed that AMPK activation in muscle is sex-dependent: 90 min of exercise activated AMPK in skeletal muscle of healthy male volunteers but in contrast to the former study, not in females. Further data are needed to study the role of oestradiol on skeletal muscle AMPK activity. The effect of exercise on AMPK is probably due, at least in part, to IL-6, which is synthesised and released from skeletal muscle in large amounts during exercise [57], and in rodents, the resultant increase in IL-6 concentration correlates with increases in AMPK activity in multiple tissues. There are no direct data of the effect of IL-6 on AMPK activity in humans but IL-6 treatment was recently shown to enhance insulin-stimulated glucose disposal in humans in vivo [58].

Role of AMPK in Cardiovascular Disease

Cardiovascular disease is a common consequence of obesity and obese patients are often treated for hypertension, atherosclerosis, and heart failure. AMPK is a key regulator of energy metabolism in the heart, too [for an extensive review on the topic, see 59]. The obvious beneficial effects of AMPK activation in ischaemia could be counterbalanced by the excessive fatty acid oxidation and reduced glucose oxidation leading to accumulation of pyruvate and protons [59]. In view of this and in view of the fact that AMPK is proposed as a possible target for obesity and diabetes treatments, it is important to know the role of AMPK in cardiac physiology and pathology in order to avoid possible side effects of future AMPK activators/inhibitors.

AMPK as an Overall Metabolic Regulator

In conclusion, AMPK has emerged as a key regulatory enzyme of cell and whole body metabolism. It influences cell metabolism in a way that favours insulin sensitivity and maintains a favourable body energy homeostasis. It is the mediator of the metabolic effects of many of the known hormones, nutrients and drugs. Thus, not only are changes in AMPK implicated in the pathogenesis of insulin-resistant states, but AMPK might also constitute a target for new treatments of these conditions. However, a note of caution is required as generalised AMP activation might result in unwanted effects (i.e. an appetite-stimulating effect and β-cell inhibition), and thus there is a need for tissue-specific modulators.

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